Tikrit Journal of Veterinary Sciences

Optical microscopy for the effect of Piroxicam administration on the placenta of pregnant Mice

Ali Khudheyer Obayes[1], Ayad Hameed Ebraheem *[2],Bader Khatlan Hameed[2]

[1]Department of Biology, College of Education , University of Samarra, Samarra, Iraq.

[2]Department of Anatomy and Histology, College of Veterinary Medicine, Tikrit University, Tikrit , Iraq

ARTICLE INFO.

Article history:

-Received: 3 / 2 / 2021

-Accepted: 17 / 5 / 2021

-Available online:

Corresponding Author:

Name: Ayad Hameed Ebraheem

E-mail: drayadibrahim@gmail.com

Tel: : 07701715182

Keywords: NSAIDs, piroxicam, placenta of mice

ABSTRACT

The placenta is a vital fetal organ that supplies crucial nutrients and oxygen to the developing fetus, as well as acting as a conduit for carbon dioxide and other fetal waste products to be removed through the maternal circulation. Non-steroidal anti-inflammatory drugs (NSAIDs) are broadly used in pregnancy to treat fever, pain and inflammation. Piroxicam are member of NSAID, used as anti-inflammation drugs. This study we used 20 pregnant (70 days old) albino Swiss mice Mus musculus Balb/c were used. pregnant mice groups were divided into four groups, Therapeutic, overdose1(T1) and overdose2 (T2) and control, each one of them were injected Intra Peritoneum by different doses of Piroxicam (0.0714, 0.1428, 0.71428) mg/kg respectively once daily for 20 days and control group was injected by normal saline .the result was revealed, engorged maternal blood vessels by RBCs, mesenchymal cell degeneration, giant cell vacuolization, Fibroid deposition in maternal blood, chorionic villi atrophy, vacuolization of syncytiotrophoplasts, Necrosis of the embryonic lacunae, Cluster of epithelial mesenchymal cells, Fibroid Odema in blood vessels, thickened walls, desquamated endothelia wall of maternal blood vessels, hyperplasia in syncytiotrophoblast and hypoplasia of syncytiotrophoblast cells . The results observed in this study showed that Piroxicam was harmful on placenta of pregnant mice. This suggests that this drug should be used with care during pregnant period in humans.

1.Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are chemically different in active groups but have the same therapeutic medication and side effects [1].

NSAIDs share the same mechanism that block the way of production of prostaglandin .The key enzyme in the prostaglandin synthesis is cyclooxygenase (COX). There are two major isoforms of COX, referred to as COX-1 and COX-2. COX-1, which has been designated as "constitutive" such as gastric cytoprotection, vascular homeostasis, platelet aggregation, and kidney function. Otherwise , COX-2 was produce during inflammation and cellular activation. [2]. Piroxicam are member of NSAID, used as anti-inflammation. [3]

Prostaglandins (PGs) produced by the maternal uterine tissues (placental tissue, fetus envelops, endometrium and myometrium) which eventually play a major roles in labour in mammalian species. [4]. Some research's, showed lack of teratogenicity was reported for COX inhibitors in human and rodents. While other anti-rheumatic drugs, such as corticosteroids are less used during pregnancy because of higher fetal toxicity [5], [6].

The perversion of drugs by pregnant women can lead to insufficient nutrition of both mother and fetus [7]. Many researches must be done to understand placental function maternal-fetal drugs transfer. [8]

The placenta is a fetal-derived organ that transports nutrition and oxygen to the developing fetus while also releasing carbon dioxide and other fetal waste products [9]. Lacunae appear within the trophoblastic complex's interior, and trophoblast invasion causes the uterine wall's maternal spiral arteries to remodel. The lacunae are formed by the branching of trophoblast into the lacunar spaces (intervillous space) and venous outlets, and the villous trees are formed by the inlets arteries into the lacunar spaces (intervillous space Fetal). capillaries transport oxygenated blood from the villi to the umbilical vein, while the umbilical arteries return deoxygenated fetal blood from the fetus to the placental villi. A continuous layer of syncytiotrophoblast separates maternal blood in the intervillous space from fetal blood in the villous capillaries, cytotrophoblast cells, basal lamina, connective tissue, and fetal endothelial cells form a discontinuous layer[10,11].

This study was designed to assessment the different doses of piroxicam on the placenta of pregnant mice.

2. Materials and methods

This study was done in medical laboratory department of biology/ Education college/ university of Samarra. Twenty four (20 female, 4 male) mature (70 days old) albino Swiss mice Mus musculus Balb/c were employed, weighing (25±3gm) obtained from college of veterinary medicine, Tikrit university. They were maintained on 12:12 light: dark bases, and 24 ±2°C with mouse pelleted food and water. Four female mice were housed in group with one male for free matting (all from the same experimental group) in plastic cages with metal cover (13*16*30) cm, with wood shavings as bedding material. The pregnant mice groups were divided into control group and the experimental were subdivides into three groups, each once was injected Intra Peritoneum (I.P). with different doses of piroxicam once daily for 19 days and control group was injected by normal saline .

2.1 Drug administration

Piroxicam ampule 200 mg/2ml. Females were injected daily Intra Peritoneum (I.P) administrated in three doses: Therapeutic dose, overdose1(T1) and overdose2 (T2) (0.0714, 0.1428, 0.71428) mg/kg for 20 days respectively [12], and Control group were injected with normal saline 0.9 mg/ L.

2.2 Surgical procedure

In ninetieth day, all females were survived for their end periods and then killed using intensive dose inhalation of chloroform inside sealed glass box. The animals were dissected, and specimens of placenta for both experimental and control immediately removed and kept in normal saline .

2.3 Histological preparation

The collected tissues Each segments of placenta was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 cº. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides [13]. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

3. Results and discussion

3.1 Control group

The histological examination was revealed normal embryonic lacunae in between , maternal blood vessel were containing RBCs and the chorionic villi had many hemosiderin Figure (1).

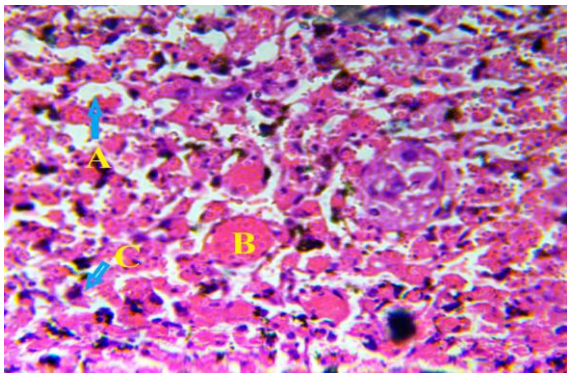

Figure (1): placenta of control group embryonic lacunae (A), maternal blood vessel (B) and giant cells (C). H&E, 40X .

3.2 Therapeutic group (T)

The placenta in periphery part of placenta was containing a maternal blood in form of pools with hemolysis of RBCs in these places, placental villi with lacunae which occupied by many mesenchymal cell, certain number of these cells were degenerated, Figure (2).

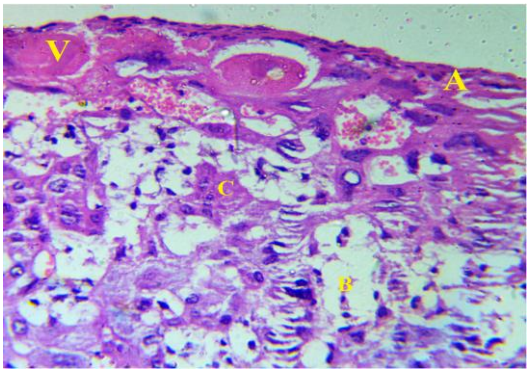

Figure (2): placenta of Tgroup show periphery of placenta (A), lacunar space with degenerated cells (B), chorionic villi (C) and maternal blood vessels (D). H&E, 40X .

The whole mesenchymal tissue of embryo was engorged containing with blood, these are intermingled with maternal blood, the villi stroma was atrophied and associated with presence of giant cells Figure (3).

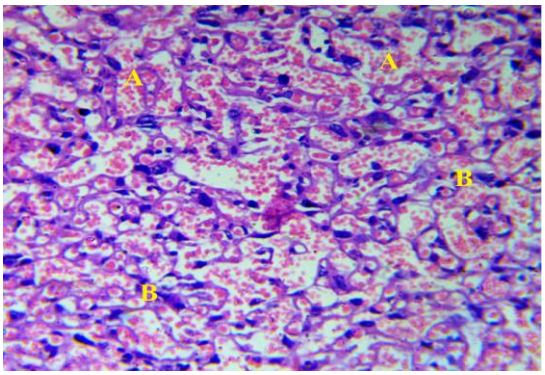

Figure (3) placenta of T group show, embryonic lacunar space engorged with blood (A), atrophy of stromal villi (B). H&E, 40X .

The maternal blood vessels were great and engorged with RBCs surrounded with lacunae filled with RBCs with the presence of cytoplasmic vacuolization in certain area, giant cell with lymphocytes are present in most of villi stroma Figure (4).

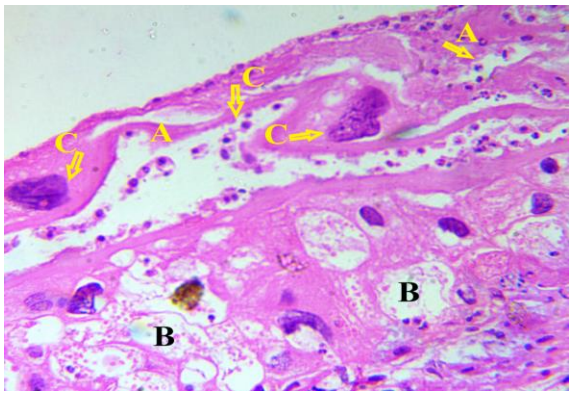

Figure (4): placenta of T group show, maternal blood vessels with blood (A), lacunae with RBCs (B), giant cells with lymphocytes (C) and stromal villi (D).H&E, 40X .

The placental membrane was heavily engorged with RBCs filled the whole lacunae and maternal spaces, the lymphocytes and giant cells (macrophages) could be detected Figure (5).

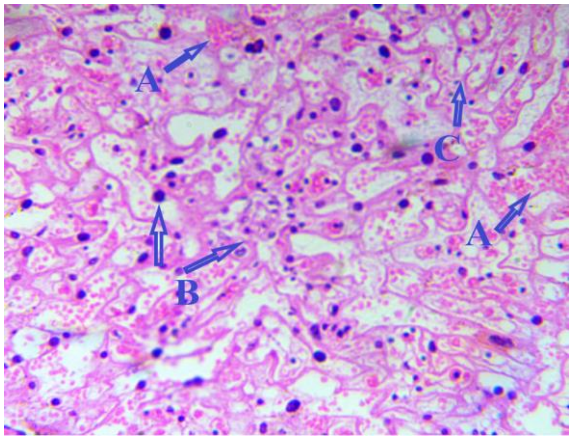

Figure (5): placenta of T group show, lacunar embryonic spaces with RBCs (A), giant cells with WBCs (B), chorionic villi atrophy (C). H&E, 40X .

3.3 Overdose 1group (T1)

The maternal blood space were present intensively congested and the embryonic lacunae have a little trophoblastic cells Figure (6).

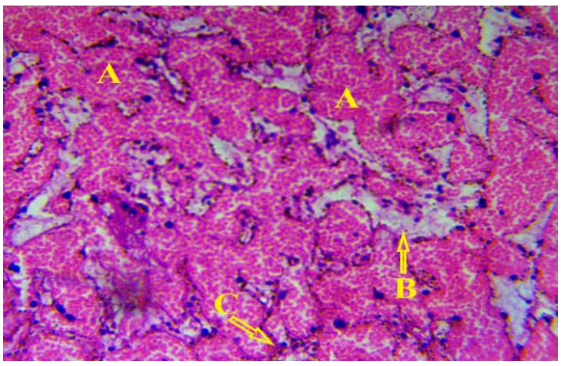

Figure (6): placenta of T1group show, congestion of maternal blood (A), lacunar embryonic spaces (B), with a few trophoblast cells (C) H&E, 40X .

The maternal blood spaces had Fibroid deposit which floating various type of cells, such as lymphocytes, giant cells and trophoblastic cells. The periphery of placenta had formed by many layers of syncytiotrophoblast Figure (7).

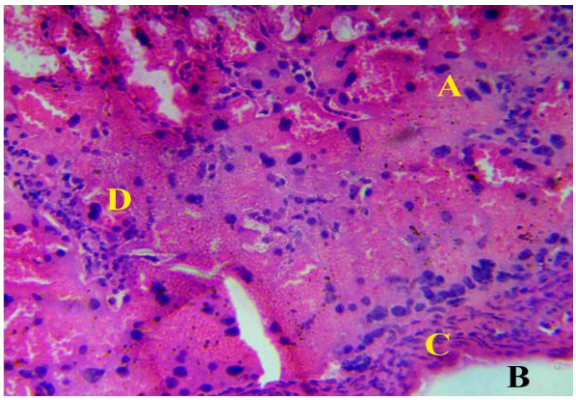

Figure (7): placenta of T1group show, maternal blood spaces with Fibroid deposition (A), periphery of placenta (B), syncytiotrophplast (C), H&E, 40X .

The placental membrane had maternal blood vessels engorged with RBCs extended to lacunar embryonic space, which infiltrated by lymphocytes where distributed in between cells and atrophied chorionic villi was appeared Figure (8).

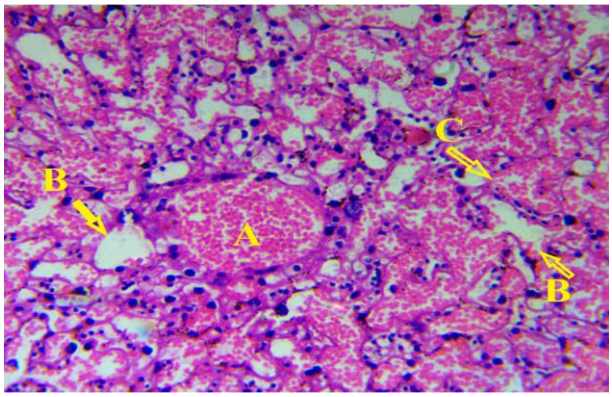

Figure (8): placenta of T1group show, maternal blood vessels engorged with blood (A), lacunar embryonic spaces (B), atrophy of chorionic villi (C). H&E, 40X .

The maternal blood spaces were extensive and occupied the most space of embryonic membrane, the surrounding area were completely engorged with atrophied chorionic villi and lacunar space. Numerous giant cells in lacunae Figure (9).

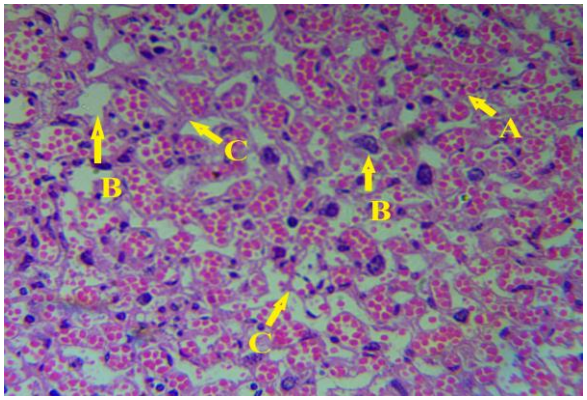

Figure (9): placenta of T1group show, intensive maternal blood spaces filled with RBCs (A), lacunar with giant cells (B), atrophy of chorionic villi (C). H&E, 40X .

Peripheral part of placenta showed vacuolization of syncytiotrophoblasts, WBCs infiltrating in between mesenchymal cells, vacuolization of giant cells and maternal blood vessels congestion Figure (10). Other sections showed clusters of epithelial cells aggregation, degeneration of mesenchymal cells with fibrils deposition and congestion of maternal blood vessel Figure (11). Necrosis of the embryonic lacunae were evident with presence of cellular debris, these are surrounded by many inflammatory cells infiltrated in stromal villi. Figure (12).

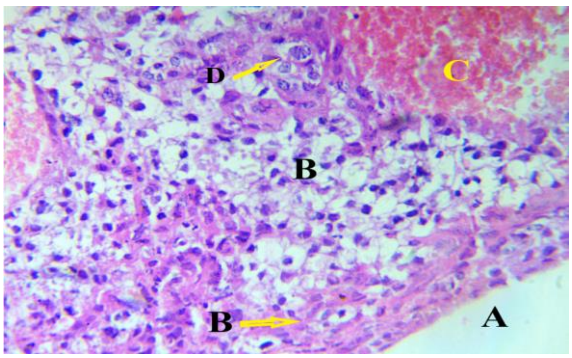

Figure (10): placenta of T1group show, periphery of placenta (A), vacuolization of syncytiotrophoblast (B), WBCs infiltration in embryonic mesenchyme membrane (B), congestion of maternal blood (C). H&E, 40X

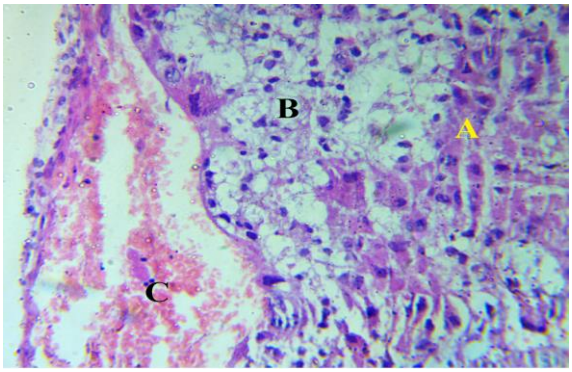

Figure (11): placenta of T1group show, cluster of epithelial cells aggregation (A), degeneration of mesenchyme membrane with fibrils deposition (B), congestion of maternal blood (C). H&E, 40X .

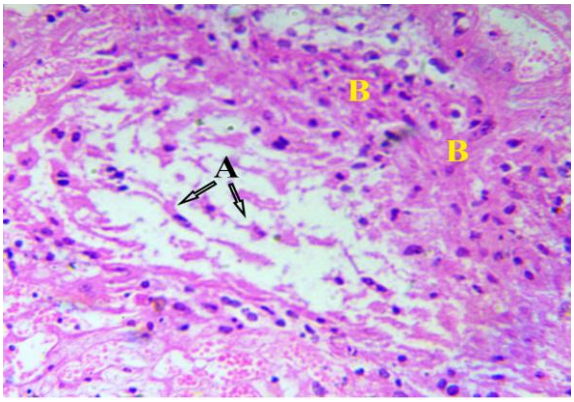

Figure (12): placenta of T1group show, Necrosis of the embryonic lacunae with presence of cellular debris (A), and these are surrounded by many inflammation cells in the stromal villi (B). H&E, 40X .

3.4 Ovedrdose2 group (T2)

The histological examination of this group was showing sever effect of drugs on

placental tissue, occupied the lacunar spaces by giant cell and lymphocytes and atrophied maternal villi with surrounded lymphocytes Figure (13). Cluster of epithelial mesenchymal cells surrounded by giant cells and lymphocytes Figure(14). Fibroid Odema in thickened desquamated endothelia wall of maternal blood vessels, which surrounded by giant cells and lymphocytes situated in embryonic lacunae spaces Figure (15). In Figure (16) showed Cluster of epithelial mesenchymal cells surrounded by giant cells and lymphocytes in between embryonic tissue, Fibroid odema in maternal blood vessel. In Figure (17) showed hyperplasia in syncytiotrophoplasts, Fibroid odema in maternal blood vessels with infiltrated by WBCs and giant cells. Other sections showed hypoplasia of syncytiotrophoplasts cells in marginal parts of placenta which surrounded by giant cells, vacuolization of epithelial mesenchymal cells Figure (18).

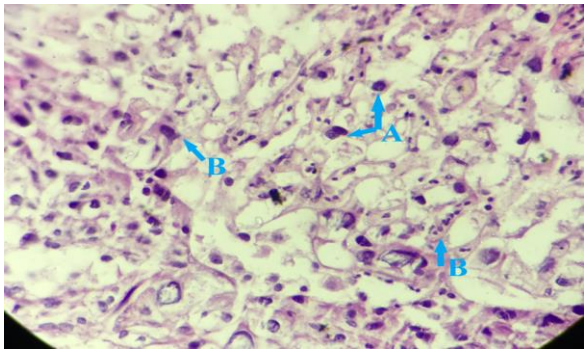

Figure (13): placenta of T2 group show, occupied the lacunar spaces by giant cell and lymphocytes (A), atrophied maternal villi with surrounded lymphocytes (B). H&E, 40X .

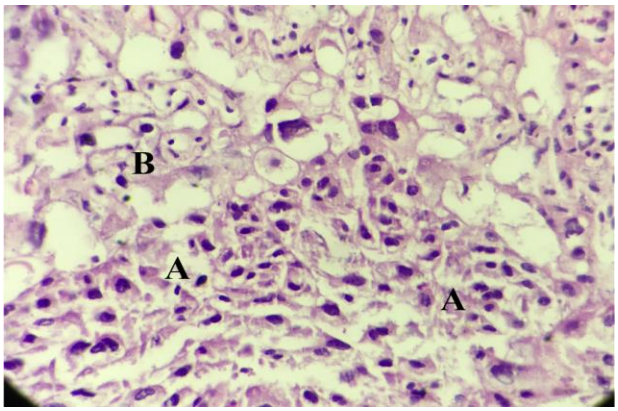

Figure (14): placenta of T2 group show, Cluster of epithelial mesenchymal cells (A), surrounded by giant cells and lymphocytes (B). H&E, 40X .

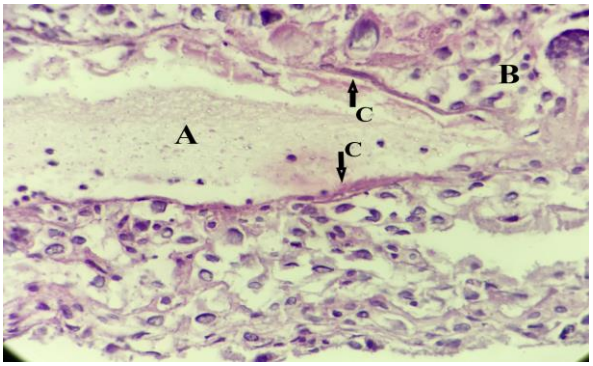

Figure (15): placenta of T2 group show, Fibroid odema (A), giant cells and lymphocytes situated in embryonic lacunae spaces (B), thickened desquamated endothelia wall of maternal blood vessels (C). H&E, 40X .

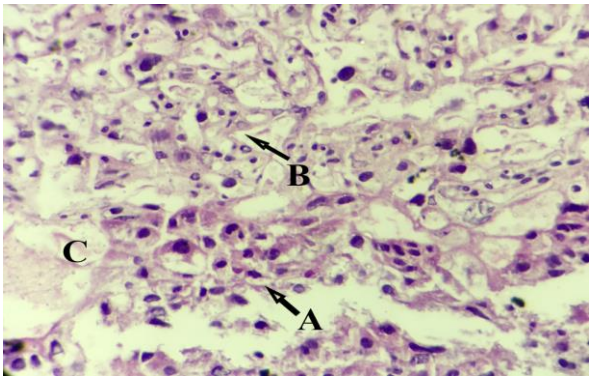

Figure (16): placenta of T2 group show, Cluster of epithelial mesenchymal cells (A), surrounded by giant cells and lymphocytes in between embryonic tissue (B), Fibroid odema (C). H&E, 40X .

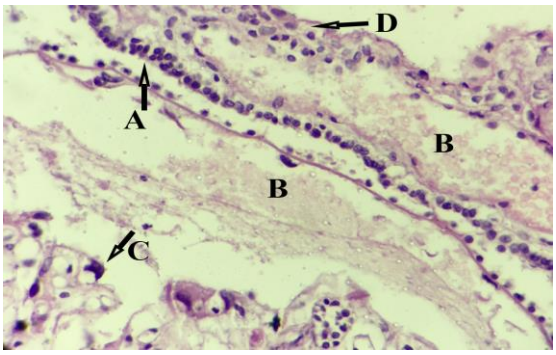

Figure (17):placenta of T2 group show, hyperplasia in syncytiotrophoblast (A), Fibroid odema in maternal blood vessels (B), with infiltrated by WBCs and giant cells (C,D). H&E, 40X .

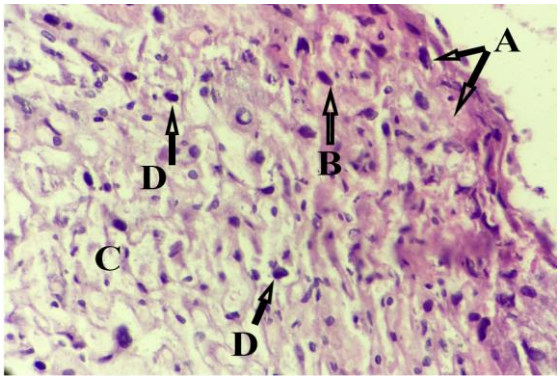

Figure (18): placenta of T2 group show, hypoplasia of syncytiotrophoblast cells in marginal parts of placenta (A), giant cells surrounded syncytiotrophoblast cells (B) and in between mesenchymal membrane (C), vacuolization of epithelial mesenchymal (D). cells H&E, 40X .

The placenta serves as a vital organ in the production and growth of the fetus. The placenta performs as the kidneys, lungs, gut and liver throughout the duration of pregnancy in order to keep the fetus alive[11]. The results of the present study showed in T group degenerated of mesenchymal cell, hemolysis of RBCs. This results agreed with El-Sayed et al. 2014. The oral administration of diclofenac are lead to prevent blastocysts membrane in the intrauterine in mice because of the ability of this drugs to block PG biosynthesis [14].

Other results of T group showed that villi stroma was atrophied which increased with dose and associated with presence of giant cells this agree with Welma et.al. (2019) who demonstrated that decreases in size and weight of placenta lead to decreases in levels of testosterone, FSH and LH in rats [15]. Estrogen is known as an inducer as growth factors necessary for placental villous, its deficiency may induce placental hypertrophy [16]. ovariectomy with estrogen and progesterone treatment [17].

The results of T1 group showed, Intensive maternal blood space and restricted mesenchymal cells in between labyrinthine pattern. While the placental volume and placental surface area increased, might be due to the labyrinth zone encouragement in their density was considered principle site in hemotrophic exchange "nutrient, hormones, ions, waste and water" between maternal and fetal parts [18].

Aggregation of lymphocytes within villi stroma, lysis RBCs agreed with the results showed the effect of anti-viral drugs in rats [19]. In addition, ethanol-induced placental hypertrophy in rats might be an adaptive response to meet placental damage and repair such as hemorrhage, stagnated maternal blood, fibrin deposition and inflammation in the labyrinth zone [20]

Other results showed fibril deposing in maternal blood spaces and increased lymphocytes, giant cells in number, this agreed with There also was a deposition of fibrin in the dilated maternal sinusoids at the central region under the amnion, increase in spongiotrophoblast and clusters of glycogen cells [15].

The maternal blood vessels engorged with RBCs extended to lacunar embryonic space, the lymphocytes where distributed in between villous stroma which agreed with effect of vasodilator in pregnant rats shows, in placenta the maternal vascular spaces were identified by lack of endothelial cell lining, and fetal blood vessels which identified by endothelial-lined vascular spaces containing varying proportions of nucleated red blood cells [21]. The results of T2 showed, the peripheral part of placenta

had many syncytiotrophoblast with giant trophoblast cells. this explain the Blood flow is a vital factor on placental function and embryo growth. Morphologic studies have shown that if physiologic changes of spiral veins occur during pregnancy, trophoblast cells will attack placenta and blood flow will increase at this site and finally placental villi disrupting will appear [22]. necrosis of the embryonic lacunae were evident with presence of cellular debris and these are surrounded by many inflammation cells in the placental villi. This agreed with the results which study the effect of cyclophosphamide in rats Hemorrhages, infarction in the decidua basal and basal zone, hyalinization of labyrinths and the white necrotic patch in the margin, phagocytic cells increase in number observed in this study [23].

Conclusion

The results observed in this study showed that Piroxicam was harmful on placenta of pregnant mice such as degeneration of mesenchymal cell, atrophied of villi stroma, maternal blood vessels engorged with RBCs, restricted in mesenchymal embryonic tissue and Necrosis of the embryonic lacunae. This suggests that this drug should be used with care during pregnant period in humans.

References

[1] Kassaw, C & ‹.Wabe, N. T. (2012). Pregnant women and non-steroidal anti-inflammatory drugs: knowledge, perception and drug consumption pattern during pregnancy in Ethiopia .*North American journal of medical sciences* .72 ‹(2)*4* ‹

[2] Antonucci, R., Zaffanello, M., Puxeddu, E., Porcella, A., Cuzzolin, L., Dolores Pilloni, M & ‹.Fanos, V. (2012). Use of non-steroidal anti-inflammatory drugs in pregnancy: impact on the fetus and newborn .*Current drug metabolism 13* ‹ .490-474 ‹(4)

[3] Sohrabvand, F., Haghollahi, F., Maasomi, M & ‹.Shariat, M. (2014). Effect of piroxicam on ART outcome: a pilot study.*International journal of fertility & sterility*.243 ‹(3)*8* ‹

[4] Welsh, T., Mitchell, C. M., Walters, W. A., Mesiano, S & ‹.Zakar, T. (2005). Prostaglandin H2 synthase-1 and-2 expression in guinea pig gestational tissues during late pregnancy and parturition .*The Journal of Physiology* .912-903 ‹(3)*569* ‹

[5] .5Burdan, F. (2005). Comparison of developmental toxicity of selective and non-selective cyclooxygenase-2 inhibitors in CRL:(WI) WUBR Wistar rats–DFU and piroxicam study.*Toxicology*.25-12 ‹(2-1)*211* ‹

[6] Perraud, J., Stadler, J., Kessedjian, M. J ‹. &Monro, A. M. (1984). Reproductive studies with the anti-inflammatory agent, piroxicam: modification of classical protocols .*Toxicology* ‹(1) *30*‹ .63-59

[7] Šlamberová, R. (2012). Drugs in pregnancy: the effects on mother and her progeny .*Physiological research .61* ‹

[8] Endicott, S & ‹.Haas, D. M. (2012). The current state of therapeutic drug trials in pregnancy .*Clinical Pharmacology & Therapeutics*.150-149 ‹(2)*92* ‹

[9] Moore, K. L., Persaud, T. V. N & ‹. Torchia, M. G. (2015 .(Before We Are Born E-Book: Essentials of Embryology and Birth Defects With STUDENT CONSULT Online Access. Elsevier Health Sciences.

[10] .10Benirschke, K., Burton, G. J & ‹. Baergen, R. N. (2012). Early development of the human placenta. In *Pathology of the human placenta*) pp. 41-53). Springer, Berlin, Heidelberg.

[11] Benirschke, K., Kaufmann, P & ‹. Baergen, R. (2006). Abortion, Placentas of Trisomies, and Immunologic Considerations of Recurrent Reproductive Failure .*Pathology of the Human Placenta*.796-762 ‹

[12] Obayes A, and Mahood, A. Histological study of the effect of Piroxicam on testes of albino mice Mus musculus. Journal of university of Anbar for Pure science, 2013;7(2.(

[13] Bancroft J, and Stevens ,C. Theory and practice of histological techniques .2nd edition .Churchill Livingstone.1982;113-114.

[14] El-Sayed, M. G. A., El-Komy, A. A. A & ‹. Ebied, D. D. I. A. (2014). Evaluation of teratogenic potential of diclofenac in pregnant female's rats .*Benha Vet. Med. J*.66-54 ‹(1)*26* ‹.

[15] Silva, W. E. D., Melo, I. M. F. D., Albuquerque, Y. M. L. D., Mariano, A. F. D. S., Wanderley-Teixeira, V & ‹. Teixeira, Á. A. C. (2019). Effect of metronidazole on placental and fetal development in albino rats .*Animal Reproduction*.818-810 ‹(4)*16* ‹

[16] Bartholomeusz, R. K., Bruce, N. W & ‹. Lynch, A. M. (1999). Embryo survival, and fetal and placental growth following elevation of maternal estradiol blood concentrations in the rat.*Biology of reproduction*-46 ‹(1)*61* ‹ .50

[17] Furukawa, S., Hayashi, S., Usuda, K., Abe, M & ‹.Ogawa, I. (2008). Histopathological effect of ketoconazole on rat placenta .*Journal of Veterinary Medical Science*.1184-1179 ‹(11)*70* ‹

[18] Al-Bayati, M. A., Ahmad, M. A & ‹. Khamas, W. (2014). The potential effect of L-arginine on mice placenta .*Adv Pharmacoepidemiol Drug Saf*.9-1 ‹(2)*3* ‹

[19] Baines, K. J., Rampersaud, A. M., Hillier, D. M., Jeyarajah, M. J., Grafham, G. K., Eastabrook, G & ‹.Renaud, S. J. (2020). Antiviral inflammation during early pregnancy reduces placental and fetal growth trajectories .*The Journal of Immunology*.706-694 ‹(3)*204* ‹

[20] Turan Akay, M & ‹.Arzu Koçkaya, E. (2005). The effects of alcohol on rat placenta .*Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease* .445-435 ‹(6)*23* ‹

[21] Witlin, A. G., Li, Z. Y., Wimalawansa, S. J., Grady, J. J., Grafe, M. R & ‹.Yallampalli, C. (2002). Placental and fetal growth and development in late rat gestation is dependent on adrenomedullin .*Biology of reproduction*.1031-1025 ‹(3)*67* ‹

[22] Kazemi, M., Sahraei, H., Azarnia, M., Dehghani, L., Bahadoran, H & ‹.Tekieh, E. (2011). The effect of morphine consumption on plasma corticosteron concentration and placenta development in pregnant rats .*Iranian Journal of Reproductive Medicine* ‹(2)*9* ‹ .71

[23] PadmaNabhan, R & ‹.Singh, S. (1984). Histopathological changes of placenta induced by cyclophosphamide in rats . *Congenital Anomalies*.8-1 ‹(1)*24* ‹

رؤية مجهرية لتأثير تناول عقار بايروكسان على مشيمة الفئران الحوامل

علي خضير عباس [1]، اياد حميد ابراهيم [2]، بدر ختلان حميد [2]

[2] قسم علوم الحياة – كلية التربية - جامعة سامراء– سامراء - العراق
[1] فرع التشريح والانسجة – كلية الطب البيطري- جامعة تكريت– تكريت - العراق

الخلاصة

تعد المشيمة من الاعضاء المهمة التي تنشأ من الجنين، وظيفتها ايصال المواد العناصر الضرورية والاوكسجين الى الجنين النامي والسبيل الوحيد للتخلص من غاز ثنائي اوكسيد الكاربون والفضلات من الجنين عن طريق الدورة الدموية الام. تعد مجموعة العقاقير المضادة للالتهاب الغير ستيرويدية اكثر العقاقير شيوعا في الاستخدام خلال فترة الحمل لعلاج الحمى ، تخفيف الالم و الالتهابات. يعد عقار البايروكيسكام من مجموعة العقاقير المضادة للالتهاب الغير ستيرويدية ويستخدم في علاج الالتهابات. تم في هذه التجربة توزيع 20 حيوان من الفئران البيض الحوامل من نوع Mus musculus Balb/c (ذات اعمار 70 يوما) الى اربعة مجاميع، المجموعة العلاجية ، مجموعة الجرعة المفرطة الاولى، مجموعة الجرعة المفرطة الثانية و مجموعة السيطرة. تم حقن (تجويف البطن) المجاميع التجريبية الثلاث بواقع جرعة واحدة يوميا بتركيز(0.0714، 0.1428 ، 0.71428) ملغ/كغ من وزن الجسم على التوالي بينما حقنت مجموعة السيطرة بالمحلول الفسلجي. تم قتل الفئران في اليوم 19من الحمل واخذت عينات المشيمة. أظهرت النتائج ما يلي احتقان الاوعية الدموية، تنكس الخلايا المشيمية، تفجي الخلايا المتعملقة، ترسب المادة الغراوية داخل الاوعية الدموية، ضمور الزغبات المشيمية، تفجي طبقة الخلايا المخلاوية، تنخر الجيبانيات الجنينية، تجمع للخلايا المتوسطة، تكون الوذمة الليفية داخل الوعاء الدموي، تثخن جدران الاوعية الدموية، انسلاخ الغلالة الداخلية للوعاء الدموي، فرط تنسج الطبقة المخلاوية وفي مناطق اخرى لوحظ ضمور في الطبقة المخلاوية. اظهرت نتائج الدراسة التأثير الضار على نسيج المشيمة في الفئران البيض.