Evaluation the Histological Effects on Brian and Skeletal Malformations in Fetuses and Neonates of Rats Treated with the Antibiotic Levofloxacin

Hanaa Mumtaz Hussein¹; Dalal Abdel-Hussein Kadhim AL–Essawi²

¹Department of Biology, Faculty of Education for Girls, University of Kufa, Iraq.
²Department of Biology, Faculty of Education for Girls, University of Kufa, Iraq.

Abstract
This study design to reveal the possible negative effects of the antibiotic Levofloxacin (Levo) on the fetus numbers (total, living, absorbed and dead) during three periods of pregnancy (20, 16, 7) days respectively and on some histological effects of brain of fetuses during two periods of pregnancy (20, 16) days respectively and the skeletal malformations in fetuses for pregnancy period (20) days and neonates after birth, the study was carried out in the animal house of the Department of biology at the College of Education for Girls - University of Kufa. This study lasted from November 2020 to March 2021, 60 adult female rats and 60 fertile male rats Rattus rattus were used in the current study, female rats were with an average age of 12 weeks and an average weight of 240 g, while male rats were at an average age of 11 weeks and the average weight of 230 g which was used for the purpose of mating with females only, and after mating between males and females rats and obtaining a sufficient number of pregnant females rats, the pregnant rats were divided into 4 groups, each containing 20 pregnant female rats. The first group was treated as a control group that was dosed with physiological salt only, the second group was dosed with the antibiotic levofloxacin at a concentration of 500 gm / kg of body weight and the third group was dosed with the antibiotic levofloxacin at a concentration of 750 gm / kg of body weight, the first 15 pregnant female rats from all groups were dissected during the two pregnancy periods (7, 16, 20) days respectively, It included each group 5 pregnant females for each pregnancy period, while the remaining 5 pregnant females from each group were left to the birth, all pregnant animals were dosed from the first day of pregnancy by a stomach tube with single dose per day. The results of the present indicated that there was a significant decrease (P<0.05) in the numbers of total and the numbers of live embryos, while the numbers of absorbed and dead embryos were significantly increased (P<0.05) in groups of animals treated with the antibiotic levofloxacin at concentrations 500 g/kg and 750 g/kg of body weight for pregnancy periods (7,16,20) days respectively compared with the numbers of all embryos and for the three pregnancy periods (7,16,20) days respectively in the control groups, and the results pointed to significant differences in two groups treated with concentrations of (500,750) g/kg of body weight during the three pregnancy periods when comparing them. The results of the study showed that the brain sections of the embryos in the pregnant rats groups in the control groups during the two pregnancy periods (16,20) days respectively did not show any pathological changes in their
tissues, while the brains of the embryos in the groups treated with the antibiotic levofloxacin at two concentrations 500 g/kg of body weight showed various pathological changes during pregnancy for periods (16 ,20) days respectively, and these histological structure effects increased by increasing drug concentration with 750 g/kg of body weight during pregnancy periods(16,20) days respectively. The study of the skeletons of embryos during the pregnancy period of 20 days and of the newborns after birth showed the occurrence of different skeletal malformations in the groups treated with the antibiotic levofloxacin at two concentrations 500 g/kg and 750 g/kg of body weight when compared with the skeletons of the embryos at the age of 20 days of pregnancy and for the newborns after birth in control groups for the same periods.

Key-words: Levofloxacin, Brian, Skeletal Malformations, Dead, Antibiotic, Pregnant.

1. Introduction

Levofloxacin(Levo) is one of the antibiotics that belongs to the family of fluoroquinolones and is used to treat several types of diseases caused by bacteria sensitive to these antibiotics, his drug belongs to the third generation of fluoroquinolones which have high activity against most gram-positive and gram-negative aerobic organisms, the scientific name of the drug is Levofloxacin, and the drug is also known by many brand names such as Levaquin, Tavanic, Felsin, Voflan and Levox, and its chemical formula is C18 H20 F N3O4. 1/2 H2O, molecular weight of it is about (370,38) (Koepe et al., 2011). The mechanism of levofloxacin action includes its ability to inhibit the transcribed DNA in bacterial cells before their division after entering them through the proteolytic channels located in the outer membrane by the process of active transport, causing inhibition of the enzymes of both DNA gyrase responsible for The super-coiling of the DNA to fit the newly formed bacterial cells and the enzyme Topoisomerase IV which separates the cloned DNA preventing bacterial growth and reproduction (Mutschier et al., 2001), Levofloxacin is rapidly absorbed from the gastrointestinal tract after administration and drug concentrations reach their maximum in plasma after 1-2 hours in stable conditions when taking a single dose orally, this drug penetrates well into most body fluids and tissues such as bone and lung tissue and its binding rate with plasma proteins in the blood is about 30-40%. Levofloxacin is excreted mainly from the body by the urinary system through removal by glomerular filtration in the kidneys(). The drug is used to treat respiratory infections such as pneumonia, as well as various bacterial urinary tract infections such as cystitis, urethritis, Prostate and conjunctivitis infections (Forsyth et al., 2018), and treatment the skin abscesses, Anthrax germs and plague in children and adults (Foxman, 2014), the use of the drug is associated with many side effects especially in the central nervous system such as headache, insomnia, sleep disturbances, dizziness and nervous pressure in the skull, and side effects appear on
the respiratory system such as asthma, cough and sore throat, and it also causes anemia, an imbalance in the numbers of platelets with low levels of prothrombin, distorted white blood cells in addition to inflammation of the blood vessels and irregular heartbeat, it also affects the urinary system especially the kidneys causing inflammation and kidney failure, and causes many effects in the digestive system such as nausea, infections of the esophagus, gallbladder, gastritis, elevated liver enzymes and gout and irritates the skin causing itching, and Johnsen-Steven syndrome.

Aim of this study was to detect the harmful effects of levofloxacin on fetuses numbers (total, living, absorbed and dead) the three pregnancy periods (7,16,20) days and on the histological structure of the brain in fetuses during the pregnancy period (16,20) days, as well as the malformations in the skeletal structures of fetuses during (20) days of pregnancy and for newborns after birth.

2. Materials and Methods

Preparation of Pregnant Female Rats

In this study, (60) adult female Albino rats of the type Rattus rattus (Sprague Dawley) were used, with an average age of 12 weeks and an average body weight of 240 g, in addition to (60) fertile male rats of the same species with average age of 11 weeks and body weight 230 g, rats were brought from the animal house of the Faculties of Science and Veterinary Medicine - University of Kufa, after making sure they are free from diseases and pregnancy, the rats were placed in special plastic cages for animals breeding, all animals were left in the animal house of the college for a week for the purpose of adaptation, the rats were exposed to similar conditions in the laboratory in terms of ventilation, lighting for 14 hours and a temperature of 30-20°, the experimental animals were provided freely with water and special animal food, for mating the adult female rats were placed with fertile male rats daily, each one female rat is placed one malerat in each of the breeding cages at six o’clock in the evening and left all night without water or food, and at eight o’clock in the morning of the next day the mated females were examined for the purpose of watching the mating plug (Vaginal plug) as shown in the figure(1-3) in the female’s vagina or on the floor of the breeding cage, the vaginal plug has been relied upon by (100%) in determining whether pregnancy has occurred or not and the first day of watching of the vaginal plug is Zero day of pregnancy (Fox et al., 2006).
Levofloxacin Drug

Levofloxacin was obtained in the form of pills in two doses (500-750) g / kg of body weight from pharmacies in the governorate, then the required concentrations in the current study were prepared according to the weights of the study animals.

Design of Study Experiment

After obtaining the required number of pregnant rats (60), all pregnant rats were treated by intra-gastric dosage orally by tube stomach tube (Gavage) for rats at a rate of once a day from the first day of pregnancy until autopsy during the two pregnancy periods (7,16,20) days respectively, pregnant female rats were divided into three main groups, each one containing 20 pregnant female rats and each main group was divided into four subgroups each containing 5 pregnant females and treated as follows:

A- The first main group (control group) (G1): It contained 20 pregnant females who were dosed with normal saline only and were divided into:

Subgroup1: It included 5 pregnant female rats, they were dosed from the first day to 16 th day of pregnancy and were dissected on the next day.

Subgroup2: It included 5 pregnant female rats, they were dosed from the first day to 7 th day of pregnancy and were dissected on the next day.

Subgroup3: It included 5 pregnant female rats, they were dosed from the first day to 20th day of pregnancy, where they were dosed at 7 am and dissected on the same day at 6 pm.

Subgroup4: It included 5 pregnant female rats were dosed from the first day of pregnancy and left until the birth.
B - The second main group (G2): contained 20 pregnant females, dosed with levofloxacin at a concentration of 500 g / kg of body weight and divided into:

Subgroup1: It included 5 pregnant female rats, they were dosed from the first day to 16th day of pregnancy and were dissected on the next day.

Subgroup2: It included 5 pregnant female rats, they were dosed from the first day to 7th day of pregnancy and were dissected on the next day.

Subgroup3: It included 5 pregnant female rats, they were dosed from the first day to 20th day of pregnancy, where they were dosed at 7 am and dissected on the same day at 6 pm.

Subgroup4: It included 5 pregnant female rats were dosed from the first day of pregnancy and left until the birth.

C - The third main group (G3): contained 20 pregnant females, dosed with levofloxacin at a concentration of 750 g / kg of body weight and divided into:

Subgroup1: It included 5 pregnant female rats, they were dosed from the first day to 16th day of pregnancy and were dissected on the next day.

Subgroup2: It included 5 pregnant female rats, they were dosed from the first day to 7th day of pregnancy and were dissected on the next day.

Subgroup3: It included 5 pregnant female rats, they were dosed from the first day to 20th day of pregnancy, where they were dosed at 7 am and dissected on the same day at 6 pm.

Subgroup4: It included 5 pregnant female rats were dosed from the first day of pregnancy and left until the birth.

Anatomy of Pregnant Female Rats

Pregnant females of rats were anesthetized at the three periods of pregnancy (7, 16, 20) days by exposing them to a quantity of diethylether, then the animals were fixed in the autopsy dish by pins, after that the abdominal cavity was opened and the uterine horns and their contents of fetuses were removed, then the numbers of total fetuses, living fetuses, absorbed and dead fetuses were counted during different pregnancy periods (7, 16, 20) days respectively.

Anatomy of Fetuses during Two Pregnancy Periods Pregnancy (16, 20) Days

After the pregnant female rats were transplanted during the two pregnancy periods (16, 20) days respectively, the uterine horns (right and left) were removed from the body and opened and
fetuses obtained, then the fetuses were separated from the placenta, after that the fetuses were anesthetized by exposure to diethyl ether, the heads of the fetuses were removed from the body and the skull was opened with sharp scissors for the purpose of extracting the fetuses’ brain, these brains were fixed by placing them in a 10% formalin solution for 48 hours for the purpose of histological study.

**Histological Study of Fetuses Brain during the Two Pregnancy Periods (16, 20) Days**

The histological sections of fetuses brain during days 16 and 20 of pregnancy were prepared according to method of Suvarna et al. (2013).

1. **Examination of Histological Sections**

   After preparing the sections of fetuses brain tissues during pregnancy for periods (16,20) days, these sections of were examined using the Optica-Italain compound microscope on 40X.

2. **Bone Skeletal Preparation by Clearing of Embryos and Newborns**

   After obtaining the fetuses at the age of (20) days by autopsying pregnant females of rats for a period of (20) days of pregnancy, as well as the newborns after birth of each from the study groups, the skeletons were prepared by clearing the body of these fetuses and newborns by following the method of McLeod (1981).

3. **Examination of the Skeletons of Fetuses and Newborns**

   After clearing the body of the fetuses at age of 20 days of pregnancy and the neonates after birth, their skeletons, which were stained with alizarin red –s stain were examined by a dissecting microscope at 5× for all groups of the current study.

4. **Photographing the histological sections of brains and skeletons of fetuses and newborns**

   The photographs of brains and skeletons of fetuses and newborns were taken for all groups in the study using a Sony camera mounted on the microscopes in which the tissue sections and skeletons were examined.
Statistical Analysis

The results of this study were statistically analyzed using the Package Social Sciences Statistical (SPSS) system version (21), and the values were as mean and standard deviation (M±SD) were extracted by using the F-test, and the Least Significant Difference (LSD) at Significant level (P<0.05) to find the significant differences between the values in the current study (Morogan et al., 2010).

3. Results and Discussion

The Effect of Study Groups on the Numbers of Fetuses during the three Pregnancy Periods (7,16, 20) days

The statistics indicated that there was a significant decrease(p<0.05) in the numbers of total fetuses and living fetuses, while the numbers of absorbed and dead fetuses increased significantly (p<0.05) in groups of rats treated with levofloxacin at concentrations 500 g/kg and 750 g/kg during different periods of pregnancy (7, 16, 20) days respectively compared with groups of control during the same three pregnancy periods (7, 16, 20) days respectively as shown in tables (1,2,3), respectively and illustrated by figures(2,5,8) respectively, and the results shown in the same tables showed that there were significant differences in the numbers of total, living, absorbed and dead fetuses between the two groups treated with two concentrations of levofloxacin (750, 500) g / kg when comparing between them and for all periods of pregnancy (7, 16, 20) day respectively, and this is what appeared in the figures (3,4,6,7,9,10)respectively, the reason of this results may be due to the harmful effects on various reproductive processes such as implantation and various fetal development processes during different periods of pregnancy through the role of levofloxacin in the synthesis of free radicals in the tissues of pregnant rats causing the degeneration and destruction of uterine tissue cells in pregnant females through oxidation of lipids in plasma membranes and other molecules inside the cells causing damage of the uterus and these toxic effects of this drug were reflected on the fetuses within the uterine horns stimulating the lack of implanted fetuses in the uterus and consequently decreased the number of total and living fetuses while the absorbed fetuses and death fetuses increased due to the lack of sufficient nutrients and oxygen due to the destruction of uterine tissues and thus the destruction of tissues for the fetuses within them, Or the reason of this result may be the lack to the weakness of self-defense means in the fetus’s bodies of antioxidants which are poorly developed in the early fetal stages, so they cannot confront the free radicals resulting from the drug
and remove their harmful effects on the tissues of the fetus causing decrease in implanted fetus numbers in the uterus and absorbed fetus numbers and death of them, thus decreasing the total and living fetus numbers (Junjie Bao et al., 2019).

Table 1 - Effect of Study Groups on fetuses numbers of Pregnant Rats during Pregnancy Period 7 days

| Fetuses Numbers Study groups | Total     | Living    | Absorbed | Dead     |
|------------------------------|-----------|-----------|----------|----------|
| Control group                | 9.6 ± 1.89| 9.6 ± 1.34| 0±0.0    | 0±0.0    |
| Treated group with levofloxacin at concentration of 500gm/kg | 6.1 ± 1.71a | 6.1 ± 0.75a | 0±0.0    | 0±0.0    |
| Treated group with levofloxacin at concentration of 750gm/kg | 4.2 ± 1.43ab | 4.2 ± 1.29ab | 0±0.0    | 0±0.0    |

L. S .D at P < 0.05

| 1.3       | 2.00 | 0   | 0   |

Mean ± standard error. Values:

L. S. D: Least significant difference.

a: Significant difference about control group.

ab: Significant difference about other groups.

P < 0.05: Probability level.

Table 2 - Effect of Study Groups on Fetuses Numbers of Pregnant Rats during Pregnancy Period 16 Days

| Fetuses Numbers Study groups | Total     | Living    | Absorbed | Dead     |
|------------------------------|-----------|-----------|----------|----------|
| Control group                | 10.5 ± 1.2| 10.5 ± 1.2| 0± 00    | 0± 00    |
| Treated group with levofloxacin at concentration of 500gm/kg | 7.5 ± 2.3a | 6.1 ± 2.5a | 1.4 ± 3.0a | 0± 00    |
| Treated group with levofloxacin at concentration of 750gm/kg | 4.6 ± 1.7ab | 3.6 ± 1.4ab | 1.0 ±2.7ab | 0± 00    |

L. S. D at P < 0.05

| 2.0       | 2.6   | 0.3  | 0   |

Mean ± standard error. Values:

L. S. D: Least significant difference.

a: Significant difference about control group.

ab: Significant difference about other groups.

P < 0.05: Probability level.
Table 3 - Effect of Study Groups on Fetuses Numbers of Pregnant Rats during Pregnancy Period 20 Days

| Fetuses Numbers Study groups | Total       | Living      | Absorbed    | Dead       |
|------------------------------|-------------|-------------|-------------|------------|
| Control group                | 10.4 ± 1.1  | 10.4 ± 1.1  | 0.0 ± 0.0   | 00 ± 00    |
| Treated group with levofloxacin at concentration of 500gm/kg | 4.2 ± 2.9a  | 2.2 ± 2.6a  | 2.0 ± 5.2a  | 00 ± 00    |
| Treated group with levofloxacin at concentration of 750gm/kg | 3.3± 2.4ab  | 1.0± 1.5ab  | 1.1± 0.3ab  | 00 ± 00    |
| **L. S.D at P < 0.05**       | 0.5         | 1.0         | 0.4         | 0          |

*Mean ± standard error. Values:*

L.S.D: Least significant difference.

a: significant difference about control group.

ab: significant difference about other groups.

P < 0.05: Probability level P < 0.05

Figure 2 - A female rat pregnant for 7 days of gestation from the control group showing: 1-Normal embryos and their distribution in the two horns of the uterus 2- Fetus.

Figure 3 - A pregnant female rat for 7 days of gestation from the group treated with levofloxacin at a concentration of 500 g/kg. It shows: 1 fetuses with abnormal number and distribution in the two horns of the uterus 2- small fetus
Figure 4 - A pregnant female rat for 7 days of gestation from the group treated with levofloxacin at a concentration of 750 g/kg shows: 1 fetuses that are abnormal in number and distribution in the two horns of the uterus 2 - small fetus.

Figure 5 - A pregnant female rat for (16) days of pregnancy) from the control group showing: 1-Normal fetuses and their distribution in the two horns of the uterus 2-Normal fetus 3-Normal placenta.

Figure 6 - A pregnant female rat for (16 days of gestation) from the group treated with levofloxacin at a concentration of 500 gm/kg, showing: 1 abnormal fetuses in the left uterine horn and the absence of fetuses in the right horn of the uterus 2 - small fetus 3 - small placenta.
Figure 7 - A pregnant female rat for (16) days of gestation from the group treated with levofloxacin at a concentration of 750 g/kg. It shows: 1 abnormal fetuses in the two cornes of the uterus 2- engorged small fetuses 3- engorged small placentas 4 bruised fetuses.

Figure 8 - Of a pregnant female rat for a period of ((20 days of gestation) from the control group showing: 1-Normal fetuses and their distribution in the two horns of the uterus 2-Normal fetus 3-Normal placenta.

Figure 9 - A pregnant female rat for a period of (20) days of gestation) from the group treated with levofloxacin at a concentration of 500 g/kg. It shows: 1 abnormal fetuses in the right uterine horn and the absence of fetuses in the left horn 2- small fetus 3- small placenta
Figure 10 - A pregnant female rat for a period of (20) days of gestation) from the group treated with levofloxacin at a concentration of 750 g/kg. It shows: 1 abnormal fetuses in the right uterine horn and the absence of fetuses in the left horn 2-small fetus 3- small placenta.

Histological Study of Fetus Brains

Histological sections of the fetus brains in groups treated with levofloxacin at a concentration of 500 g/kg during pregnancy of (16,20) days showed various patho-histological changes in the structure of the brain such as few necrosis in astrocytes, thickening in oligodendrocytes, necrosis of cells neurological, necrosis of nervous tissue as shown in figures(12, 13, 14,15) respectively, while these pathological effects of brain tissue increased severity in groups of fetuses whose their mothers were treated with the levofloxacin drug at a concentration of 750 g / kg of the same two pregnancy periods (16,20) days respectively as shown in the figures (17,18,19,20) respectively compared with the control groups of the same two pregnancy periods (16,20) days respectively as in the figures(11,16) respectively, these histological results of the brain of fetuses may be back to that levofloxacin drug when crossed from the pregnant female rats to the fetuses through the placenta it will induce toxic effects in different body tissues that may result from tissue ischemia that occurs because the drug blocks the activity of the electron transport chain in mitochondrial organelles resulting in decreased production of energy molecules (ATP) and inhibition of respiration in cells due to the lack of oxygen supply to fetal tissues causing programmed death of cells, apoptosis, necrosis and other pathological effects (Meijum et al., 2016), or the histological effects of the drug on the brains of the fetuses may be due to the increased production of free radicals that play an important role in lipid peroxidation in cellular membranes which have harmful effects on tissues, in addition to the oxidation processes of large intracellular molecules such as proteins and nucleic acids causing necrosis, destruction and programmed cell death of cells thus destroying and damaging different tissues in the
body in fetuses, or the reason of these pathological effects in the brain tissues may also be due to the role of levofloxacin in inhibiting endogenous cellular antioxidant factors that are immature in fetuses during pregnancy (Budani et al., 2020) therefore the increase in oxidative stress which results from the increasing in the generation of free radicals caused by this drug in the cells of the fetal tissues will negatively affect them due to the lack of development of antioxidant mechanisms that counteract the results of oxidative processes causing oxidative destruction of the various fetal tissues due to the loss of balance between oxidation processes and the antioxidant means that absorb the products of oxidation processes and rid the body of their harmful effects which increases with increasing drug concentration and period of pregnancy and this is what was shown by the study of Ayokanmi et al. (2015), some studies also mentioned that levofloxacin causes harmful side effects on most body systems, but it tends to cause more negative effects in the central nervous system (Maharani et al., 2019), as studies have shown that the neurotoxicity caused by levofloxacin occurs due to distortion of amino acids or impairment of the neurotransmitter GABA later causing necrosis in nerve tissue cells and this was shown by a study (2012)Ismai a et al., another study showed that levofloxacin has toxic effects on the central nervous system so that it is difficult to distinguish between the effects of the drug and others effects resulting from severe infections that the central nervous system is exposed to them (Larissa et al., 2017).

Figure 11 - A cross-section of a normal brain of a rat fetus from the control group for a gestation period of 16 days) showing: 1- astrocytes 2- microglial neurons 3- oligodendrocytes 4- neurons (Hemotoxylin-Eosin- stain 400x).
Figure 12 - A cross-section of the brain of a rat fetus from the group treated with levofloxacin at a concentration of 500 gm/kg for a period of 16 days gestation. It shows: 1- Slight necrosis of astrocytes 2- Thickening of microglia neurons 3- Thickening of few glia neurons Dendritic 4- Necrosis of Neurons 5- Necrosis of Nervous Tissue (Hemotoxylin-Eosin stain - 400X).

Figure 13 - A cross-section of the brain of a rat fetus from the group treated with levofloxacin at a concentration of 500 g/kg for a period of 16 days gestation. It shows: 1- More necrosis in astrocytes 2- Severe thickening of microglia neurons 3- Greater thickening of neurons Oligodendrocytes 4- Major necrosis of neurons -5 Hemorrhages in the nervous tissue 6- Necrosis of nerve tissues (Hymotoxylin-Eosin- stain 400x).

Figure (14): A cross-section of the brain of a rat fetus from the group treated with levofloxacin at a concentration of 750 g/kg for a period of 16 days gestation showing: 1- Increased necrosis in astrocytes 2- Slight necrosis in microglial neurons 3- Little necrosis in glial neurons Oligodendrocytes 4- Increased necrosis of neurons (Hymotoxylin-Eosin-400x stain)
Figure 15 - A cross-section of the fetal brain of a rat from the group treated with levofloxacin at a concentration of 750 g/kg for a period of 16 days gestation showing: 1- Increased necrosis in astrocytes 2- Increased necrosis in microglia neurons 3- Increased necrosis in neurons Oligodendrocytes 4 - large necrosis of neurons (Hemotoxylin-Eosin-400x stain).

Figure 16 - A cross-section of a normal brain of a rat fetus from a control group for a period of 20 days gestation showing: 1- astrocytes 2- microglial neurons 3- oligodendrocytes 4- neurons (Hemotoxylin-Eosin stain-400x).

Figure (17): A cross-section of the fetal brain of a rat from the group treated with levofloxacin at a concentration of 500 g/kg for a period of 20 days gestation showing: 1- Large necrosis in astrocytes 2- Large necrosis in microglia 3- Shrinkage in oligodendrocytes 4- Large necrosis of oligodendrocytes 5 - Large necrosis of neurons 6 - Necrosis of nerve tissue (Hemotoxylin-Eosin stain -400x)
**Figure 18** - A cross-section of the fetal brain of a rat of the group treated with levofloxacin at a concentration of 500 g/kg for a period of 20 days gestation showing: 1- Very large necrosis in astrocytes 2- Very large necrosis in microglia neurons 3- Severe contraction in Oligodendroglial neurons 4- Very large necrosis of neurons 5- Severe necrosis of nervous tissue (Hematoxylin-Eosin stain -400x).

**Figure 19** - A cross-section of the fetal brain of a rat from the group treated with levofloxacin at a concentration of 750 g/kg for a period of 20 days gestation showing: 1- Severe necrosis of astrocytes 2- Severe necrosis of glial neurons 3- Severe necrosis of young glial cells Oligodendrocyte 4- Severe necrosis of neurons- 5 Necrosis of nerve tissue (Hematoxylin-Eosin stain -400X).

**Figure 20** - Across -section of the fetal brain of a rat of the group treated with levofloxacin at a concentration of 750 g/kg for a period of 20 days gestation. It shows: 1- Very severe necrosis of astrocytes 2- Very severe necrosis of glial neurons 3- Very severe necrosis in Oligodendritic glial neurons 4- Very severe necrosis of neurons 5- Severe necrosis of nervous tissue 6- Hemorrhage in the nervous tissue (Hematoxylin-Eosin- stain 400X)

**The Malformations of Fetuses and Newborns Skeletons**

The results of the study of skeletons in fetuses of pregnant female rats treated with a concentration of 500 gm/kg of levofloxacin for a period of 20 days pregnancy revealed various skeletal abnormalities represented by deformation of the skull bones, loss of caudal vertebrae as in
the figure (22), as well as in fetuses treated with a concentration of 750 gm/kg of levofloxacin. In the treated groups, these deformations were represented by shortening the length of the skeleton, causing small fetuses and newborns, as it was observed that a shortening occurred in the length of the fetuses and the front, hind and caudal limbs with the loss of the caudal vertebrae as in the figure (23) in fetuses whose their mothers were the treated at a concentration of 500, 750 mg / kg, and also newborns after birth suffered from deformities represented by the loss of the caudal vertebrae at a concentration of 500 mg / kg and these effects increased in the treated groups at a concentration of 750 gm kg, in which the newborns suffered from severe deformities in the bones of the caudal vertebrae, severe deformities in the bones of the skull in the figure (25,26) and lack of ossification of some bones in the body in newborn which were shown in the figure (27) When compared with the control groups as in the figures (21,24) respectively, this result of our current study can be justified and as some studies showed that levofloxacin penetrates at a high rate in bone tissue (Rimmele et al., 2004), as the study of (2000) Huddleston et al showed that fluoroquinolones have a negative effect on cartilage growth and cartilage ossification in children causing abnormalities in cartilage shape, bone formation within cartilage and a marked decrease in the number of cartilage cells and this drug has no role in the bone fracture healing process when used to treat induced bone fractures in rats and it was impaired the healing during first stages during early bone fracture repair, but the drug stimulated damage to the course of bone fracture healing, and another study was conducted to evaluate the degree of benefits to the disadvantages caused by levofloxacin when used for treatment in people with arthritis and bone infections, this study concluded that levofloxacin caused many adverse drug reactions in patients and the drug treatment took a significantly long period compared to other antibiotics used in the study (Asseray et al., 2000).

Figure 21 - Normal skeleton of a cleared rat fetus from the control group during `20 days of gestation. Alizarin red stain - (5X)
Figure 22 - Distorted skeleton of a cleared rat fetus from the group treated with levofloxacin at a concentration of 500 g/kg for 20 days of gestation showing: 1- Deformation of the skull bones 2- Loss of some caudal vertebrae. Alizarin red stain - (5X)

Figure 23 - Distorted skeleton of a cleaned rat fetus from the group treated with levofloxacin at a concentration of 750 g/kg for 20 days of pregnancy showing: 1- Deformation of the bones of the skull and jaw 2- Loss of some foot bones. Alizarin red stain - (5X)

Figure 24 - Normal Skeleton of a Newborn rat born from the control group after birth. Alizarin red stain - (5X)
Figure 25 - Deformed skeleton of a shedding rat after birth from the group treated with levofloxacin at a concentration of 500 g/kg showing: 1- Deformation of the skull bones 2- Loss of some caudal vertebrae. Alizarin red stain - (5X)

Figure 26 - Distorted skeleton of a shedding newborn rat after birth from the group treated with levofloxacin at a concentration of 750 g/kg showing: 1- Lack of ossification of the skull (cartilage remains) 2- Deformation of the bones of the lower jaw 3- Deformation of some bones of the front end 4- Loss of some caudal vertebrae. Alizarin Red stain- (5X)

Figure 27 - Deformed skeleton of a shedding newborn rat after birth from the group treated with levofloxacin at a concentration of 750 g/kg showing: 1- Lack of ossification of the skull and upper jaw (cartilage remains) 2- Loss of some phalangeal bones of the upper extremity 3- Lack of ossification of the ribs 4- Loss of some bones The phalanges of the hind limb 5- lack of ossification of some bones of the hind limb 6- some caudal vertebrae. Alizarin red stain- (5X)
4. Conclusion

This study revealed that antibiotic levofloxacin at different concentrations stimulated negative and harmful effects on the fetus numbers which included total fetus numbers, living fetus numbers, absorbed fetus numbers, and dead fetus numbers, on histological structures of brains of the fetuses during the different pregnancy periods and skeletal malformations in fetuses and newborns after birth.

References

Asseray, N., Bourigault, C.; Boutoille, D; Happi, L.; Corvec, S.; Touchais, S.; Bemer, P. and Navas, D. (2016). Levofloxacin at the usual dosage to treat bone and joint infections: A cohort analysis. International Journal of Antimicrobial Agents., 47(6):1-11

Ayokanmi, O. and Olaniyi, S. (2015). Influence of different doses of levofloxacin on antioxidant defense system and markers of renal and hepatic dysfunction in rats. Advances Toxicol., (2015): 7.

Budani, M.C. and Tiboni, G.M. (2020). Effects of supplement ion with natral antioxidants on oocytes and perimplantation embryos. Antioxidants. 9: 612-637.

Forsyth, V.S. Armbruster, C.E.; Smith, S.N.; Pirani, A.; Springman, A.; Walters, M. S.; Nielubowicz, G.R.; Himpsl, S.D.; Snitkin, E.S.; Mobley, H.L.T. (2018). Rapid Growth of Uropathogenic Escherichia coli During Human Urinary Tract Infection. M bio. 9(2): 1-13

Fox, T.; Barthold, S.; Davisson, M.; New Comer, C.; Qumby, F. and Smith, A. (2006). The mouse in biomedical research: Normative. Biol. Husbandry. Med. 2nd edition., Elsevier.

Foxxman, B. (2014). Urinary Tract Infection Syndromes Occurrence, Recurrence, Bacteriology, Risk Factors, and Disease Burden. Infect Dis Clin N Am. 28: 1–13.

G Swapna, B Pravallika, J Poojitha Review on Drug-drug interaction studies on Amiodarone and Levofloxacin. Research journal of Pharmacology and Pharmacodynamics 11(4), 147-152, 2019

Huddleston, P., Steckelberg, J.M.; Hanssen, A.D.; Rouse, M.; Bolander, M.E. and Patel, R. (2000). Ciprofloxacin Inhibition of Experimental Fracture-Healing. The Journal of Bone and Joint Surgery 82(2):161-173.

Ismail Kocyigit, Sumeyia Dortdudag, Murat Sipahioglu, Aydin Unal, Hasan Esat Yucel, Bulent Tokgoz, Eray Eroglu, Oktay Oymak and Cengiz Delirium: Is it a dangerous utas (2012) Levofloxacin – induced drug in patients with renal dysfunction? Renal Failure, 34: 5, 634-636.

Junjie Bao, Yong Zou, Yuanyuan Liu, Li Yuan, Robert E Garfield, Huishu Liu, Nicotine protects fetus against LPS-induced fetal growth restriction through ameliorating placental inflammation and vascular development in late pregnancy in rats. Bioscience reports 39(7), 2019

Koeppe, M.O.; Cristofoletti, R.; Fernandes, E.F.; Stropirts, S.; Jungier, H.; Kopp, S.; Midha, K.K.; Shah, V.P.; Stavchansky, S.; Dressman, J.B. (2011). Bio-waiver Monographs for Immediate Release Solid Oral Dosage From: Levofloxacin. Journal of pharmaceutical sciences,100(5), 1628-1636.

McLeod, M.J. (1980). Differentail staining of cartilage and bone in whole mouse fetuses by alcian bluealizarin reds. Teratology., 22: 299 – 300.
Meijun Sony, Hongcheng Wu, Jingbo Jiang (2016): Antibiotic drug Levofloxacin inhibits proliferation and induces apoptosis of lung cancer cells through inducing mitochondrial dysfunction and oxidative damage) Dec 1, 2016. in Biomedicine and Pharmacotherapy SCI (6) 3.73.

Mutschler, Ernst; Schäfer-Korting, Monika (2001). Drug effects. (8 ed.). Stuttgart: Scientific publishing company. 814f. ISBN 978-3-8047-1763-3.

Rimmele, T.; Boselli, E. and Breilh, D. (2004). Diffusion of levofloxacin into bone and synovial tissues. Journal of Antimicrobial Chemotherapy., 53(3): 533-535.