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Phenotypic and Histological Changes in White Rat Fetuses Induced by Cefixime and Levofloxacin

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Abstract:

This study aimed to evaluate the phenotypic and histological effects of the antibiotics cefixime and levofloxacin on white rat fetuses (*Rattus norvegicus*) during the stages of embryonic development. A total of 35 female rats were divided into seven groups and treated with therapeutic and double doses of the drugs, either individually or in combination, during days 6 to 11 of gestation. The results revealed phenotypic abnormalities such as limb and spinal deformities, growth retardation, and fetal resorptions, particularly in high-dose and combination-treated groups. Histological examinations showed notable alterations in the liver (e.g., inflammatory cell infiltration and giant cells), kidneys (impaired formation of tubules and interstitial tissue), and placenta (thickening and rupture of placental barriers). This study highlights the potential risks of using these antibiotics during pregnancy and calls for a reassessment of their safety when prescribed to women of reproductive age.

Keywords: Cefixime, Levofloxacin, Rat fetuses, Embryotoxicity, Congenital anomalies, Histological alterations.

التغيرات المظهرية والنسيجية في أجنة الفئران البيضاء المحدثة بالسيفيكسيم والليفوفلوكساسين

صالح نوري القيسي ، رشيد خميس شعبان

مستخلص:

هدفت هذه الدراسة إلى تقييم التأثيرات المظهرية والنسيجية للمضادات الحيوية السيفيكسيم والليفوفلوكساسين على أجنة الجرذان البيضاء *Rattus norvegicus* خلال مراحل التطور الجنيني. تم تقسيم 35 انثى جرذ حامل إلى سبع مجموعات عولجت بجرعات علاجية ومزدوجة ومضاعفه من الأدوية، إما منفردة أو مجتمعة، خلال الأيام من 6 إلى 11 من الحمل. وكشفت النتائج عن تشوهات مظهرية مثل تشوهات الأطراف والعمود الفقري، وتأخر النمو، وارتشاف الجنين، وخاصة في المجموعات ذات الجرعات العالية والمجموعات المعالجة بالجرعات المزدوجه أظهرت الفحوصات النسيجية تغيرات ملحوظة في الكبد والكلى ،تمثلت بارتشاح الخلايا الالتهابية والخلايا العملاقة، ضعف تكوين الأنابيب والأنسجة الخلالية، تغيرات نسجيه في المشيمة تمثلت بسماكة وتمزق حواجز المشيمة. تبرز هذه الدراسة المخاطر المحتملة لاستخدام هذه المضادات الحيوية أثناء الحمل وتدعو إلى إعادة تقييم سلامتها عند وصفها للنساء في سن الإنجاب.

الكلمات المفتاحيه : سيفيكسيم، ليفوفلوكساسين، أجنة الفئران، السمية الجنينية، التشوهات الخلقية، التغيرات النسيجية.

Introduction:

In recent decades, the use of antibiotics has increased significantly, not only in clinical and therapeutic applications but also in veterinary and agricultural fields. This widespread usage has raised serious health and environmental concerns, particularly regarding the impact of these compounds on living organisms during embryonic development. In this context, cefixime emerges as a broad-spectrum third-generation cephalosporin antibiotic. It demonstrates high efficacy against a wide range of Gram-positive and Gram-negative bacteria and functions by inhibiting bacterial cell wall synthesis through binding to penicillin-binding proteins. Its relative resistance to beta-lactamase enzymes has made it an effective treatment option for respiratory, urinary, and skin infections (Swathi *et al.*, 2023). However, multiple reports have indicated that cefixime may be associated with potential hepatic and renal toxicity, especially when administered in high doses or over prolonged periods. This is supported by several studies showing elevated liver enzymes (ALT, AST) and histological changes in hepatic and renal tissues in rats exposed to both therapeutic and excessive doses of cefixime (Shaker, 2020; Haboob, 2018).

In parallel, levofloxacin is one of the fluoroquinolone antibiotics that acts by inhibiting the enzymes DNA gyrase and topoisomerase IV, thereby preventing bacterial DNA replication and leading to cell death. Despite its high efficacy, levofloxacin like cefixime has been associated with several adverse effects, ranging from gastrointestinal disturbances and hepatic and renal toxicity to genetic and phenotypic effects in some animal models (Unnisa & Madhukar, 2020; Fiani *et al.*, 2021). Notably, the increasing use of these two drugs, either individually or in combination, whether in clinical practice or as part of experimental studies, raises fundamental questions regarding their potential impact on embryonic development in mammals, particularly given existing evidence of their ability to cross the placental barrier and potentially affect the histological formation of fetal organs.

Based on the above, this study aims to scientifically and objectively highlight the phenotypic and histological

changes that may occur in white rat fetuses (*Rattus norvegicus*) as a result of exposure to cefixime and levofloxacin during critical stages of embryonic development.. Rigorous analytical methods were employed, including histological examinations and phenotypic observations, to accurately assess potential damage. The study is grounded in a robust scientific foundation, incorporating findings from previous studies on the toxicity of cefixime (Shokoohi, 2017; Raddam, 2021) and levofloxacin, as well as contemporary standards for embryotoxicity assessment in toxicological pharmacology.

The importance of this research extends beyond evaluating the risks associated with two widely used clinical drugs; it contributes to a deeper understanding of drug effect dynamics during organogenesis an increasingly vital area of research in light of rising medication use during pregnancy. Moreover, its findings may support efforts to reinforce drug safety standards and reassess clinical guidelines regarding antibiotic use in women of reproductive age. The integration of phenotypic and histological data within a well-controlled experimental model offers an in-depth scientific perspective on toxicological mechanisms and may help pave the way toward safer therapeutic alternatives in the future.

Experimental Animals:

A total of 35 female white rats weighing between 180–200 grams were used in this experiment. The animals were bred in the animal house of the College of Veterinary Medicine, University of Tikrit, and housed in plastic cages with dimensions of 28 × 13 cm. The animals were left for one week to acclimatize before the start of the experiment. All appropriate laboratory conditions were maintained, including a stable temperature of 25°C and a 12-hour light/12-hour dark cycle, with proper ventilation. The animals were fed a standard pellet diet. The experiment began on 20/9/2024 and concluded on 20/10/2024.

Experimental Design:

Female white rats (Rattus norvegicus) weighing 180–200 g were put in a 2:1 ratio (two females per male) with proven fertile males overnight for mating. On the following morning, the presence of sperm in vaginal smears was confirmed using a light micro-

scope to determine successful mating. The day spermatozoa were observed was designated as gestational day 1 (GD1). This method of pregnancy verification is used in rodent reproduction studies and enables accurate timing of gestational stages (Moore *et al.*, 2015). Pregnancy progression was also monitored by observing behavioral changes and by measuring weight gain in the females, as described by Turner *et al.* (2011).

The study was designed using 35 female white rats randomly divided into seven groups, with five animals per group. Weight homogeneity was considered during group allocation based on the following treatments.

- **Group 1 (Control):** Received distilled water only.
- **Group 2:** Treated with cefixime at a dose of 30 mg/kg from day 6 to day 11 of gestation (six doses during pregnancy). Day 6 of pregnancy (GD6) was selected for treatment initiation because it marks the initiation of the organogenesis phase of rat embryonic development when the embryo is most vulnerable to teratogenic activity. The time used is in agreement with practices employed in developmental toxicity experiments (Alhussaini & Alghamdi, 2022)
- **Group 3:** Treated with cefixime at a dose of 60 mg/kg from day 6 to day 11 of gestation (six doses during pregnancy).
- **Group 4:** Treated with levofloxacin at a dose of 38 mg/kg from day 6 to day 11 of gestation (six doses during pregnancy). Group 2 and group 3 were administered the cefixime doses of 30 mg/kg and 60 mg/kg, respectively, selected from the experiment by (Shaker *et al.*,2020) which tried the therapeutic and double cefixime dose on albino rats. The levofloxacin doses in Groups 4 and 5 (38 mg/kg and 76 mg/kg) were determined in accordance with the developmental toxicity study of Takahashi *et al.* (2011), which was performed to investigate its effect on fetal and postnatal development in rats.
- **Group 5:** Treated with levofloxacin at a dose of 76 mg/kg from day 6 to day 11 of gestation (six doses during pregnancy).
- **Group 6:** Treated with both cefixime and levofloxacin at doses of 30 mg/kg and 38 mg/kg, respectively, from day 6 to day 11 of gestation.
- **Group 7:** Treated with double dos-

es of both cefixime and levofloxacin at 60 mg/kg and 76 mg/kg, respectively, from day 6 to day 11 of gestation.

• Embryo Extraction and Histological Tissue Preparation

• On gestation day 20, pregnant rats were euthanized using an overdose of isoflurane anesthesia, in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020). A sterile midline laparotomy was performed to expose the uterine horns. The fetuses were extracted from the uteri using blunt forceps to prevent tissue trauma. All fetuses were examined for external malformations, weighed, and measured immediately. This process ensures the collection of fetuses prior to spontaneous delivery and at a consistent level of development suitable for histological and phenotypic analyses.

Following dissection, liver, kidney, and placenta tissues were fixed immediately in 10% neutral buffered formalin for 16–20 hours at room temperature. The tissues were dehydrated in a series of graded ethanol (70%, 80%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax. Serial sections, 5 µm in thickness, were cut using the aid of a rotary microtome. The tissue sections were mounted onto glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E) for light microscopy.

Results:

• **Phenotypic Changes in Embryos**

The findings related to phenotypic changes in embryos revealed clear differences among the experimental groups. Embryos in the control group appeared normal in terms of number and balanced distribution within the uterine horns, with an average length of approximately 3 cm. Their anatomical features such as the head, trunk, limbs, and tail were well-defined, indicating normal embryonic development (Figure 1). In the group treated with a therapeutic dose of cefixime (30 mg/kg), the embryos appeared in normal numbers and with near-normal morphology, but were smaller in size compared to the control, and some cases of resorption (miscarriage) were observed (Figure 2). In the group treated with a double dose of cefixime (60 mg/kg), prominent malformations were noted, including spinal curvature and limb shortening, along with an increased incidence of resorption (Figure 3).

In contrast, the group treated with a therapeutic dose of levofloxacin (38 mg/kg) did not show significant phenotypic differences compared to the control group, indicating minimal effect at this concentration (Figure 4). However, the double dose of levofloxacin (76 mg/kg) led to several malformations, including reduced fetal size, abdominal skin fissures, and fusion of the hind limbs, accompanied by multiple resorptions (Figure 5).

In the groups treated with a combination of cefixime and levofloxacin, more pronounced changes were observed. The therapeutic combination (30 and 38 mg/kg, respectively) resulted in skin malformations, elongation of the head and limbs, and an abnormally long trunk (Figure 6). The double-dose combination (60 and 76 mg/kg) was associated with severe malformations, including spinal curvature, skin abnormalities in the abdominal region, elongation of the head, and repeated fetal resorptions, reflecting a cumulative toxic effect from the combined treatment(Figure7).

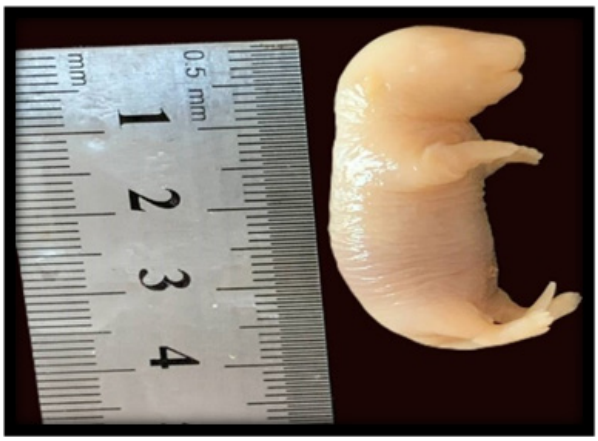

Figure (1): Lateral view of a control rat embryo showing normal shape and size.

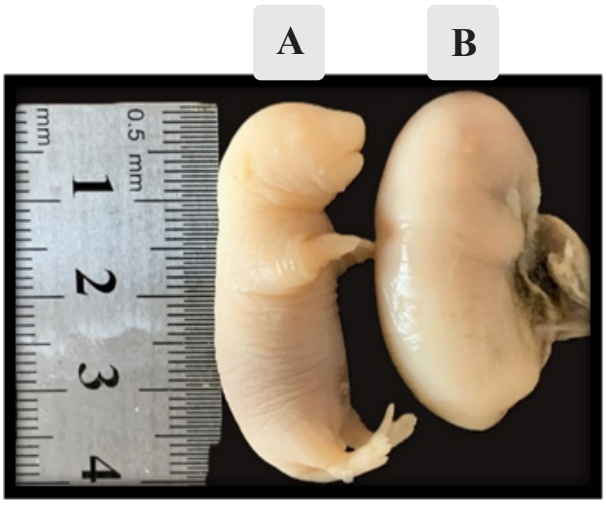

Figure (2): Lateral view of albino rat embryos (A) control embryo, (B) embryo treated with a therapeutic dose of cefixime for six days, showing smaller size compared to the control.

Figure (3): Lateral view of rat embryos (A) control embryo, (B) embryo treated with a double dose of cefixime for six days, showing spinal curvature and limb shortening.

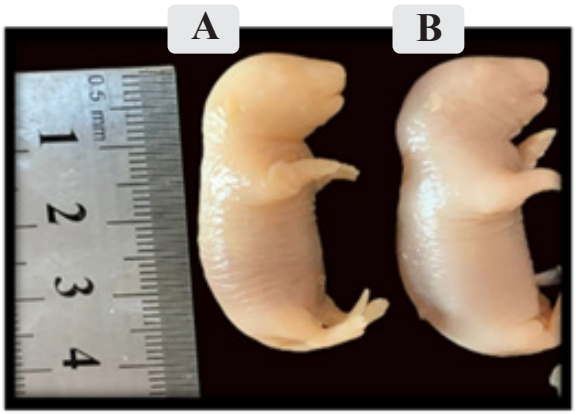

Figure (4): Lateral view of albino rat embryos (A) control embryo showing normal appearance, (B) embryo treated with a therapeutic dose of levofloxacin for six days, showing no deformities.

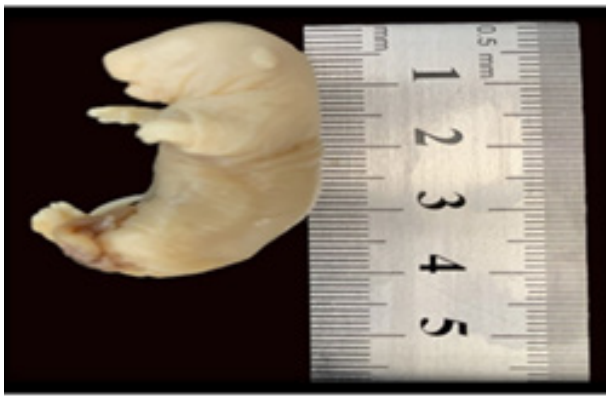

Figure (5): Lateral view of white rat fetuses treated with a double dose of levofloxacin, showing fusion of the hind limbs and curvature of the vertebral column

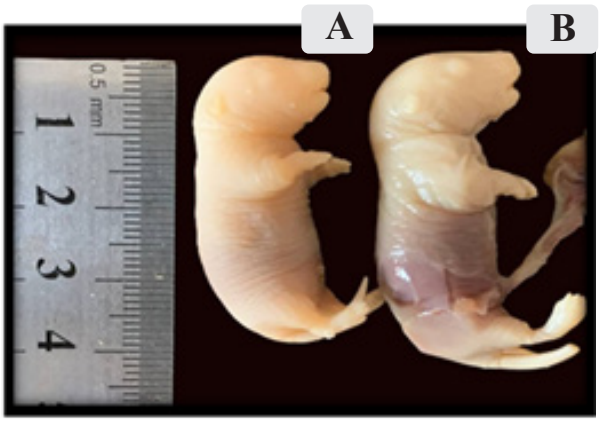

Figure (6): Lateral view of white rat fetuses (A) control fetus, (B) fetus treated with a therapeutic dose of cefixime and levofloxacin showing abdominal skin fissures, an elongated dorsal cranial region, and extended hind limbs compared to the control group.

Figure (7): Lateral view of white rat fetuses treated with a double dose of cefixime and levofloxacin, showing skin malformation, spinal curvature, elongated head, and asymmetry of the forelimbs and hind limbs.

- **Histological Changes of the Liver:**

Histological examinations of the liver in 20-day-old rat fetuses revealed variations in hepatic tissue structure among the studied groups. In the control group, the liver showed normal architecture characterized by the presence of a central vein (CV) and a regular distribution of hepatic cells (HC), with no pathological signs (Figure 8). In the group treated with a therapeutic dose of cefixime (30 mg/kg), the examination showed the presence of the central vein and hepatic cells along with a notable appearance of giant cells, indicating the onset of abnormal tissue response (Figure 9). In the double-dose cefixime group (60 mg/kg), the overall liver structure persisted, but irregularities in the organization of hepatic cells were observed, reflecting a worsening of histological alterations (Figure 10).

Conversely, the group treated with a therapeutic dose of levofloxacin (38 mg/kg) displayed a histological pattern relatively similar to the control group, with preservation of the central vein and hepatic cells without clear abnormalities (Figure 11). However, the double dose (76 mg/kg) resulted in more evident changes, most notably the prominence of the central vein against a background of affected hepatic tissue (Figure 12).

When both drugs were combined, the therapeutic combination (30 and 38 mg/kg) led to an increase in the number of giant cells and disruption in the formation of hepatic tissue (Figure 13). The double-dose combination (60 and 76 mg/kg) revealed marked infiltration of inflammatory cells, along with observable central vein and hepatic cells, indicating a more severe cumulative toxic effect on the fetal liver (Figure 14).

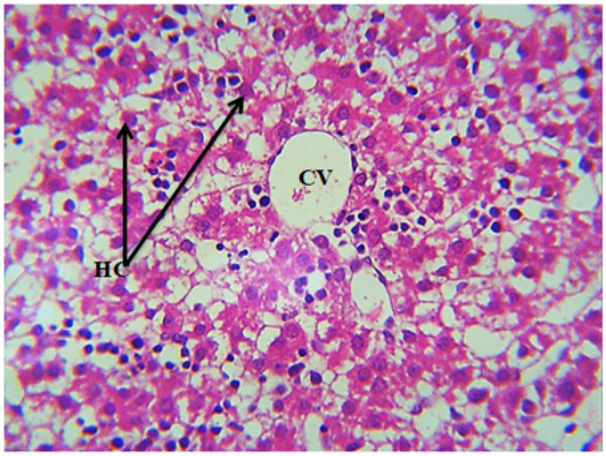

Figure (8):
Liver section of a 20-day-old fetus from the control group showing the central vein (CV) and hepatic cells (HC). H & E stain, 400X.

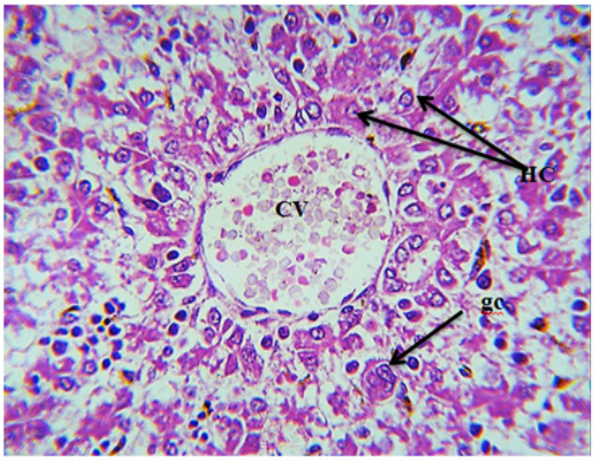

Figure (9):
Liver section of a 20-day-old fetus from the group treated with a therapeutic dose of cefixime, showing the central vein (CV), hepatic cells (HC), and the presence of giant cells (GC). H & E stain, 400X

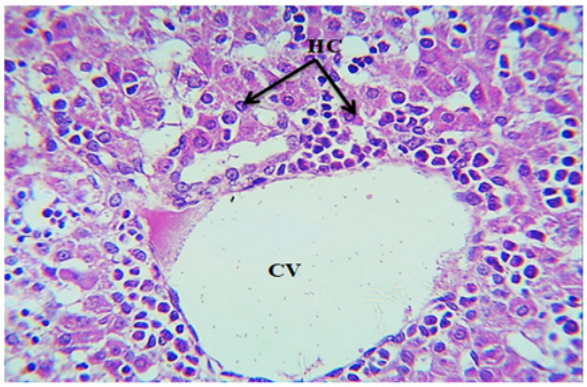

Figure (10): Liver section of a 20-day-old fetus from the group treated with a double dose of cefixime, showing the central vein (CV) and hepatic cells (HC). H & E stain, 400X.

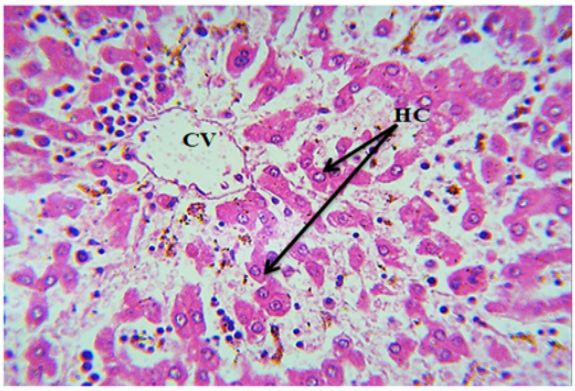

Figure (11): Liver section of a 20-day-old fetus from the group treated with a therapeutic dose of levofloxacin, showing the central vein (CV) and hepatic cells (HC). H & E stain, 400X.

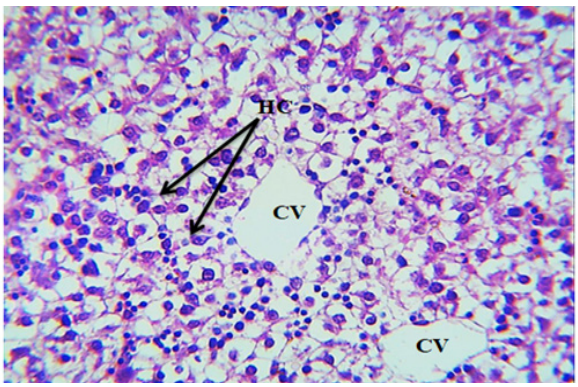

Figure (12): Liver section of a 20-day-old fetus from the group treated with a double dose of levofloxacin, showing the central vein (CV) and impaired development of hepatic cells (HC). H & E stain, 400X.

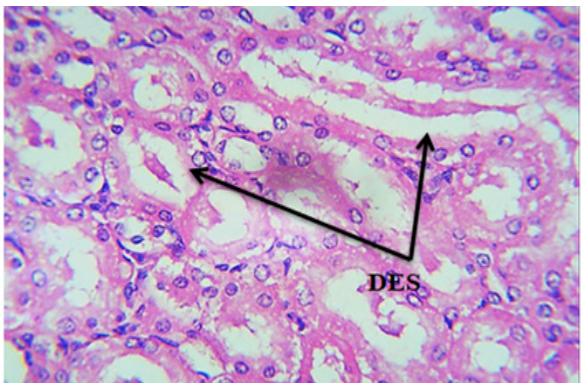

Figure (13): Liver section of a 20-day-old fetus from the group treated with a therapeutic dose of cefixime and levofloxacin, showing an increased number of giant cells (GC) and impaired development of hepatic cells (HC). H & E stain, 400X.

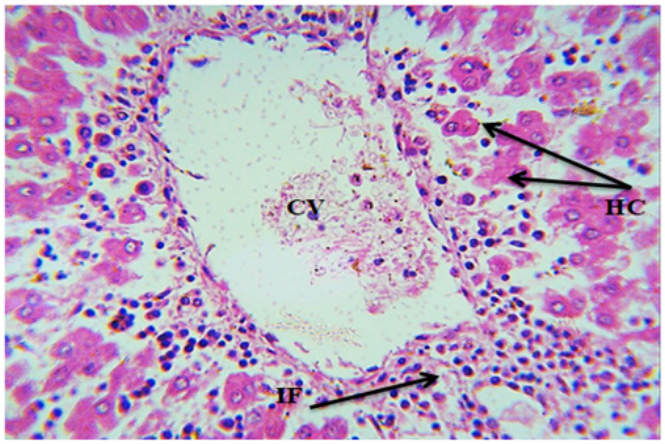

Figure (14): Liver section of a 20-day-old fetus from the group treated with a double dose of cefixime and levofloxacin, showing infiltration of inflammatory cells (IF), along with observable hepatic cells (HC) and central vein (CV). H & E stain, 400X.

• Histological Changes in the Kidney:

Histological examinations of the kidneys in 20-day-old rat fetuses revealed clear structural differences among the experimental groups. The control group exhibited normal tissue organization, characterized by the proper formation of glomeruli (G) and urinary tubules (UT) (Figure 15). The group treated with a therapeutic dose of cefixime (30 mg/kg) maintained the basic structure of the glomeruli and tubules, with only slight changes (Figure 16). However, the double-dose cefixime group (60 mg/kg) showed a noticeable impairment in the development of urinary tubules, indicating a more pronounced negative effect on the developing kidney (Figure 17).

Similarly, the group treated with a therapeutic dose of levofloxacin (38 mg/kg) preserved the general renal tissue architecture without apparent alterations (Figure 18), while the double dose of levofloxacin (76 mg/kg) resulted in mild histological changes in both glomeruli and tubules (Figure 19). The groups treated with a combination of cefixime and levofloxacin exhibited, at the therapeutic dose (30 and 38 mg/kg), weakened development of the renal interstitial tissue along with irregular appearance of glomeruli and tubules (Figure 20). This combined drug effect became more pronounced in the double-dose group (60 and 76 mg/kg), where glomeruli and tubules were present but accompanied by more distinct histological changes, suggesting a cumulative adverse impact on fetal kidney development(Figure21).

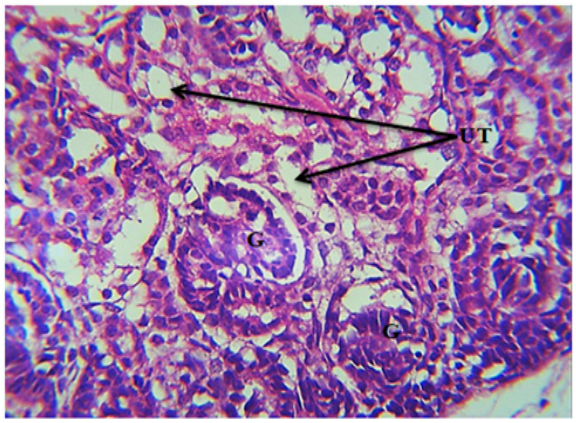

Figure (15):
Kidney section of a 20-day-old fetus from the control group showing the glomerulus (G) and urinary tubules (UT). H & E stain, 400X.

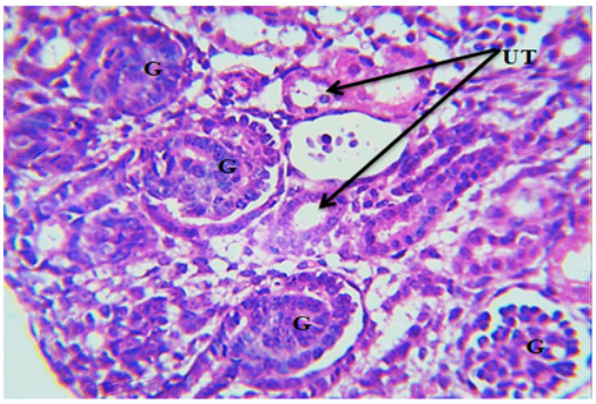

Figure (16):
Kidney section of a 20-day-old fetus from the group treated with a therapeutic dose of cefixime, showing the glomerulus (G) and urinary tubules (UT). H & E stain, 400X.

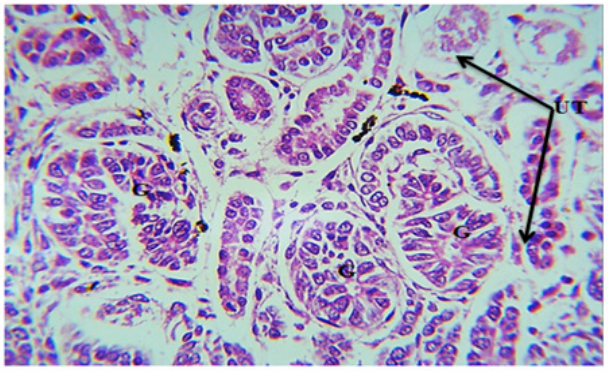

Figure (17):
Kidney section of a 20-day-old fetus from the group treated with a double dose of cefixime, showing the glomerulus (G) and impaired development of urinary tubules (UT). H & E stain, 400X.

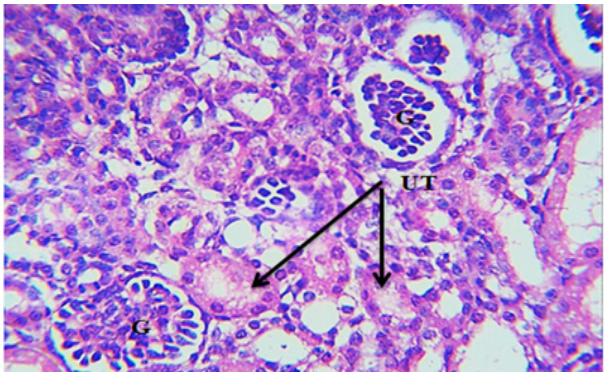

Figure (18):
Kidney section of a 20-day-old fetus from the group treated with a therapeutic dose of levofloxacin, showing the glomerulus (G) and urinary tubules (UT). H & E stain, 400X.

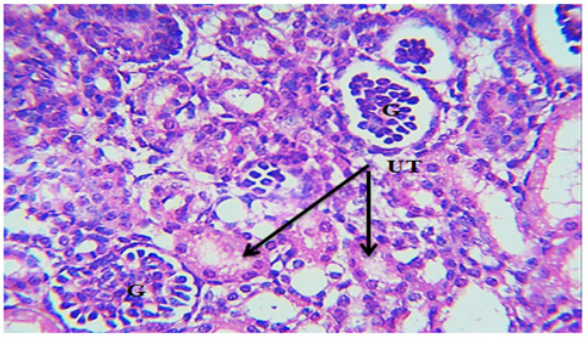

Figure (19):
Kidney section of a 20-day-old fetus from the group treated with a double dose of levofloxacin, showing the glomerulus (G) and urinary tubules (UT). H & E stain, 400X.

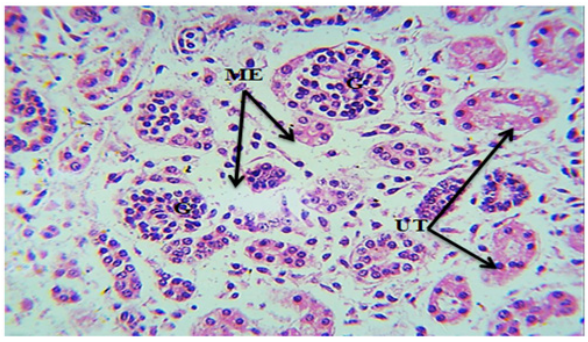

Figure (20):
Kidney section of a 20-day-old fetus from the group treated with a therapeutic dose of cefixime and levofloxacin, showing the glomerulus (G) and urinary tubules (UT), with noticeable impairment in the development of renal mesenchymal tissue (ME). H & E stain, 400X.

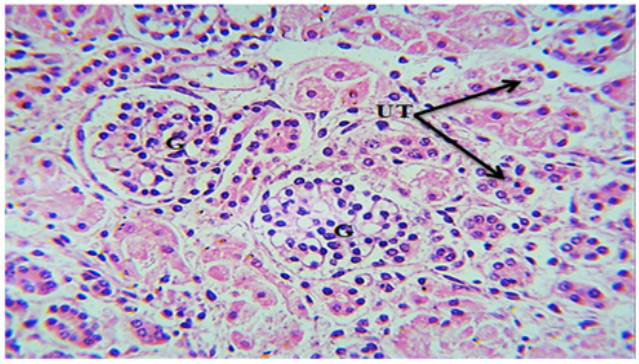

Figure (21):
Kidney section of a 20-day-old fetus from the group treated with a double dose of cefixime and levofloxacin, showing the glomerulus (G) and urinary tubules (UT). H & E stain, 400X

• **Histological Changes in the Placenta:**

Histological analysis of placental tissues from 20-day-old rat fetuses revealed notable differences among the examined groups. In the control group, the placental structure appeared normal, with well-organized maternal and embryonic blood components and clearly defined placental barriers (Figure 22). In the group treated with a therapeutic dose of cefixime (30 mg/kg), distinct thickening of the placental barriers was observed, indicating an early pathological response (Figure 23). The double-dose cefixime group (60 mg/kg) exhibited rupture of these barriers and increased blood content, reflecting significant histopathological damage to the placenta (Figure 24).

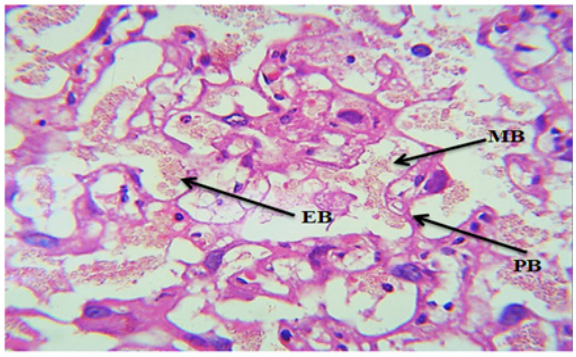

Figure (22):
Placental section from the control group showing embryonic blood (EB), maternal blood (MB), and placental barriers (PB). H & E stain, 400X.

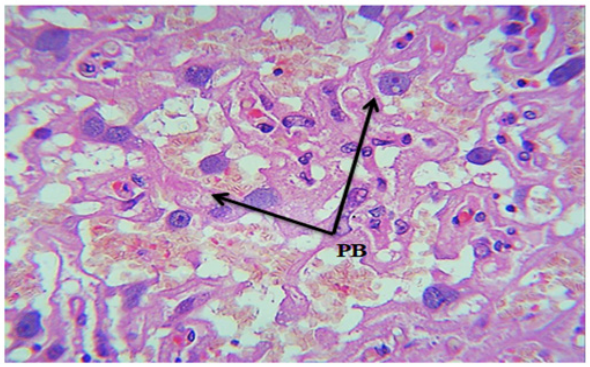

Figure (23):
Placental section from the group treated with a therapeutic dose of cefixime, showing thickening of the placental barriers (PB). H & E stain, 400X.

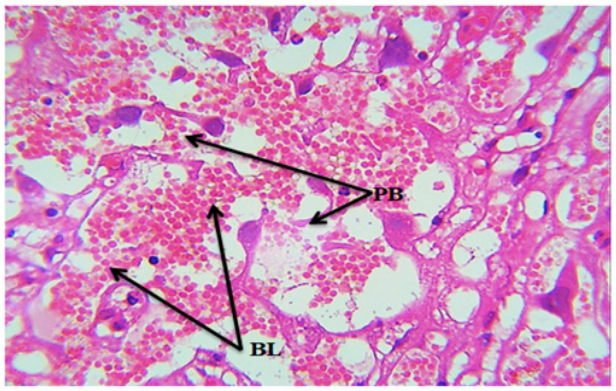

Figure (24): Placental section from the group treated with a double dose of cefixime, showing rupture of the placental barriers (PB) and increased blood content (BL). H & E stain, 400X.

In contrast, the levofloxacin-treated groups, both at therapeutic (38 mg/kg) and double doses (76 mg/kg), maintained normal placental tissue structure, with intact barriers and normal maternal and fetal blood distribution (Figures 25 and 26). However, in the groups treated with a combination of cefixime and levofloxacin, the therapeutic dose group (30 and 38 mg/kg) displayed increased thickening of placental barriers along with reduced placental blood flow (Figure 27). These changes were exacerbated in the double-dose combination group (60 and 76 mg/kg), where the barrier thickening persisted alongside a marked reduction in blood supply, indicating a compounded adverse effect on placental efficiency (Figure 28).

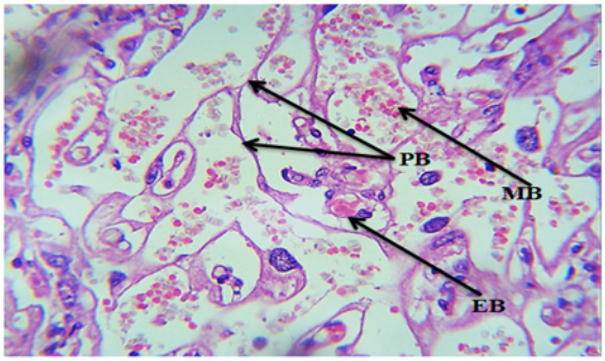

Figure (25):
Placental section from the group treated with a therapeutic dose of levofloxacin, showing placental barriers (PB), maternal blood (MB), and embryonic blood (EB) in their normal structure. H & E stain, 400X.

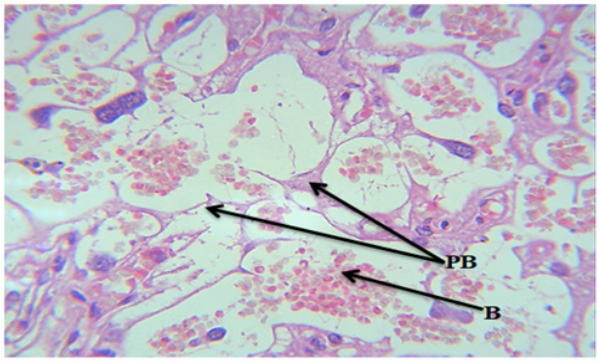

Figure (26):
Placental section from the group treated with a double dose of levofloxacin, showing placental barriers (PB) and placental blood (B) in their normal structure. H & E stain, 400X.

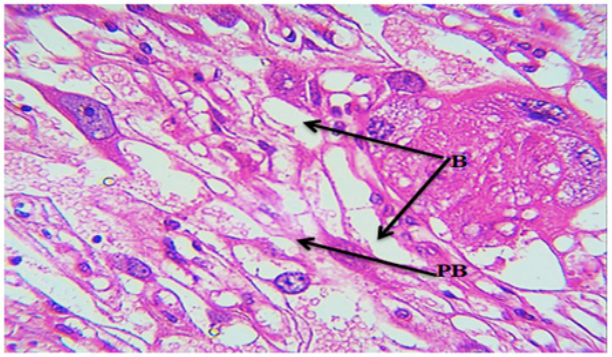

Figure (27):
Placental section from the group treated with a therapeutic dose of cefixime and levofloxacin, showing increased thickness of the placental barriers (PB) and reduced placental blood supply (B). H & E stain, 400X.

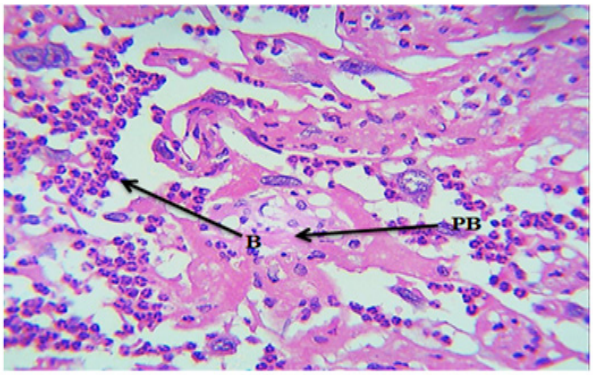

Figure (28):
Placental section from the group treated with a double dose of cefixime and levofloxacin, showing increased thickness of the placental barriers (PB) and reduced placental blood supply (B). H & E stain, 400X.

Discussion:

- **Effect of Treatment on Phenotypic Changes in Embryos:**

The study results demonstrated that exposure to cefixime and levofloxacin whether individually or in combination led to phenotypic alterations in rat fetuses, varying according to the drug type and dosage. The control group showed normal embryonic development in terms of length and structural differentiation (Figure 1), reflecting healthy growth in the absence of harmful pharmacological agents. In contrast, a therapeutic dose of cefixime (30 mg/kg) caused growth retardation and resorptions (Figure 2), indicating a suppressive effect on cellular division or placental perfusion (Beaudoin *et al.*, 2013). When the dose was doubled (60 mg/kg), malformations became more evident, such as limb shortening and spinal curvature (Figure 3), which are indicative of disrupted cellular differentiation in the embryonic axis and limb buds (Sparrow *et al.,* 2012; Capdevila & Izpisúa Belmonte, 2001).

As for levofloxacin, no apparent teratogenic effects were observed at the therapeutic dose (38 mg/kg) (Figure 4), consistent with findings by Myers et al. (2010), who reported the safety of low-dose exposure. However, the double dose (76 mg/kg) resulted in marked signs of toxicity, including abdominal skin fissures, limb fusion, and resorptions (Figure 5), likely due to oxidative stress and inhibition of DNA repair enzymes in embryonic cells (Cuzzolin *et al.,* 2014).

The combination treatment exhibited a cumulative toxic effect even at therapeutic doses, with deformities noted in the head, skin, and trunk (Figure 6), possibly caused by drug interaction in placental or hepatic metabolism, leading to the accumulation of toxic intermediates (Hass, 2006). These deformities became more severe with double doses (Figure 7), manifesting as disruptions in the Wnt and BMP signaling pathways critical for embryogenesis (Niehrs, 2010). Additionally, the high rate of resorptions suggests activation of the in-utero natural selection mechanism to eliminate non-viable embryos (Clark *et al.,* 1996).

These findings clearly indicate that cefixime and levofloxacin, especially when used in combination or at high doses, may possess teratogenic poten-

tial that increases the risk of congenital malformations or pregnancy loss. This underscores the necessity of exercising caution when prescribing these antibiotics during pregnancy, particularly during early organogenesis.

- **Effect of Treatment on Histological Changes in the Liver**

Histological examinations of the liver in 20-day-old rat fetuses revealed pathological responses that varied depending on the type and dose of the administered antibiotic. In the control group (Figure 8), hepatic cells appeared normally arranged around the central vein, reflecting a healthy physiological development of the fetal liver tissue (Zorn, 2008). In the cefixime-treated group at a therapeutic dose (30 mg/kg), giant hepatic cells were observed (Figure 9), indicating impaired cell division or delayed maturation, possibly due to a drug-induced effect on the cell cycle (Fisher *et al.*, 2004). In the double-dose cefixime group (60 mg/kg), the tissue showed a similar pattern without additional structural changes (Figure 10), suggesting persistent damage without further morphological progression, potentially indicating suppressed regeneration of hepatic cells (Lemasters *et al.*, 2002).

In the levofloxacin group at a therapeutic dose (38 mg/kg), the tissue appeared nearly normal (Figure 11), indicating that this dose was insufficient to cause noticeable histological disruption, consistent with findings by Kato *et al.* (2009). However, in the double-dose levofloxacin group (76 mg/kg), the liver tissue showed unclear structure with loss of cellular detail (Figure 12), suggesting extensive cellular degeneration or a loss of fundamental structural organization (Slikker *et al.*, 2004).

In the group treated with both drugs at therapeutic doses (30 and 38 mg/kg), a large number of giant cells were found, along with poorly organized hepatic tissue (Figure 13), indicating a cumulative effect that impairs liver tissue maturation due to overlapping toxic influences (Carpenter *et al.,* 2002). In the double-dose combination group (60 and 76 mg/kg), inflammatory cell infiltration was observed (Figure 14), indicating an active immune response to tissue damage and a clear sign of toxicity that may extend beyond the fetal stage (Sakamoto *et al.,* 2011).

• **Effect of Treatment on Histological Changes in the Kidney**

Histological examinations of the kidneys in 20-day-old rat fetuses revealed that the normal structural organization of glomeruli and urinary tubules in the control group (Figure 15) reflects complete maturation of the fetal renal system, consistent with the final stages of kidney development as described by Schedl (2007). In the group treated with a therapeutic dose of cefixime (30 mg/kg), the renal structure remained normal (Figure 16), indicating that this dose did not cause visible histological damage, in line with the notion that some cellular changes may remain below the threshold of conventional microscopic detection (George & D'Cruz, 2008). However, in the double-dose cefixime group (60 mg/kg), impaired formation of urinary tubules was observed while glomeruli remained intact (Figure 17), suggesting that tubular cells are more sensitive to the drug's effects, possibly due to disruption in differentiation signals associated with the Wnt and Pax2 pathways (Dressler, 2006).

In the levofloxacin-treated group at a therapeutic dose (38 mg/kg), no significant changes were noted (Figure 18), supporting the findings of Takahashi *et al.* (2011), which affirm that moderate doses of this drug do not induce marked renal toxicity in fetuses. In contrast, the double-dose group (76 mg/kg) showed the presence of glomeruli and tubules but without additional structural descriptions (Figure 19), suggesting the possibility of subcellular or ultrastructural disturbances that cannot be easily detected via routine histology consistent with fluoroquinolone-induced DNA damage mechanisms (Cuzzolin *et al.*, 2001).

In the group treated with both drugs at therapeutic doses (30 and 38 mg/kg), weakened development of renal interstitial tissue was evident despite the presence of glomeruli and tubules (Figure 20), indicating a combined effect on mesenchymal cells potentially linked to disruptions in growth factors like TGF-β and VEGF (Kobayashi *et al.,* 2008). Meanwhile, in the double-dose combination group (60 and 76 mg/kg), the basic renal architecture persisted without apparent changes (Figure 21), which may reflect early cell-cycle arrest (senescence) due to mitochondrial damage, as described by

Gibson & MacLaughlin (2015).

These findings highlight the sensitivity of fetal kidneys to pharmaceutical agents, particularly during advanced stages of development, with a clear impact of cefixime on tubular formation and cumulative effects under combination treatment. This underscores the importance of caution when prescribing these drugs during pregnancy.

- **Effect of Treatment on Histological Changes in the Placenta:**

Histological examinations of the placenta in 20-day-old rat fetuses showed that the control group exhibited a normal structure, with maternal and fetal blood clearly separated by intact placental barriers (Figure 22), reflecting the proper physiological function of the placental barrier (Burton & Fowden, 2015). In the group treated with a therapeutic dose of cefixime (30 mg/kg), thickening of the placental barriers was observed (Figure 23), which may hinder material exchange and represents a possible defensive tissue response to cellular deposition or increased collagen production, as noted by Benirschke *et al.* (2012). At the double dose (60 mg/kg), rupture of the barriers and increased blood accumulation in the placenta were recorded (Figure 24), indicating a breakdown in the placental barrier and loss of its function, potentially leading to hazardous blood mixing between the mother and fetus (Cotechini *et al.,* 2014).

In contrast, the group treated with a therapeutic dose of levofloxacin (38 mg/kg) showed no significant changes, with the placental barriers appearing normal (Figure 25), which is consistent with reports confirming the limited histological toxicity of this drug at low doses (Terhune *et al.*, 2016). Even at the double dose (76 mg/kg), the placental structure remained normal without pathological indicators (Figure 26), supporting findings by Arun & Kavanagh (2012) that fluoroquinolone effects on the placenta may not intensify in the absence of accompanying inflammatory conditions.

However, the combination of cefixime and levofloxacin at therapeutic doses (30 and 38 mg/kg) showed cumulative effects, such as thickening of placental barriers and reduced placental blood flow (Figure 27), suggesting drug interaction that affects placental vasculature and may lead to fetal hypoxia (Redline, 2007). These changes

became significantly more severe in the double-dose combination group (60 and 76 mg/kg), where excessive thickening of the barriers and a substantial reduction in fetal blood supply were observed (Figure 28), indicating severe placental dysfunction similar to early-stage preeclampsia or a chronic toxic stress response (Roberts & Post, 2008).

These findings highlight the histological impact of cefixime on the placenta and show that levofloxacin alone does not cause significant tissue damage, even at high doses. However, when both drugs are combined, a synergistic negative effect emerges, severely impairing placental function, warranting caution regarding drug interactions during pregnancy.

Conclusions:

1. Cefixime induces clear phenotypic and histological effects in fetuses when administered at high doses, including growth retardation, limb and spinal deformities, as well as hepatic, renal, and placental alterations.

2. Levofloxacin exhibits relatively lower toxicity at therapeutic doses; however, higher doses result in both phenotypic and histological changes, particularly affecting the liver and placenta.

3. Combined administration of cefixime and levofloxacin even at therapeutic levels leads to a cumulative or synergistic negative effect, intensifying damage to fetal tissues, which is evident in external malformations and disorganized tissue structures.

4. The placenta was among the most affected organs under combination treatment, supporting the hypothesis that both drugs can cross the placental barrier and exert direct effects on the fetal environment.

5. These results underscore the importance of caution when prescribing these drugs during pregnancy and highlight the need for broader studies to evaluate the embryotoxic potential of commonly used antibiotics.

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tivity. The time used is in agreement with practices employed in developmental toxicity experiments (Alhussaini & Alghamdi, 2022)

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