The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
EISSN: 2959-4820
Volume 1, Issue 3, July-September 2023, Page No: 85-93
Website: https://najsp.com/index.php/home/index
SJIFactor 2023: 3.733 **ISI 2023: 0.383**

Ondansetron's effect on the incidence of birth defects in the fetuses of pregnant albino mice *Mus musculus*

Arwa Adress Alnuimy *
Biology Department, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq

*Corresponding author arwabio2020@uomosul.edu.iq.

Received: June 29, 2023	Accepted: August 04, 2023	Published: August 07, 2023

Abstract

Background: Ondansetron It is a 5-HT3 serotonin receptor antagonist that is used to prevent nausea and vomiting caused by cancer chemotherapy, radiotherapy, and surgery, as well as to treat nausea and vomiting during pregnancy, also known as morning sickness, is usual in the eighth week of pregnancy, with an average incidence rate of 80%, and disappears in the majority of pregnant women by the sixteenth week. Hyperemesis, an acute type of the disease, affects 1 in 200 to 300 pregnancies and is linked to dehydration and nutritional deficiencies. **Objectives:** The aim of this research is to see how ondansetron, a cure for nausea and vomiting, affects pregnant mice fetuses. **Methods**: The dosage of ondansetron 8 mg/kg B.W, prepared by dissolving it in 20 ml distilled water, 20 pregnant females were given the aquatic solution from the 7th to the 18th day of pregnancy. **Results:** There is a percentage of congenital abnormalities, with facial anomalies, spina bifida, cleft palate, limb, and tail deformities being the most common. The study also found that this drug has histological effects on the lung, represented by congestion, bleeding, and the occurrence of emphysema. **Conclusion**: Since pregnancy is such a vital period in a woman's life, she must be cautious when taking medications, like ondansetron, because research on it is rare.

Keywords: Birth Defect, Fetuses, Pregnant Mice, Ondansetron.

Cite this article as: A. A. Alnuimy, "Ondansetron's effect on the incidence of birth defects in the fetuses of pregnant albino mice *Mus musculus*," *The North African Journal of Scientific Publishing (NAJSP)*, vol. 1, no. 3, pp. 85–93, July-September 2023

تأثير عقار اوندنسيترون في إحداث العيوب الخلقية لأجنة الفئران البيض الحوامل *Mus musculus*

أروى ادريس النعيمي *
قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

الملخص

Ondansetron وهو مضاد لمستقبلات السيروتونين *HT3-5* يستخدم لمنع الغثيان والقيء الناجمين عن العلاج الكيميائي للسرطان والعلاج الإشعاعي والجراحة، وكذلك لعلاج الغثيان والقيء أثناء الحمل، كما أنه علاج لغثيان الصباح الذي يحدث عادة في الأسبوع الثامن من الحمل، بمتوسط معدل حدوث 80٪، ويختفي عند غالبية النساء الحوامل بحلول الأسبوع

السادس عشر. يؤثر القيء المفرط وهو نوع حاد من المرض، على 1 من كل 200 إلى 300 حالة حمل ويرتبط بالجفاف ونقص التغذية. كان الهدف من هذا البحث هو دراسة تأثير عقار أوندانسيترون كعلاج للغثيان والقيء على أجنة الفئران الحوامل، أعطيت جرعة أوندانسيترون 8 ملجم / كجم من وزن الجسم والذي حضر بإذابته في 20 مل من الماء المقطر، أعطيت 20 أنثى حامل المحلول المائي من اليوم السابع إلى اليوم الثامن عشر من الحمل، وجدت الدراسة عددًا من التشوهات الخلقية، منها تشوهات الوجه، وتشوهات السنسنة المشقوقة ، والحنك المشقوق، والأطراف، وتشوهات الذيل هي الأكثر شيوعًا، كما وجدت الدراسة أن لهذا العقار تأثيرات نسجية على الرئة تمثلت بالاحتقان والنزف وحدوث النفاخ الرئوي، لهذا ونظرًا لأن الحمل فترة حيوية في حياة المرأة، يجب أن تكون حذرة عند تناول الأدوية وتلجأ للاستشارة الطبية.

الكلمات المفتاحية: العيوب الخلقية، الأجنة، الفئران الحوامل، عقار اوندانسيترون، الرئة.

1- Introduction

Ondansetron is a serotonin 5-HT3 receptor antagonist that is used to treat nausea and vomiting caused by cancer chemotherapy, radiation therapy, and surgery [1], as well as to prevent nausea and vomiting during pregnancy [2], Nausea and vomiting occur at a rate of 80% in pregnant women, peaking in the eighth week and disappearing in the majority of pregnant women by the sixteenth week, and hyper vomiting can occur at a rate of one case per 200-300 pregnancies, accompanied by dehydration and a lack of nutrition [3, 4], Nausea and vomiting may occur during pregnancy as a result of changes in hormone levels such as progesterone, estrogen, and thyroid-stimulating hormone, or as a result of the digestive system's peristaltic movement; however, the exact cause is unknown. Because of the potential risks to the mother and fetus, there is always apprehension about using anti-nausea and vomiting medications during pregnancy. Nonetheless, early treatment of these symptoms helps to prevent their exacerbation as well as psychological issues that may affect pregnant women [5], Regardless of the medication used by the mother, all pregnancies are at risk of developing birth defects, and 15% of them end in miscarriage [6], Ondansetron acts on nausea and vomiting by blocking signals from the emetic center in the brain and is commonly used in the treatment of these two disorders, but there is uncertainty about its protection and efficacy since it crosses the placenta [7], Furthermore, it does not always prevent nausea and vomiting, which necessitates intravenous fluids [8].Ondansetron is a type of ondansetron that is used to treat a variety, Ondansetron Hydrochloride Dihydrate is the European name for the drug. Ondansetron Hydrochloride is the USP name for this drug. Ondansetron is a form of ondansetron that is (as Dihydrate Dihydrate), 1,2,3,9-tetrahydro-9-methyl-3 - (2-methyl-1 H-imidazole-1-yl) methyl) -4H-carbazol-4-one is the methyl name for ondansetron. Figure 1 shows the chemical composition of the base and the hydrochloride, as well as the dehydrate types. S (+) and R (-) stereoisomers are mixed in a racemic mixture. A selective 5-HT3 serotonin receptor blocker, Ondansetron hydrochloride dehydrate has a molecular weight of 365.86 g/mol and a molecular weight of C18H19N30.HCl.2H2O, while the free ondansetron base has a molecular fraction of 298 g/mol. [9].

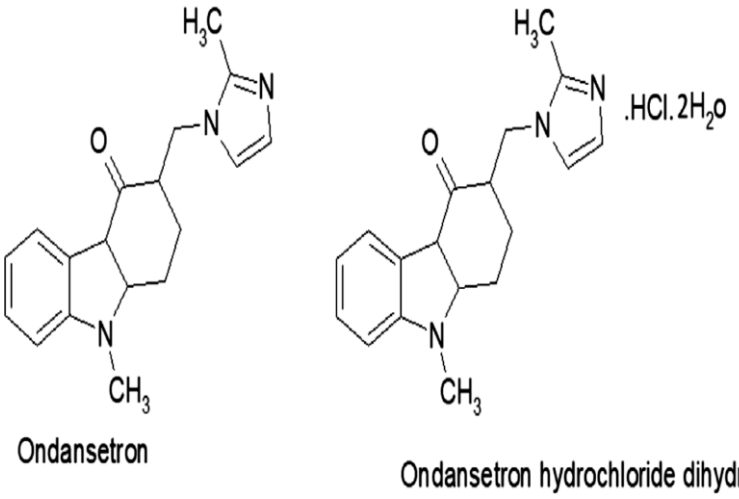


Figure 1 Chemical structure of the ondansetron-free base and ondansetron hydrochloride dehydrate.

2. Materials and methods

2.1: Animals

The Swiss albino mice Balb C mice weighing on average (25±2) grams were obtained. The mice were housed in plastic cages with metal mesh covers in a well-ventilated room with a temperature of (24± 2) m and a 12-hour light / dark cycle (10), and a special diet of mice and water was provided (11). For fertilization, three females were placed in a single cage with one male overnight, and fertility was assessed by observing sperms in the vaginal plug depending "on the method" (12), The first day of pregnancy occurred on the day of mating, and the zero-day pregnancy occurred the next day.

2.2: The method of treatment

The usage of ondansetron 8 mg/kg B.W, prepared by dissolving it in 20 ml distilled water, provided that the lethal dose of ondansetron in mice when given orally is (10-30) mg/kg BW (13), pregnant mice were given an ondansetron aqueous solution by mouth through a gavage needle.,

2.3: Design of an experiment

The date of mating was written on the pregnant females who owned the vaginal plug, and (20) pregnant females were given the aquatic solution from the seventh day of pregnancy (Organogenesis) until the 18th day of pregnancy (the day of the anatomy).

2.4: Groups of experimenters

Thirty pregnant female mice were used in the current study. They were split into two teams. The first control group had ten mice, while the second group received an aqueous solution of ondansetron solution 8 mg/kg BW and had twenty pregnant mice.

2.5: Examination of fetuses

Pregnant females were anesthetized with chloroform on the eighteenth day of pregnancy, and embryos were extracted after embryonic membranes were removed then its lung was also extracted and fixed with a 10% neutralized formalin fixative for (48-24) hours (14) and examined with an Olympus-type dissecting microscope, and the histological study of the lung was carried out based on (15).

Results and discussion

Physical analysis revealed the appearance of many congenital defects in the embryos of mice whose mothers had been given the drug ondansetron, a dosage of 8 mg/kg of body weight for 11 days, from the seventh to the eighth day, which is the time of organ development (organogenesis), and this dose was below therapeutic doses as compared to the normal lethal dose. It ranges from 10 to 30 mg/kg of body weight in mice, Abnormalities in various structures were discovered, including hydrocephalus in the neck region, the loss of fingers and buds, abdominal atrophy and congestion, and a thick short caudate (Picture: a), Loss of eyes, a divergence of the ear pinna, elongation of the combs of the front limbs, adhesion of the fingers, and blueness of the abdominal region with a straight caudate were also included in the study's results. (Picture: b),The results showed (Picture: c) the appearance of exophthalmia, wrinkled skin, and meningomyelocele, The findings also revealed facial malformation, eye loss, ear pinna deviation, clumping of front limb fingers, and ventral region atrophy (Picture: d),There was enlargement of the head, ovoid, and Excencephalon, as well as bulging eyes, a Cleft lip, and a short tail (Picture: e) ,The loss of front limb fingers and their presence in buds, the curvature of the left hind limb, sticking to the right hind limb fingers, and a thick tail were all included in the study's results (Picture: f), so the results of our study showed that the use of the treatment during the period of organogenesis has caused several "congenital anomalies, including malformations of the eyes, ears, limbs, head, facial area, trunk, skeletal system, and nervous system defects ondansetron can cross the placental barrier (7), As a result, it is supposed to cause deformations, even if they are minor, as shown by a reference study, which found that it did cause deformations, but only at minor rates (16), Other research found that the drug caused various types of birth defects at different rates. According to a report (17), which spanned the years 1997 to 2010, 1248 women received care during pregnancy, with 58 "congenital" birth defects occurring at a rate of one per cent , The research (18) found that the drug causes a variety of deformities, including leg, eye, ear, brain, and skeletal defects, which are similar to our findings, The research also revealed that the treatment is the source of meningomyelocele deformation, which is followed by a wide range of other damages, such as complete paralysis and deformities affecting the lower extremities and trunk, including loss of sensation (19).

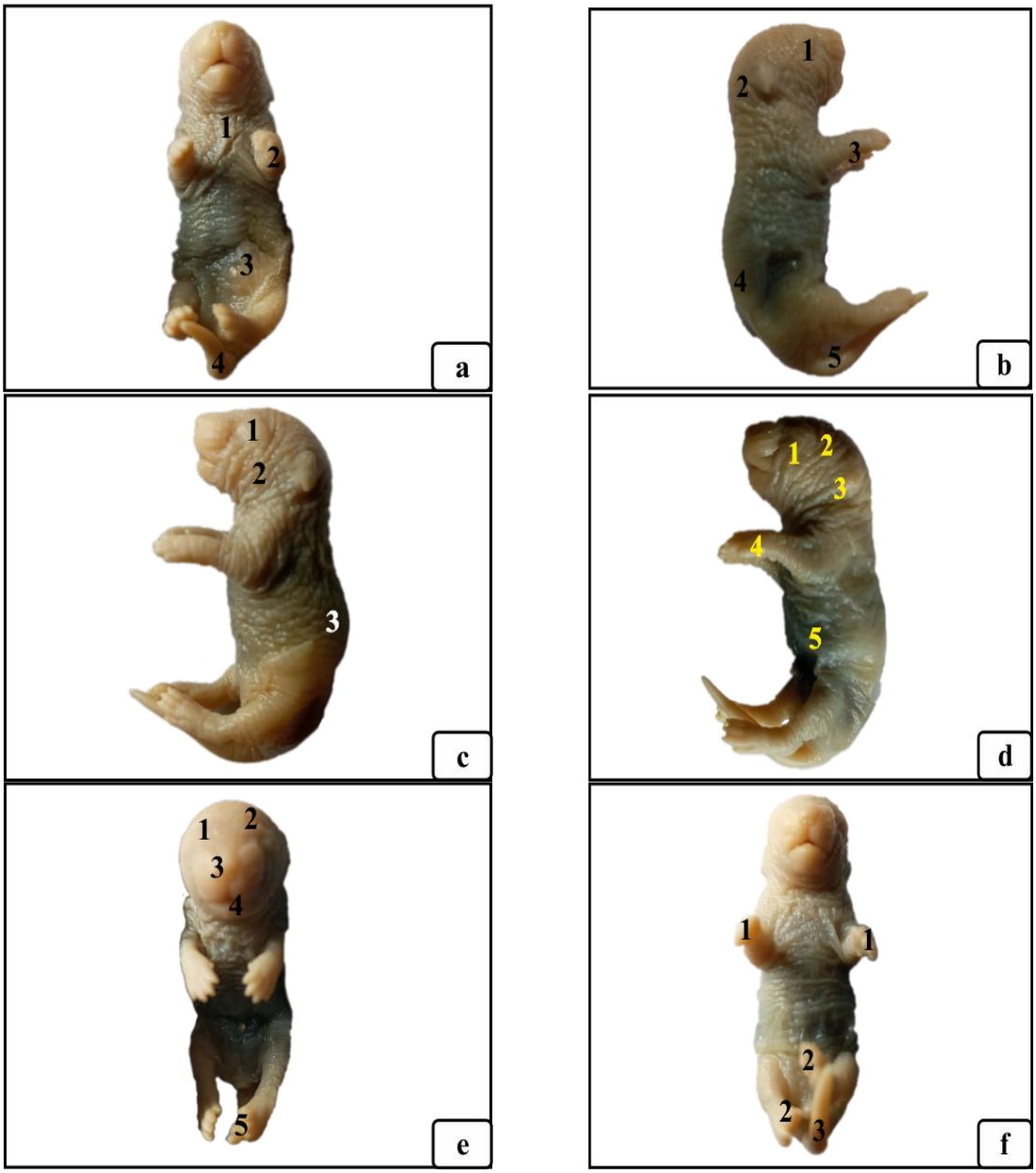
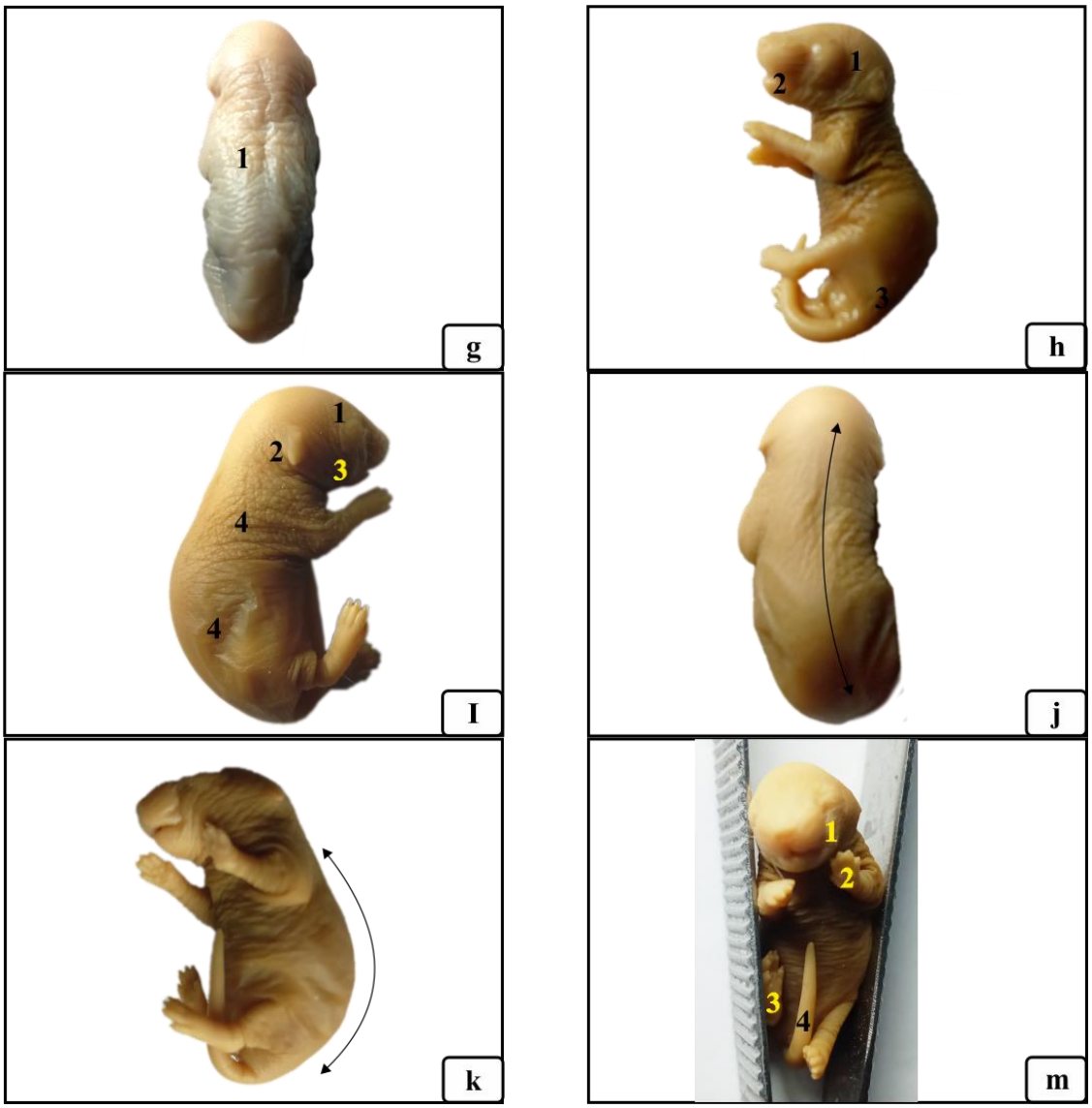
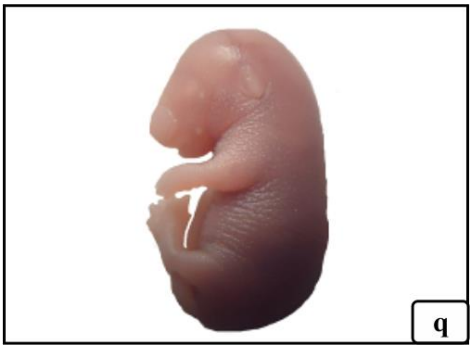


Figure 2 shows the deformed fetuses as a result of the mother's dosing with an aqueous solution of ondansetron appear in it , (Picture : a) hydrocephalus neck region (1), loss of fingers (2), abdominal atrophy and congestion(3), and a thick short caudate(4), (Picture: b) Loss of eyes (1), a divergence of the ear pinna(2), elongation of the combs of the front limbs, adhesion of the fingers (3), and blueness of the abdominal region(4) a straight caudate (5), (Picture: c) exophthalmia(1), wrinkled skin(2), and meningomyelocele(3), (Picture: d) facial malformation (1), eye loss(2), ear pinna deviation (3), clumping of front limb fingers (4),ventral region atrophy (5), (Picture: e) enlargement of the head(1), ovoid (2), Excencephalon (3),bulging eyes (4), a Cleft lip (4), a short tail(5), (Picture: f) loss of front limb fingers and their presence in buds(1), curvature of the left hind limb (2), sticking to the right hind limb fingers (2), a thick tail (3).

The study also indicate the appearance of spina bifida (Picture: g),The study's findings revealed the emergence of exophthalmia ,mandibular shortness and enlargement of the sacral region (Picture: h), flatness and elongation of the nasal region, protrusion of the ear pinna, mandibular shortness and wrinkling and sagging of the skin (Picture: I),as well as the occurrence of spine curvature (Picture: j),there was also loss of the front brain, middle brain congestion, and trunk curvature (picture: k) , and the cleft palate and fingers tend to join the front left and right posterior curvature, as well as a long tail

that reaches the abdominal area (picture: m), in comparison to the control group (picture: q), as well as skeletal system deformities and differentiation of spina bifida (20), Spina bifida is a congenital disorder in which the spine does not develop properly during fetal development or is born with an open neural tube defect (21), These disorders cause an obstruction in the flow of cerebrospinal fluid through the posterior cerebral orifice, resulting in research (22), and our findings are consistent with some studies that show a slight increase in cleft palate (23), The process of normal palate formation occurs when the palatal flap fuses and the midline epithelial line decomposes through transmission or programmed death, and the cleft palate does not happen, and thus the shelves can split in the developing face (24), The cause of limb deformities and tail atrophy may be attributed to programmed cell death, which caused the occurrence of limb deformities and atrophy in some of them, or possibly the absence of the apical region of the ectodermal as the appearance of abnormalities in the tailbone region is due to a defect in the formation of the tail bud mesoderm due to a lack of gene function responsible for its formation (25), Another study did not link treatment with any of these abnormalities (26), and the results of our study did not agree with several other studies was indicated that the treatment does not cause birth defects (27).

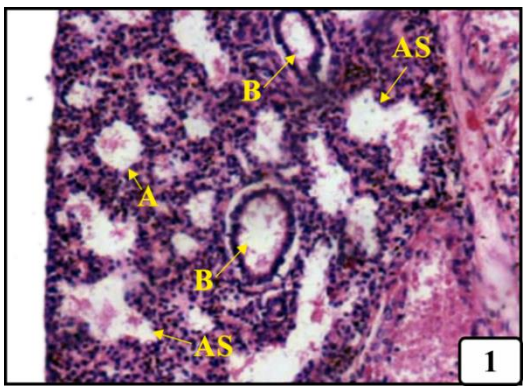


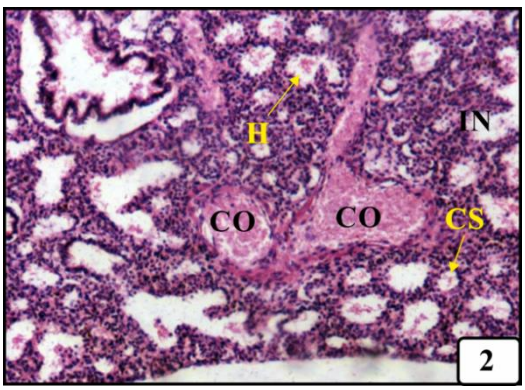


(Picture: q): control group

Figure 3: The following figure shows the congenital malformations of fetuses whose mothers were dosed with an aqueous solution of ondansetron, (Picture: g) spina bifida, (Picture:h) exophthalmia (1), mandibular shortness(2) enlargement of the sacral region(3), (Picture: I) flatness and elongation of the nasal region (1), protrusion of the ear pinna(2), mandibular shortness(3) wrinkling and sagging of the skin (4), (Picture: j) spine curvature (arrow), (picture: k) loss of the front brain, middle brain congestion, and trunk curvature(arrow), cleft palate and fingers tend to join the front left and right posterior curvature, as well as a long tail that reaches the abdominal area (picture: m), (picture: m) cleft palate (1) fingers, tend to join the front left(2) right posterior curvature (3) long tail that reaches the abdominal area (4).

The study discovered that treatment caused a number of pathological histological changes in the fetus's lungs, including congestion, hemorrhage, collapse of some alveoli, dilation and congestion of blood vessels, desquamation of the lining epithelium of some bronchioles, and the study indicated the emergence of thinking of epithelium bronchioles. Extraverted red blood cells in embryonic lung tissue, folded epithelium in the bronchiole, and proliferation among the histological alterations detected in these tissues, "emphysematous lung tissue was damaged."(Form:3), as for histopathological changes , the interference between antioxidant processes, which results in the generation of reactive oxygen species and damages lung tissue, may be the root cause of the histopathological alterations in the lungs (28) For instance, "the stimulation of macrophages that produce IL-8 results in the infiltration of inflammatory cells" Desquamation may result from an increase in the amount of inflammatory macrophage cells, which in turn activates inflammatory cells and increases their number (29;30), and the cause of the dose of pulmonary emphysema, which is a form of chronic obstructive pulmonary disease that leads to For alveolar malformations (31; 32), emphysema affects the bronchioles, causing them to swell and their walls to collapse (33).





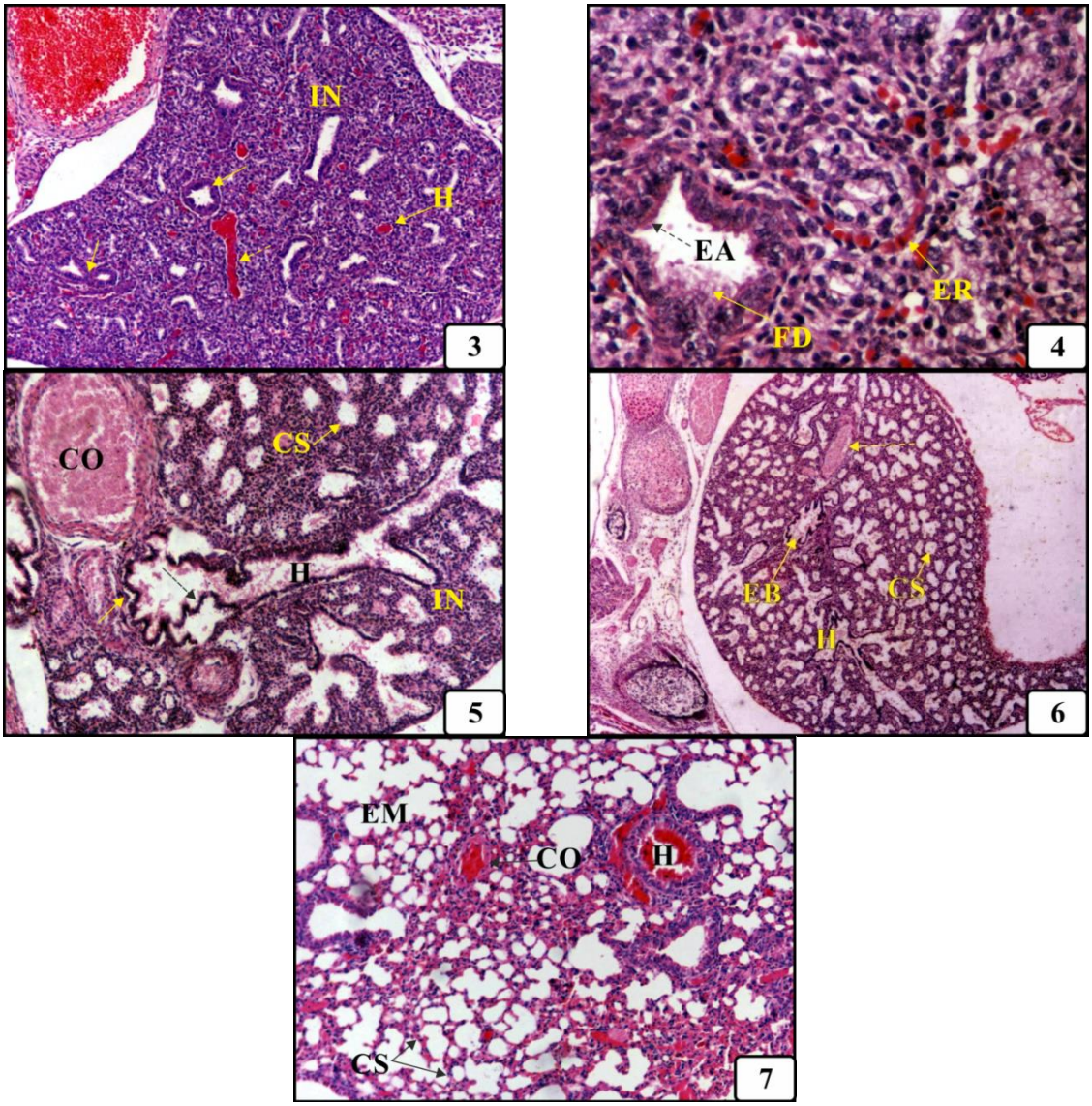


Figure 4 Fetus lung tissues histological section of all (pic:1) groups control group illustrates Alveoli (A) Bronchioles (B) Alveolar sac (AS) , (pic:2) Histological section of the lung of an embryonic mouse the mother's dosing with an aqueous solution of ondansetron show Congestion (CO) Hemorrhage (H)Collapse of some alveoli (CS) infiltration cell Infiltration (IN) , (pic:3) show dilation and congestion of blood vessels (dashed arrow) desquamation the lining epithelium of some bronchioles (arrow) (H) (IN), (pic:4) thinking of bronchiole epithelium (dashed arrow) Extravagated red blood cells (ER) Fibrin deposition (FD) Expansion alveolar sacs (EA) , (pic:5) Bronchiole with folded epithelium and proliferation (dashed arrow) separation of the lining of the bronchioles (arrow) (H) (IN) (CS) (pic:6) dilation and congestion of blood vessels (dashed arrow) (CS) (EA) (H), (pic:7) Emphysematous dilation (EM) (CO) (H).

Conclusion

Pregnancy is a vital time in a woman's life during which she undergoes several changes and is subject to a variety of unpleasant symptoms, such as vomiting, nausea, and a variety of other issues that necessitate medication, at the end Since there are few trials on some medications, including ondansetron, pregnant women must exercise caution and be wary of this and other treatments. To maintain the wellbeing of paradise and newborns, procedures should not be taken without medical advice.

Acknowledgment

The researcher thanks and gratitude the University of Mosul, College of Education for Pure Sciences, Department of Biology for their support, this research was not funded by any entity.

References

[1] G. Koren, "Motherisk update. Is ondansetron safe for use during pregnancy?" *Canadian family physician Medecin de famille canadien* vol. 58,10 2012: 1092-3.

[2] B. Pasternak, H. Svanström, and A. Hviid, Ondansetron in pregnancy and risk of adverse fetal outcomes. The New England journal of medicine, 368(9), 2013, 814–823. https://doi.org/10.1056/NEJMoa1211035.

[3] M. Davis, Nausea and vomiting of pregnancy: an evidence-based review. The Journal of perinatal and neonatal nursing, 18(4), 2004, 312–328. https://doi.org/10.1097/00005237-200410000-00002.

[4] E. Ferreira, M. Gillet, J. Lelièvre, and J. Bussière, Ondansetron use during pregnancy: a case series. Journal of population therapeutics and clinical pharmacology = Journal de la therapeutique des populations et de la pharmacologie clinique, 19(1), 2012, e1–e10.

[5] B. Thomas, P. Rouf, M. Al-Hail, D. Al-Saad, A.Tharannum, et al. Medication used in Nausea and Vomiting of Pregnancy – A Review of Safety and Efficacy. Gynecol Obstet (Sunnyvale) 5: 270.2015, doi:10.4172/2161-0932.1000270.

[6] P. Nguyen, and A. Einarson.Managing nausea and vomiting of pregnancy with pharmacological and nonpharmacological treatments. Women's health (London, England), 2(5), 2006, 753–760. https://doi.org/10.2217/17455057.2.5.753.

[7] P. Pasricha, N. Pehlivanov, A. Sugumar, and J. Jankovic. Drug Insight: from disturbed motility to disordered movement--a review of the clinical benefits and medicolegal risks of metoclopramide. Nature clinical practice. Gastroenterology and hepatology, 3(3), 2006,138–148. https://doi.org/10.1038/ncpgasthep0442

[8] Abramowitz, A., Miller, E. S., and Wisner, K. L. (2017). Treatment options for hyperemesis gravidarum. Archives of women's mental health, 20(3), 363–372. https://doi.org/10.1007/s00737-016-0707-4.

[9] G. Rajawat, T. Belubbi, M. Nagarsenker, B. Abrahamsson, R. Cristofoletti, D. Groot, P. Langguth, A. Parr, J. Polli, M. Mehta, V. Shah, T. Tajiri, and J. Dressman. Biowaiver Monograph for Immediate-Release Solid Oral Dosage Forms: Ondansetron. Journal of pharmaceutical sciences, 108(10), 2019, 3157–3168. https://doi.org/10.1016/j.xphs.2019.05.033.

[10] 10- A. Saleem, S. Ray, B. Ahmed. Influence of hyperglycemia on oxidative stress and DNAase-mediated genomic fragmentation coupled with apoptotic and necrotic cell deaths in Kidney.Can.J.App.Sci.1(3):2011, 129-143.

[11] E. Balducci-Roslindo, K. Silvério, M. Jorge, and H. Gonzag. Effect of isotretinoin on tooth germ and palate development in mouse embryos. Brazilian dental journal, 12(2),2001, 115–119.

[12] A. Wallace, P. Raven, M. Yamaguchi, M. Ando, R. Yamamot. Smoking is a risk factor forthe progression of idiopathic membranous nephropathy.2014, PLoS One; 9: e100835.

[13] US National Library of Medicine. Hazardous substance data bank (HSBD) .ondansetron (CASRN: 99614-02-5),2015.

[14] R. Ye, A. Ren, L. Zhang, Z. Li, J. Liu, L. Pei, and X. Zheng, X. Tea drinking as a risk factor for neural tube defects in northern China. Epidemiology (Cambridge,Mass.), 22(4),2011, 491–496. https://doi.org/ 10.1097/EDE. 0b013e 31 821b4526

[15] K. Suvarna, C. Layton, and j. Bancroft. Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences.2018.

[16] S. Carstairs. Ondansetron Use in Pregnancy and Birth Defects: A Systematic Review. Obstetrics and gynecology, 127(5),2016, 878–883. https://doi.org/10.1097/AOG.0000000000001388.

[17] B. Pasternak, H. Svanström, D. Mølgaard-Nielsen, M. Melbye, and A. Hviid. Metoclopramide in pregnancy and risk of major congenital malformations and fetal death. JAMA, 310(15), 2013, 1601–1611. https://doi.org/10.1001/jama.2013.278343

[18] S. Parker, C. Van Bennekom, M. Anderka, A. Mitchell, and National Birth Defects Prevention Study. Ondansetron for Treatment of Nausea and Vomiting of Pregnancy and the Risk of Specific Birth Defects. Obstetrics and gynecology, 132(2),2018, 385–394. HTTP:// doi.org/ 10.1097/ AOG. 0000 0000 0000 2679.

[19] L. Avagliano, V. Massa, T. George, S. Qureshy, G. Bulfamante, and R. Finnell. Overview on neural tube defects: From development to physical characteristics. Birth defects research, 111(19), 2019, 1455–1467. https://doi.org/10.1002/bdr2.1380

[20] P. Beuriat, I. Poirot, F. Hameury, A. Szathmari, C. Rousselle, I. Sabatier, F. di Rocco, and C. Mottolese, Postnatal Management of Myelomeningocele: Outcome with a Multidisciplinary Team Experience. World neurosurgery, 110, 2018, e24–e31. https://doi.org/10.1016/j.wneu.2017.09.169.

[21] M. Sahni, and A. Ohri, A.Meningomyelocele, Bookshelf ID: NBK536959 PMID: 30725644.2019.

[22] J. Nagler, J. Levy, J. and R. Bachur, Stridor in an infant with myelomeningocele. Pediatric emergency care, 23(7), 2007, 478–481. https://doi.org/10.1097/01.pec.0000280523.61064.15.
[23] K. Huybrechts, S. Hernández-Díaz, L. Straub, K. Gray, Y. Zhu, E. Patorno, R. Desai, H. Mogun, and B. Bateman. Association of Maternal First-Trimester Ondansetron Use with Cardiac Malformations and Oral Clefts offspring. JAMA, 320(23),2429–2437. HTTP:// doi.org/ 10. 1001 / Jama .2018.18307.
[24] A. Nakajima, Y. Ito, E. Tanaka, R. Sano, Y. Karasawa, M. Maeno, K. Iwata, N. Shimizu, and C. Shuler. Functional role of TGF-β receptors during palatal fusion in vitro. Archives of oral biology, 59(11), 2014, 1192–1204. https://doi.org/10.1016/j.archoralbio.2014.07.007
[25] J. Collin, M. Otto, M. Identification of novel roles of the cytochrome P450 system in early embryogenesis; Effect on vasculogenesis and retinoic acid homeostasis, Amer, Soci, microbe; 23(17):2003,6103-6116.
[26] A. Zambelli-Weiner, C. Via, M. Yuen, D. Weiner, and R. Kirby. First trimester ondansetron exposure and risk of structural birth defects. Reproductive toxicology (Elmsford, N.Y.), 83, 2019, 14–20. https://doi.org/10.1016/j.reprotox.2018.10.010.
[27] Health Products Regulatory Authority (HPRA). (2018). Available at: www.imb.ie.
[28] S. Tedesco, H. Doyle, J. Blasco, G. Redmond, and D. Sheehan. Oxidative stress and toxicity of gold nanoparticles in Mytilus edulis. *Aquatic toxicology (Amsterdam, Netherlands)*, *100*(2), 2010, 178–186. https://doi.org/10.1016/j.aquatox.2010.03.001.
[29] R.Rubin, D. Strayer, E.Rubin . Rubin's pathology: clinicopathologic foundations of medicine, 5th ed; 45(2): 2008, 283-284. Lippincott Williams and Wilkins.
[30] H. Tazelaar, J. Wright, and A. Churg, A. Desquamative interstitial pneumonia. *Histopathology*, *58*(4), 2011, 509–516. https://doi.org/10.1111/j.1365-2559.2010.03649.x.
[31] N. Rustagi, S. Singh, N. Dutt, A. Kuwal, K. Chaudhry, S. Shekhar, R. Kirubakaran,.Efficacy and Safety of Stent, Valves, Vapour ablation, Coils and Sealant Therapies in Advanced Emphysema: A Meta-Analysis. Turk Thorac J. Jan 01;20(1):2019,43-60.
[32] D. Dunlap, R. Semaan, C. Riley, and F. Sciurba. Bronchoscopic device intervention in chronic obstructive pulmonary disease. *Current opinion in pulmonary medicine*, *25*(2), 2019, 201–210. https://doi.org/10.1097/MCP.0000000000000561.
[33] P. Pahal, A. Avula, and S. Sharma. Emphysema. In *StatPearls*. StatPearls Publishing.2022.