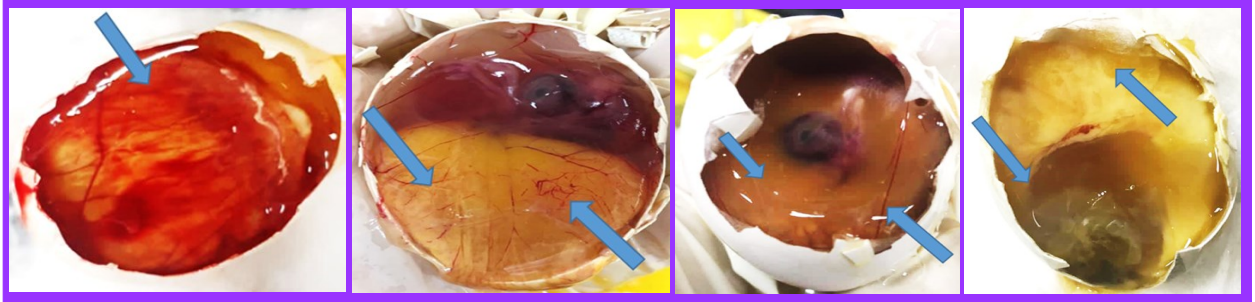

BJP

Bangladesh Journal of Pharmacology

Research Article

Morphological and histopathological alterations caused by meloxicam in chick fetuses

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info; www.bdpsjournal.org
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
Bangladesh J Pharmacol 2022; 17: 147-153
ISSN: 1991-0088

Morphological and histopathological alterations caused by meloxicam in chick fetuses

Mervat Ahmed AbdRabou[1] and Aljohara M. Al- Otaibi[2]

[1]Department of Biology, College of Science, Jouf University, P.O. Box: 2014, Sakaka, Saudi Arabia; [2]Department of Biology, College of Science, Princess Nourah Bint Abdulrahman University, 13225, Saudi Arabia.

Article Info

Received: 14 October 2022
Accepted: 1 November 2022
Available Online: 19 November 2022
DOI: 10.3329/bjp.v17i4.62233

Cite this article:
AbdRabou MA, Al-Otaibi AM. Morphological and histopathological alterations caused by meloxicam in chick fetuses. Bangladesh J Pharmacol. 2022; 17: 147-153.

Abstract

During pregnancy, the administration of certain drugs can cause harm to the unborn child. The purpose of the present study was to investigate the impact of meloxicam on morphological and histopathological changes in chick embryo. Forty-eight eggs were divided into four groups. After 12 days of incubation (37.8°C, humidity - 65%), the groups were given meloxicam at varying doses. Fetuses were then removed after 48 hours. The results show that administration of high doses of meloxicam produced weakly or absence of vitelline vascularization. Also, meloxicam application instigated fetal deformities such as growth retardation, subcutaneous hemorrhage, very thin skin and exencephaly. Many histopathological alternations were noted in the retina, liver and kidney tissues of chick fetus of treated groups with meloxicam compared to the control group. The high doses of meloxicam caused many morphological and histopathological changes in the chick fetus. The safety of meloxicam was not established in this study.

Introduction

Due to the uncertainty of the safety and efficacy of drugs in pregnancy, it can cause patients and providers anxiety. Despite this, the need for these drugs is still high due to the increasing number of maternal chronic diseases (Sun et al., 2020). However, the mother can still take these medications if she has medical conditions that requires regular treatment. Some of these conditions include hypertension and epilepsy (Sachdeva et al., 2009).

One of the most common nonsteroidal anti-inflammatory drugs used for treating inflammatory and painful conditions in humans and animals is meloxicam. The prolonged use of this drug can cause adverse effects (Karkoszka et al., 2022). Nonsteroidal anti-inflammatory drugs are commonly used to treat various conditions such as fever and pain (Dzięcioł et al., 2022). When used by pregnant women, these drugs can cause adverse effects, especially when they cross the placenta. They can also increase the risk of premature fetal closure (Elkomy et al., 2018).

Prenatal exposure to nonsteroidal anti-inflammatory drugs cause fetal adverse effects, such as damage to the skeleton, kidney, lungs, and brain (Antonucci et al., 2012). Meloxicam is a drug that can decrease inflammation and provide analgesia. It can also prevent the synthesis of cyclooxygenase (Urban, 2000). It also inhibits the activity of cyclooxygenase-2. It is also one of the few drugs that can treat patients with severe asthma (Oliveira et al., 2009).

The use of the drug during pregnancy can cause various problems to fetuses, such as death or abnormalities. It can also affect the functioning of the placenta, which can lead to a child with a lack of weight and growth. In addition, it can cause the uterus to contract severely, which can result in premature labor and dead of the

fetus (Stephansson et al., 2011).

The current work aimed to demonstrate the effect of meloxicam on the morphological and histopathological changes in chick fetus.

Materials and Methods

The study was completed at the Jouf University, laboratories of the Department of Biology from September to November, 2020.

Incubation

Forty-eight eggs were incubated at 37.8 ± 0.1°C for 12 days. The eggs were rotated every 2 hours during the incubation period. After 24 hours, two eggs were selected randomly and examined by the window method to identify the embryonic disc to perform fertilized eggs.

Method of injection

The outer shell of the egg was sterilized with alcohol (70%), and marked with a label. A hole was made on the blunt pole of the egg using a sharp needle to inject meloxicam. Meloxicam was liquefied in saline solution, and the hole was covered (AbdRabou, 2021).

Preparation of drug

The meloxicam was obtained from a Pharmacy in Sakaka City, Saudi Arabia. The powder's different doses were dissolved in saline.

Groups of study

The eggs were separated into 4 sets, 12 eggs in each group. One group (control) received 0.1 mL saline. Other groups received 0.001 mg/0.1 mL (Group 1), 0.01 mg/0.1 mL (Group 2) and 0.02 mg/0.1 mL (Group 3) of meloxicam post 12 day of incubation. Each time 0.1 mL of fluid was injected.

The eggs were re-incubated then were examined post 48 hour post injection.

Morphological studies

Post 48 hours of injection of meloxicam, by window procedure, the eggs of all sets were opened and fetuses photographed for morphological check. The viability of the fetuses was evaluated by the locomotion.

Histopathological studies

Small pieces of fetal retia, kidney and liver tissue were picked up and fixed neutral buffer formol for 24 hours and prepared for paraffin serial sections (5 µm for the histopathological study and stained with hematoxylin and eosin (AbdRabou et al.,2021).

Quantitative observations

The number of dead and live fetuses in each group was determined. The lengths and weights of the fetuses were also taken into account.

Statistical analysis

SPSS version 21 software used to analyze the data. The statistical significance difference between sets was determined by the ANOVA test.

Results

Morphological studies

Figure 1 shows photographs of the vitelline vascularization of fetuses post 48 hours from meloxicam injection. Normal vitelline vascularization was noted in control group, weak vitelline vascularization in G1 group, decrease and absence of vitelline vascularization in Group 2 and Group 3. Figure 2 shows photographs of the fetuses post 48 hours from meloxicam injection. There was normal embryo in control group, reduction of size in Group 1, subcutaneous hemorrhage and very thin skin in Group 2, exencephaly and subcutaneous hemorrhage in Group 3.

Quantities studies

Table I shows the percentage of live and dead fetuses after 48 hours of meloxicam injection. The high percentage of dead fetuses was in Group 3 (58.3%). This percentage deceased in the Group 2 (41.7%), Group 1 (27.3%) respectively, but there was zero in control

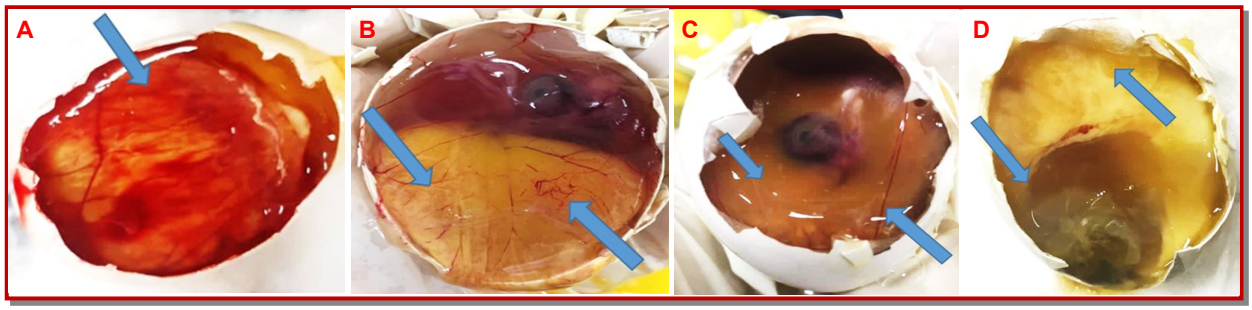

Figure 1: Photographs showing vitelline vascularization inside the egg after 48 hours of meloxicam injection. Normal vitelline vascularization (↑) in the control group (A); weak vitelline vascularization (↑) in Group 1 (B); decrease (↑) and absence (↑) of vitelline vascularization in Group 2 and Group 3 (C & D)

Table I

% Live and dead chick fetuses after meloxicam injection

Groups	Infertile	Fertile	Live	Dead
Control	0	100	100	0
1	8.3	91.7	72.7	27.3
2	0	100	58.3	41.7
3	0	100	41.7	58.3

group.

Table II shows a significant reduction in the fetuses length of groups Group 2 (6.2 ± 0.1 cm) and Group 3 (5.3 ± 0.2 cm) respectively associated with the control group (6.4 ± 0.1 cm). No significant decrease in the fetuses length of Group 1 group (6.3 ± 0.2 cm). A significant reduction in the mean of fetuses weight of groups Group 1 (7.7 ± 0.5 g), Group 2 (5.9 ± 0.4 g), and Group 3 (4.9 ± 0.4 g) respectively associated to control group (9.0 ± 0.7 g).

Table II

Effects of meloxicam of fetal length and weight

Groups	Length (cm)	Significant	Weight (g)	Significant
Control	6.4 ± 0.1	-	9.0 ± 0.7	-
1	6.3 ± 0.2	0.083	7.7 ± 0.5	0.000[a]
2	6.2 ± 0.1	0.007[a]	5.9 ± 0.4	0.000[a]
3	5.3 ± 0.2	0.000[a]	4.9 ± 0.4	0.000[a]

'a' mean statistically significant

Histopathological studies

Figure 3 shows the transverse section in retina tissue of chick embryo. Moderate-defined layering of retina, but the pigmented epithelium layer not appear in Group 1, in Group 2 morphological differences between retial layers were observed, but in Group 3 there is complete damage in all retinal layers. In kidney tissue of chick embryo of Group 1 showed more necrotic area, myelomatosis or neoplastic over growth of cells of the plasma cell series and acute tubular necrosis with shrinking of glomeruli, In Group 2 there was massive regions of necrosis with complete loss of renal parenchyma. In Group 3, severe highly massive infiltration of inflamamatory cells in the renal cortex, degeneration of most renal tubules. In Group 1, the liver tissue of chick embryo showing some pathological lesion represented by dilation in central vein, aggregation of macrophages cells at the central vein and eritrocytes (Figure 4). In the liver tissue of Group 2, aggregation of macrophages cells were increased at the central vein, large and irregular lumen of the sinusoidal capillaries and eritrocytes were observed. In Group 3 severe damaged all hepatocytes, more necrotic areas were observed (Figure 5).

Discussion

The use of drugs during pregnancy cause embryonic a lot of malformations. Some of these abnormalities caused by translations or chromosomal aberrations, while others are linked to environmental factors. Exposure to nutritional deficiencies can also increase the risk of developing these conditions (Temiz et al., 2009).

High doses of meloxicam can cause retardation and other developmental issues in the developing as neural system development. It led to the neural tube defect in chick embryo (Geliflimine, 2010).

After 30 weeks of pregnancy, taking non-steroidal anti-inflammatory medications can increase the risk of a fetal artery being prematurely closed. It can also lead to dangerous effects on the fetus, including oligohydramnios and kidney failure. If the mother is received it before birth, the effects of these drugs can effect on various organs and tissues, such as the skeleton, brain, lungs, the digestive system, and cardiovascular system of fetuses (Antonucci et al.,2012).

The application of different doses of meloxicam post 12 day of incubation in the present study established that

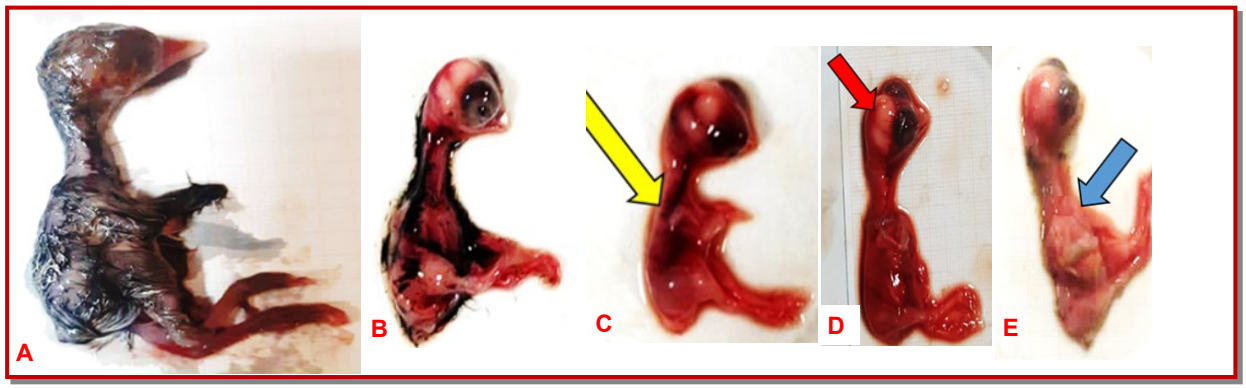

Figure 2: Photographs of chick fetuses after 48 hours of meloxicam injection. Normal embryo in control group (A); reduction in size in Group 1 (B); subcutaneous hemorrhage and very thin skin in Group 2 (↑) (C); exencephaly (↑) and very thin skin (↑) in Group 3 (D & E)

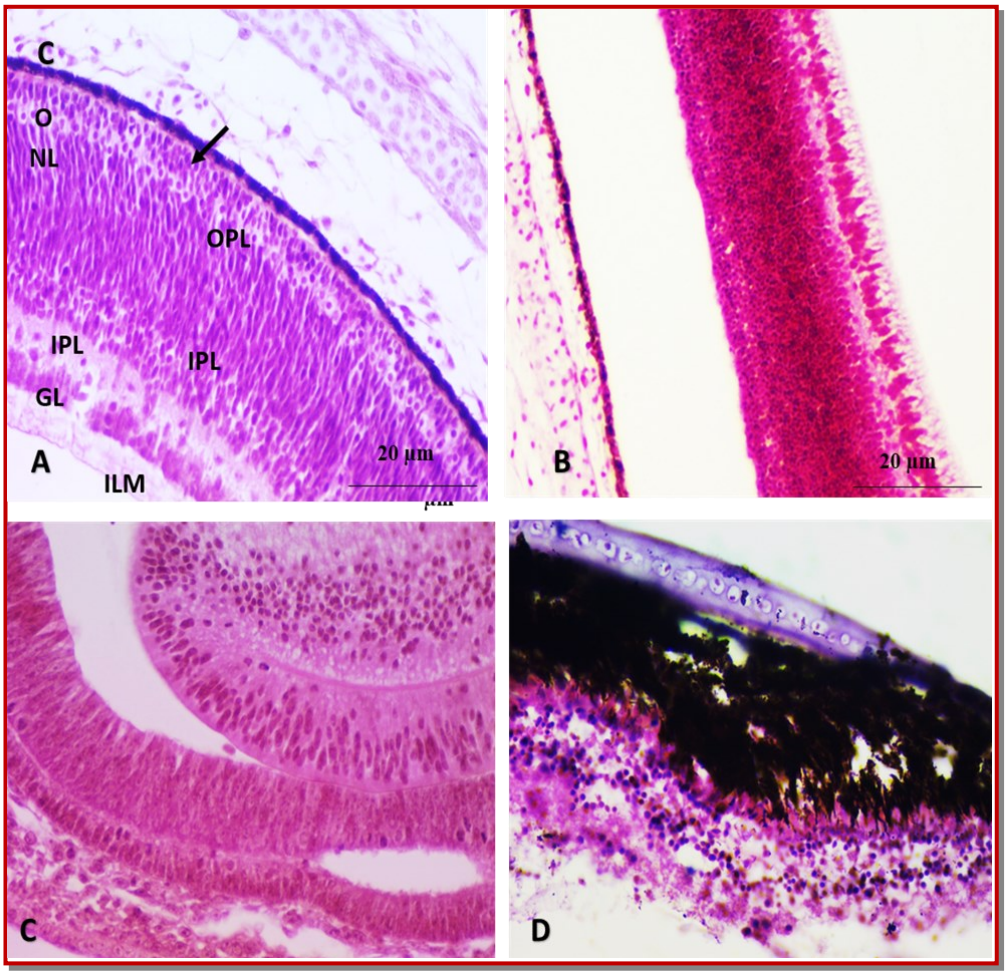

Figure 3: Transverse section in retina tissue of chick embryo. Normal histological structure of the retina pigmented epithelium of simple columnar epithelium layer (PL), very thin layer of rods and cones (arrow head) inner limiting membrane (ILM). Outer nuclear layer (ONL). Outer plexiform layer (OPL). Inner nuclear layer (INL). Inner plexiform layer (IPL). Ganglion cell layer (GL). Nerve fiber layer and (Inner limiting membrane (basal lamina) (ILM) (A); moderate-defined layering of retina, but the pigmented epithelium layer not appear in Group 1 (B); morphological differences between layers were observed in Group 2 (C); complete damage in all retinal layers in Group 3 (D). H&E, Bar 20 µm

it instigated a weak and lack of vitelline vascularization associated with control group. This product in agreement with studies of Cetinkal et al. (2010) and Abd-Rabou (2021), they used meloxicam in different doses in chick embryo. Also, the administration of meloxicam in the present study caused reduction of size of fetuses, subcutaneous hemorrhage, very thin skin, exencephaly in treated groups compared to the control group. The use of these drugs can also affect the placenta role. It can reduce the blood supply to the fetus and decrease the nutrients that it receives from the mother. It also lead to severely contraction of the uterus, which cause premature labor and damage to the fetus (Burdan et al., 2005). These results agree with the result of Abdrabou (2021). Meloxicam can cause hemorrhage around the internal organs, cerebral dilatation and microcephaly (Burdan et al., 2005). Taking meloxicam during pregnan-cy can increase the risk of fetuses' malformations, such as heart septal defects (Ofori et al., 2006).

In the current study receiving meloxicam led to growth retardation in the fetuses because of low body height and weight. These results agree with AbdRabou (2021) and Erdem and Guzeloglu (2010), they found a significant reduction in the height, weight and size of newborns which mother-treated with meloxicam. Meloxicam also caused neural tube defect in the embryo chick (Ami et al., 2016).

The high doses of meloxicam caused many histopathological alternations in the retial tissue. Severe damaged and degenerated all renal cells, more necrotic area, myelomatosis or neoplastic over growth of cells of the plasma cell series and acute tubular necrosis with shrinking of glomeruli, massive regions of necrosis with complete loss of renal parenchyma were noted in Group 1 and Group 2, but severe highly massive infiltration of inflammatory cells in renal cortex region, degeneration and destruction of most renal tubules

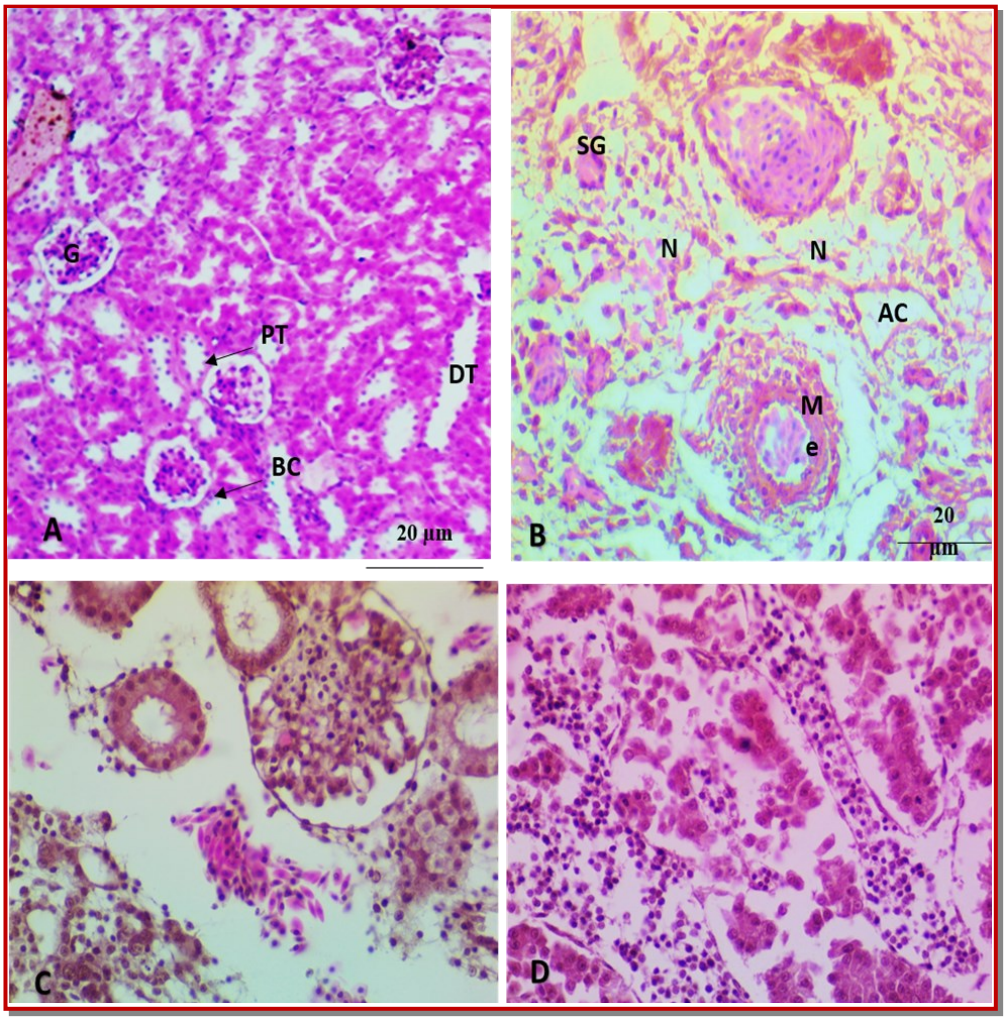

Figure 4: Transverse section in kidney tissue of chick embryo. Normal Bowman's capsule (BC), glomeruli (G), proximal tubules (PT) and distal tubules (DT) in control group (A). Severe damaged and degenerated all renal cells, more necrotic area (N), myelomatosis (Me) or neoplastic over growth of cells of the plasma cell series and acute tubular necrosis (AC) with shrinking of glomeruli (SG) were observed in Group 1 (B); massive regions of necrosis with complete loss of renal parenchyma in Group 2 (C); severe highly massive infiltration of inflammatory cells in renal cortex region and degeneration and destruction of most renal tubules in Group 3 (D). H&E, Bar 20 µm

were noted in Group 3 group. The liver tissue of chick embryo in the present study showed some pathological lesion represented by dilation in central vein, aggregation of macrophages cells at the central vein, eritrocytes, large and irregular lumen of the sinusoidal capillaries and eritrocytes were noted in Group 1 and Group 2 groups, but severe damaged all hepatocytes, more necrotic area were observed with high doses (Group 3). The use of 15 mg/kg meloxicam for 15 days significantly increased the number of mononuclear cells infiltration and pseudolobular formation in the parenchymal tissue. Meloxicam is absorbed in the small intestine and stomach. It is also carried by the body through its binding to albumin protein. It becomes inactive once it has been metabolized in the liver (Burukoglu et al., 2016). Meloxicam administration of 0.2 and 0.6 mg/kg increased the aspartate aminotransferase levels and caused vasocongestion and liver damage, necrosis and karyorrhexis in hepatocyte cells (Sturmer et al., 2021).

The adminstration of nonsteroidal anti-inflammatory drugs caused kidney faliure to 2.5 million person In the US (Al-Rekabi et al., 2001). A study conducted on Wistar rats revealed that the use of 1.2 and 2.4 mg/kg meloxicam for 28 days significantly increased lipid peroxidation in the liver and kidneys. It also caused various health conditions such as gastric and intestinal ulcers and hemorrhagic gastroenteritis (Mahaprabhu et al., 2011). In prolonged use of Meloxicam, this drug can cause nehprotoxic and hepatotoxic effects (Sulaiman et al., 2010). Because of the meloxicam hazards on fetuses in the present study, so it may be not suitable for treatment during pregnancy.

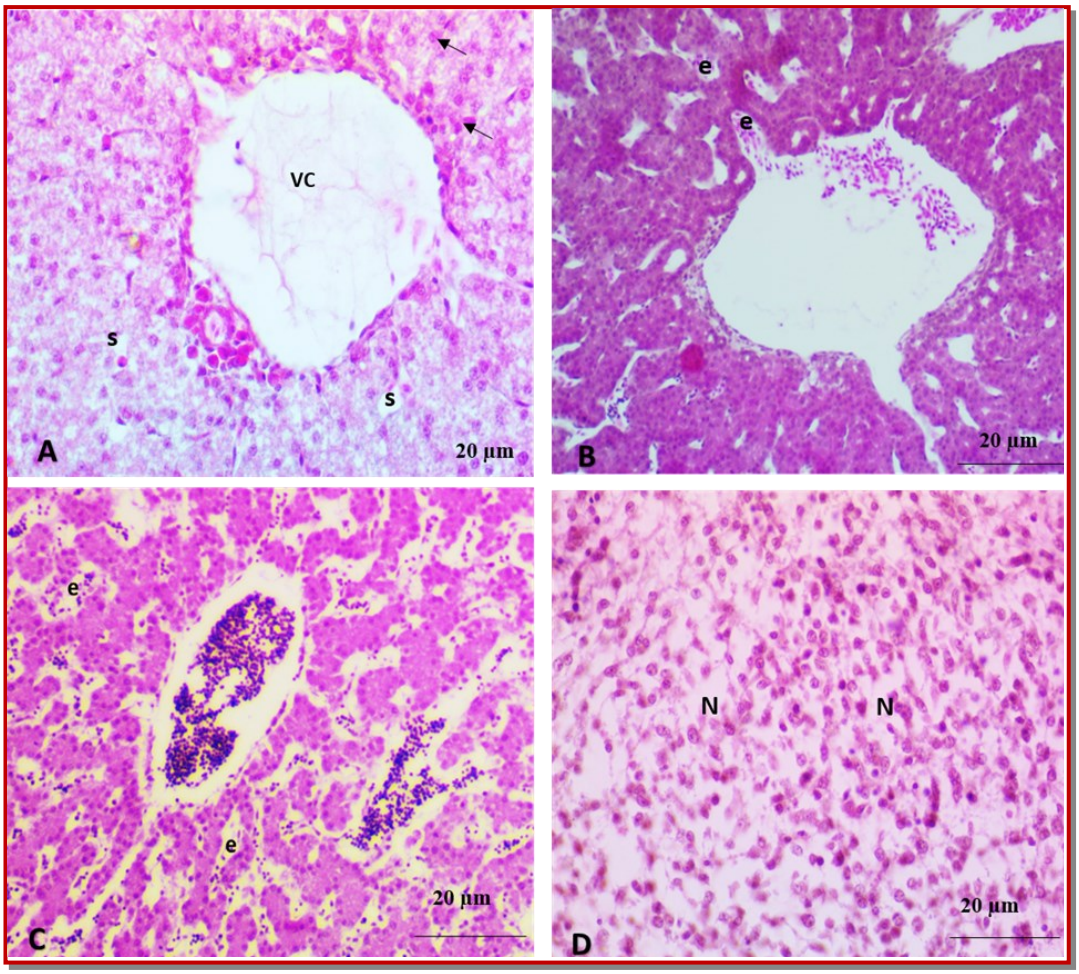

Figure 5: Transverse section in liver tissue of chick embryo (control) showing sinusoids (S), endothelial cells (→), vena centralis (VC) (A); dilation in central vein, aggregation of macrophages cells at the central vein, eritrocytes (e) were observed in Group 1 (B); aggregation of macrophages cells were increased at the central vein in Group 2, note the large and irregular lumen of the sinusoidal capillaries and eritrocytes (e) were observed (C); severe damaged all hepatocytes, more necrotic area (N) were observed in Group 3 (D). H&E, Bar 20 µm

Conclusion

The administration of meloxicam with high doses caused a weak and lack of vitelline vascularization. It also caused many fetal malformations, most notable subcutaneous hemorrhage, exencephaly, growth retardation and fetal death. Moreover, it caused many histopathological alternations in the fetal retina, renal and liver tissues.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

References

AbdRabou MA, Al-Ghamdi FA, Al-Otaibi AM , Gewily DI. Histopathological, histochemical and immunological studies on fetal pancreatic tissue of rats treated with carisoprodol. Int J Pharmacol. 2021; 17: 506-16.

AbdRabou MA. Effect of different doses of meloxicam on the development of chick embryo. Int J Pharmacol. 2021; 17: 474 -81.

Al-Rekabi F, Abbas D, Hadi N. Effects of subchronic exposure to meloxicam on some hematological, biochemical and liver histopathological parameters in rats. Iraqi J Vet Sci. 2009; 23: 249-54.

Ami N, Bernstein M, Boucher F, Rieder M, Parker L. Folate and neural tube defects: The role of supplements and food fortification. Paediatr Child Health. 2016; 21: 145-49.

Antonucci R, Zaffanello M, Puxeddu E, Porcella A, Cuzzolin L, Pilloni MD, Fanos V. Use of non-steroidal anti-inflamma-

tory drugs in pregnancy: Impact on the fetus and newborn. Curr Drug Metab. 2012: 13: 474-90.

Burdan F. Comparison of developmental toxicity of selective and non-selective cyclooxygenase-2 inhibitors in CRL: (WI) WUBR Wistar rats-DFU and piroxicam study. Toxicology 2005; 211: 12-25.

Burukoglu D, Baycu C, Taplamacioglu F, Sahin E, Bektur E. Effects of nonsteroidal anti-inflammatory meloxicam on stomach, kidney, and liver of rats. Toxicol Ind Health. 2016; 32: 980-86.

Cetinkal A, Colak A, Topuz K, Demircan MN, Simsek H. The effects of meloxicam on neural tube development in the early stage of chick embryos. Turk Neurosurg. 2010; 20: 111-16.

Dzięcioł M, Szpaczek A, Uchańska O, Niżański W. Influence of a single dose of meloxicam administrated during canine estrus on progesterone concentration and fertility: A clinical case study. Animals 2022; 12: 655.

Elkomy AA, Salem MA, Kandeil AM, Hassan A, Elhemiely AA. Teratogenic effect of meloxicam on pregnant rats: Implication of organogenesis period. Benha Vet Med J. 2018; 35: 317-27.

Erdem H , Guzeloglu A. Effect of meloxicam treatment during early pregnancy in *Holstein heifers*. Reprod Domestic Anim. 2010; 45: 625-28.

Geliflimine EN. The effects of meloxicam on neural tube development in the early stage of chick fetuses. Tur Neurosurg. 2010; 20: 111-16.

Karkoszka M, Rok J, Banach K, Kowalska J, Rzepka Z, Wrześniok D. The assessment of meloxicam phototoxicity in human normal skin cells: *In vitro* studies on dermal fibroblasts and epidermal melanocytes. Molecules 2022: 27: 4215.

Mahaprabhu R, Bhandarkar AG, Jangir BL. Ameliorative effect of *Ocimum sanctum* on meloxicam induced toxicity in Wistar rats. Toxicol Int. 2011; 18: 130-36.

Ofori B, Oraichi D, Blais L, Rey E, Bérard A. Risk of congenital anomalies in pregnant users of non-steroidal anti-inflammatory drugs: A nested case-control study. Birth Defects Res. Part B: Dev Reprod Toxicol. 2006; 77: 268-79.

Oliveira ÉD., Azevedo RD, Bonfilio R, de Oliveira DB, Ribeiro GP, de Araújo MB. Dissolution test optimization for meloxicam in the tablet pharmaceutical form. Braz J Pharm Sci. 2009; 45: 67-73.

Sachdeva P, Patel BG, Patel BK. Drug use in pregnancy, a point to ponder. Indian J Pharm Sci. 2009; 71: 1-7.

Stephansson O, Granath F, Haglund A. Ekbom A, Kieler H. Drug use during pregnancy in Sweden-assessed by the prescribed drug register and the medical birth register. Clin Epidemiol. 2011; 3: 43-50.

Sturmer T, Elseviers MM, De Broe ME. Non-steroidal anti-inflammatory drugs and the kidney. Curr Opin Nephrol Hypertens. 2001; 10: 161-63.

Sulaiman AH, Jarjees MT. The effect of diclofenac and meloxicam on liver and kidney of male rats. A histomorphometrical study (Doctoral dissertation, PhD Thesis]. 2010. Collage of Medicine, University of Mosul).

Sun AJ, Li S, Zhang CA, Jensen TK, Lindahl-Jacobsen R, Eisenberg ML. Parental comorbidity and medication use in the USA: A panel study of 785 000 live births. Hum Reprod. 2020; 35: 669-75.

Temiz C, Temiz P, Demirel A, Sayın M, Umur AŞ, Özer FD. Effect of sodium phenytoin concentration on neural tube development in the early stages of chicken embryo development. J Clin Neurosci. 2009; 16: 307-11.

Urban M . Cox-2 specific inhibitors offer improved advantages over traditional NSAIDs. Orthopedis 2000; 23: 61-64.

Author Info
Mervat Ahmed AbdRabou (Principal contact)
e-mail: mababdraboh@ju.edu.sa