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Study Of Histological Effects Of Liver, Kidney And Structural Abnormalities Of Pregnant Rat Fetuses Treated With Lercanidipine

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Abstract: The current study aimed to demonstrate the effect of lercanidipine on the livers, kidneys, and skeletal deformities of pregnant rat fetuses during the two stages of pregnancy (16 and 20) days, respectively, this study was carried out in the animal house of the College of Science at the University of Kufa, starting from 1/10/2022 to 1/3/2023. In the current experiment, 45 female and male albino rats were used, it was distributed to 30 adult females and 15 fertile male rats for the purpose of mating only, and after mating the rats and the occurrence of pregnancy and obtaining a sufficient number of pregnant rats by (30) pregnant rats, which were divided into 3 main groups, each of which contained 10 pregnant rats, the first group represented a control group and was dosed with physiological solution only, while the second group was dosed with lercanidipine at a concentration of 10 mg / kg of body weight, while the third group was dosed with lercanidipine at a concentration of 20 mg / kg of body weight, females were dosed orally from the first day of pregnancy in one dose per day. After the end of the experiment, five pregnant rats from each group were neutered during the gestation period (16) days, while the other five were neutered during (20) days of gestation, the results of the current study showed that the treatment of pregnant females with the drug lercanidipine during pregnancy caused pathological changes in the structure of the livers of the fetuses, such as (destruction of the wall of the central vein, necrosis and degeneration of liver cells, necrosis of the hepatic tissue, and infiltration of inflammatory cells), as well as in the histological structure of fetal kidneys, which were represented by (Shrinkage of the renal glomerulus, widening of the Bowman's space, necrosis of the cells of the inner lining of the renal tubule, hemorrhage in the urinary tubules), during the gestation period of 16 days, these pathological effects increased in the tissues of these organs with an increase in the gestation period (20) days and for both livers and kidneys, compared with the control groups and for the two gestation periods (16 and 20) days, respectively,

Keywords*: *liver, kidney, pregnant rat, lercanidipine

1. Introduction

Blood pressure is the force by which the blood pushes the walls of the blood vessels, as the blood moves to supply the organs and tissues of the body with nutrients, water, oxygen, and enzymes, and it is called the blood circulation. The heart muscle contracts, pushing blood and its contents into the aorta and then to the rest of the arteries [1] , medical statistics show the great importance of maintaining normal blood pressure, which is estimated to be between 90-120 mm Hg systolic and 60-80 mm Hg diastolic [2], any increase over this limit is stressful for the heart and kidneys, and one of the

most common and dangerous cardiovascular diseases is high blood pressure, which is called the silent killer. Because it does not show any obvious symptoms, and a person can be infected with it for years without realizing it, and it may cause early infertility in men or a stroke. The diastolic pressure is always a lower value than the systolic pressure, and the pressure reading is written as a fraction of 120/80, and the higher value represents the systolic pressure [3] .

The use of blood pressure lowering drugs may be considered an urgent necessity for people who suffer from high pressure because of its serious health effects if they are not fatal. The biochemical and physiological changes that a pregnant woman goes through with her fetus when the health condition is stable, so how about when the pregnancy process is accompanied by high blood pressure, many medicines are available to treat high blood pressure, but it is difficult to choose the right medicine that does not harm the mother and her fetus. No matter how effective the medicines are, they are not without negative effects and side effects on public health [4], the fetus is connected to the mother by the placenta, as this organ provides oxygen and nutrients to the fetus during its growth and rids it of waste products in its blood. This means that the passage of medications taken by the mother through that organ to the fetus is not excluded [5]. there are many chemicals such as drugs or other environmental pollutants that are dangerous disfiguring agents, as are some diseases that the pregnant mother suffers from, such as diabetes, which occurs before or during pregnancy and is known as gestational diabetes among the diseases that increase the possibility of congenital malformations of fetuses, or internal factors due to genetic mutations or chromosomal abnormalities that stimulate these malformations during the different stages of pregnancy [6].

Lercanidipine is one of the antihypertensive drugs of the family of calcium channel blockers, the third generation of dihydropyridines, as this group prevents calcium ions from entering the cells through their channels, thus reducing heart rate and the effect of vasodilators, lercanidipine has a long half-life and a high lipid affinity, and it works to lower blood pressure gradually and for a long time due to its high vascular selectivity. The drug is of importance in the treatment of gastric damage caused by non-steroidal anti-inflammatory drugs, it reduces the oxidation of low-density lipoprotein cholesterol in patients with high blood pressure and type 2 diabetes [7], there is not enough information regarding the safety of using the drug, so it is forbidden to use it during pregnancy, and the drug can be transmitted with breast milk, so it is not recommended to take it during the lactation period [8].

2. Methodology

Laboratory Animals

The current study required the use of (45) albino rats, the type of Rattus Rattus (Sprague Dawley), of which (15) were male, their weight ranged between (200-250) g and their age ranged between (12-13) weeks. Males to mate with females to obtain pregnant rats, which is one of the requirements of the current study, while (30) female rats were used, their weights ranged between (185-200) g and their ages ranged between (11-12) weeks. The animals were brought from the animal house of the College of Pharmacy, University of Karbala, females and males were placed separately before the start of the experiment in special cages for the breeding of laboratory animals, and the diet was used as food for the rats, and special bottles were used to drink water, and the rats were given water and diet in a freeway, The laboratory conditions were appropriate and similar for the animals throughout the experiment period, with an average temperature of 24 °C, lighting, ventilation, and appropriate humidity. The cages were cleaned by changing the sawdust with which the cages were covered and sterilized twice a week. The animals were left for ten days in the animal house to adapt to the place. And to ensure that they are free of pregnancy and diseases before the start of the experiment.

Animal of Mating:

Fertile males were placed with adult females, at the rate of one male for every two females, in the breeding cages, at six o'clock in the evening, after giving food and water, and at eight thirty in the morning of the next day, fertile males were placed with adult females, at the rate of one male for every two females, in the breeding cages, at six o'clock in the evening, after giving food and water, and at eight thirty in the morning of the next day female rats were examined for the purpose of determining pregnancy, by taking vaginal smears using the lube, which was sterilized by heating and then cooled with physiological saline, after which a vaginal swab was taken and placed on the glass slides prepared in advance, then the samples were stained with methylene blue then, they were examined using a light microscope to see the sperm [9], the females in one cage were taught to distinguish them from each other, and the potentially pregnant females were isolated after seeing the sperm in their vaginal smear, then the cages were informed of the date of pregnancy and the name of the researcher, in addition to the coded information related to the research, in preparation for the start of dosing.

Design of experiment

In the current study, one basic experiment was designed to study the histological changes of lercanidipine in the histological structures of the kidneys and livers of pregnant rat fetuses and the structural abnormalities during the two gestational periods (16 and 20) days, respectively, as this experiment included the use of (30) adult female rats and (15) fertile male rats, after the female rats were married by male rats and the required number of pregnant adults, which were (30) pregnant rats, were divided into 3 main groups, which contained each Of which, 10 pregnant rats, the first

group was dosed with physiological solution only and was treated as a control group, while the second group was treated with lercanidipine at a concentration of 10 mg / kg of body weight, while the third group was treated with lercanidipine at a concentration of 20 mg / kg of body weight, pregnant females were dosed orally with one dose per day from the first day of pregnancy by the gastric dosing method (Intragastric) using a Stomach tube (Gavage). The first five pregnant rats from each of the three groups were inoculated during the pregnancy stage (16) days, while the other five were inoculated from Each group within (20) days of pregnancy.

Dissection of pregnant female rats and their fetuses at the two stages of pregnancy (16 and 20) days.

The right and left uterine horns were extracted after anesthetizing pregnant rats using diethyl ether, after fixing the animal on the dissection platform with staples to dissect pregnant rats during the stages of pregnancy (16, 20) days, respectively, the embryos that were anesthetized with diethyl ether were obtained, and after fixing the embryo on the dissection base and opening the abdominal cavity, the kidneys and livers were removed, which were washed with physiological saline and dried, then the organs were placed in 10% formalin to prepare them for histological study.

Histological sections Preparation

The method of Suvarna (2013)[10] was adopted for the purpose of preparing tissue sections of livers and kidneys of fetuses during 16 and 20 days of gestation for histological study.

Examination of histological sections

After preparing histological sections of the livers and kidneys of pregnant rat fetuses during pregnancy stages (16, 20) days, respectively, the histological sections were examined by a compound microscope at (40x) magnification.

Preparing the skeletons for embryos

Embryos were used in this experiment for a period of 16 days and 20 days in all study groups for the purpose of preparing the skeletons after drying them, and the method of Medode (1980) [11] was followed.

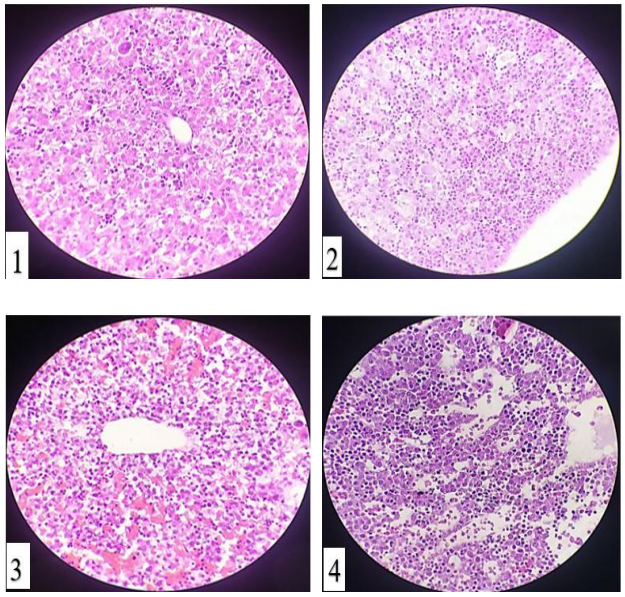
Photographer

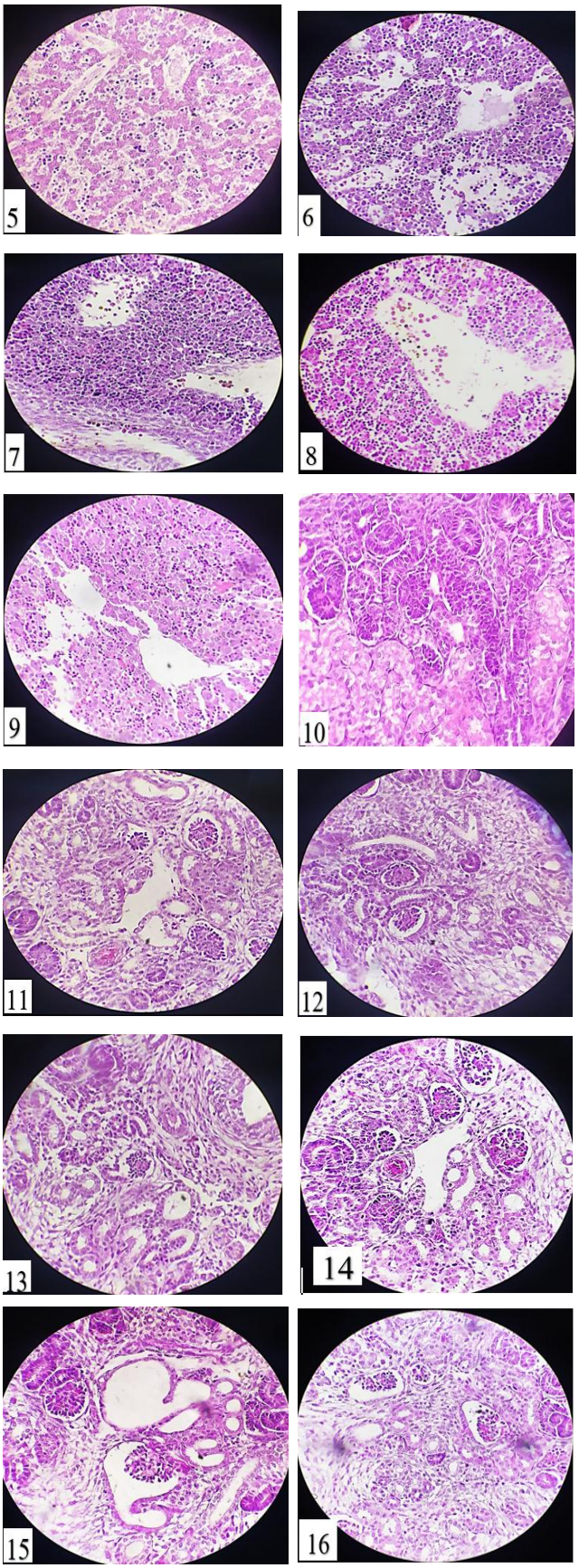
Photographs of the histological sections of the embryonic organs (kidneys and livers) and structural abnormalities were taken during the two periods of pregnancy (16 and 20) days, respectively, using the same optical microscope that was used for histological examination of the studied organs after providing it with a digital camera.

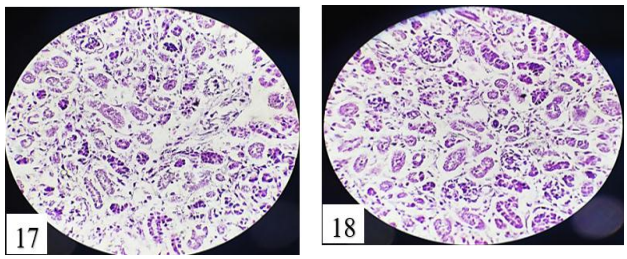
3. Results:

First: - Histological study of the livers and kidneys of fetuses

The results of the histological study of sections of the kidneys and livers of fetuses whose mothers were treated for a period of (16, 20) days of pregnancy with the drug lercanidipine at two different concentrations 10 mg and 20 mg, respectively, showed different histological changes in the liver such as necrosis of the liver tissue, the destruction of the wall of the central vein, severe necrosis of the liver cell, widening of the sinusoids, infiltration of inflammatory cells, widening of the central vein, and hemorrhage in the liver tissue. (9,8,7,6,5,4,3,2) respectively, Compared with the histological structure of the livers of the fetuses in the control groups, as in the picture (1), the kidney tissue also suffered abnormal effects - pathological for the fetuses whose mothers were treated with the drug lercanidipine at two different concentrations of 10 and 20 mg / kg during the gestation period of 16 days, such as bowman's capsule wall collapsed, renal glomerulus contraction, necrosis, hemorrhage and necrosis in the urinary tubules, renal cell necrosis, and renal tissue hemorrhage as shown in the pictures (18, 17,16, 15,14, 13, 12, 11) respectively, these histological-pathological effects in the tissues of the kidneys increased in intensity with increasing drug concentration and the duration of pregnancy for a period of 20 days (18, 17, 16, 15) when compared with the results of microscopic examination of the liver tissue of the control group, which did not show any negative changes in and during the same two periods of pregnancy. (10 and 20 days), respectively, as shown in the picture (10).

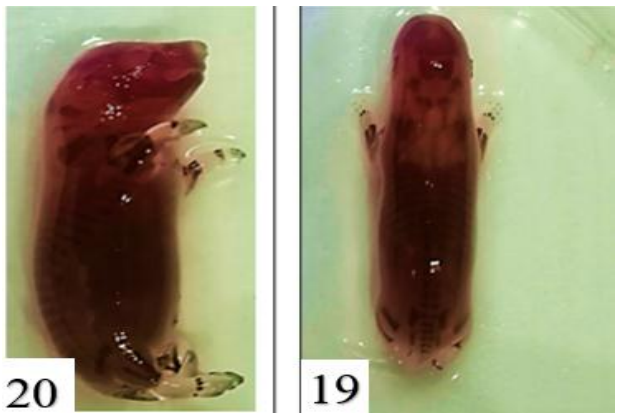


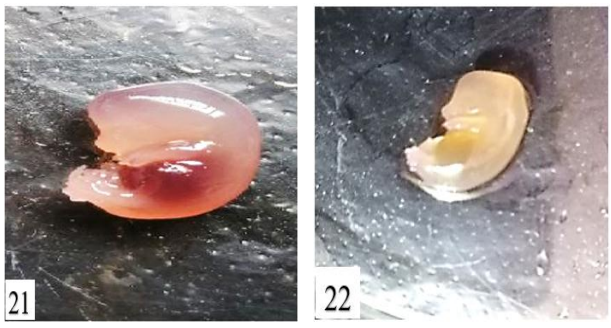


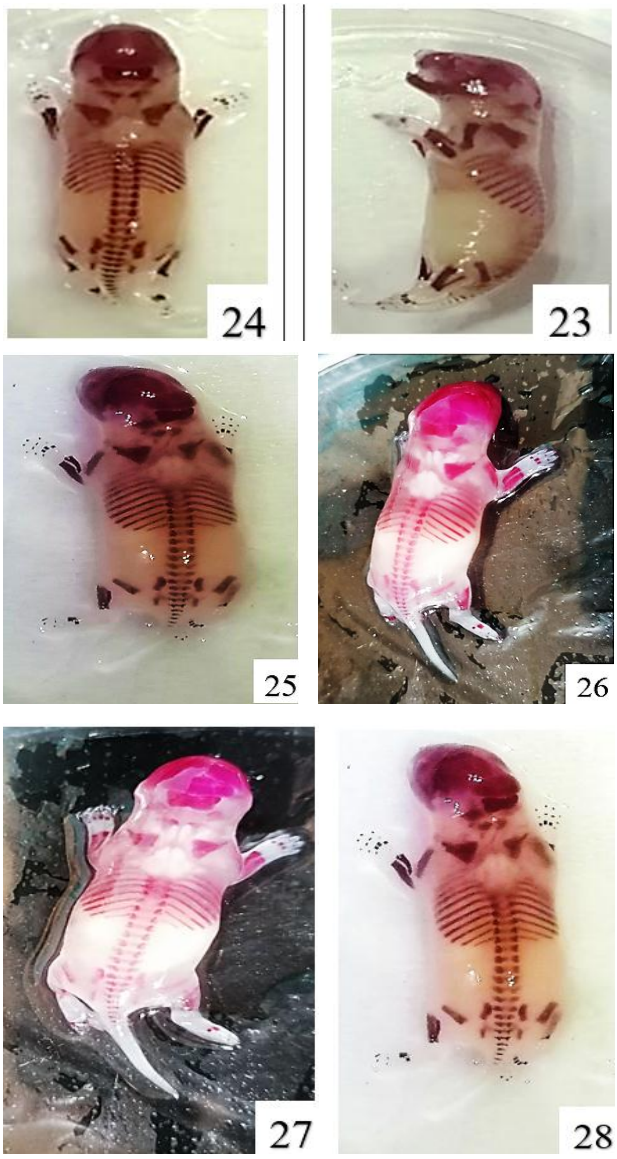


Second: - Studying the structural deformities of the fetuses during the two periods of pregnancy (16 and 20 days)

The results of drying the skeletons of the current study in the fetuses of pregnant rats treated with lercanidipine at concentrations (10), 20 (mg / kg) during (16, 20) days of pregnancy showed various deformities in the skeleton such as the small size of the fetus in general and the lack of ossification of its skeleton on the day of (16) from pregnancy as shown in the pictures (21, 22), while the deformities on the 20th day of pregnancy were deformation of the cranial bones, the loss of a number of phalanges of the upper and lower limbs, the loss of some caudal vertebrae and the shortening of the skeleton, causing a small size of the fetuses, as shown in the pictures (28, 27, 26, 25) respectively, when compared with the skeletons of fetuses in the control group for the same two gestational days (16, 20) days as shown in pictures (24, 23, 20,19), respectively.







3. Discussion

First: - The effect of lercanidipine on the histological structure of the livers and kidneys of fetuses during the two stages of pregnancy (16,20) day.

The results of the histological study of the livers and kidneys of fetuses of pregnant animals treated with lercanidipine at two concentrations (10 and 20) mg / kg, from the first day of pregnancy until the periods 16 and 20 days of pregnancy numerous negative histological changes suggestive of the danger of using the drug during pregnancy, whose effect on tissues increased as the duration of pregnancy increased and the concentration of the drug increased when compared with the histological structure of the livers and kidneys of pregnant rat fetuses in control groups and for the same gestational periods, 16 days and 20 days respectively, numerous negative histological changes suggestive of the danger of using the drug during pregnancy, whose effect on tissues increased as the duration of pregnancy increased and the concentration of the drug increased when compared with the histological structure of the livers and kidneys of pregnant rat fetuses in control groups and for the same gestational periods, 16 days and 20 days, respectively, it is possible to explain these results of the current study that the drug has the ability to pass through the placenta, causing cases of crash and deterioration during its passage between the cells and tissues of the embryos which is characterized by its weak ability to confront these harmful effects with the lack and weakness of its defensive devices, which are characterized by the beginning of their formation as a catalyst during the passage of pathological and toxic effects, because the drug has an effect on energy production in mitochondria, causing tissue ischemia (lschemia) due to a defect in energy production (ATP) and synthesized proteins due to irregular concentrations of calcium (C++) between mitochondria and the endoplasmic reticulum [10], likewise, the drug under study may affect the electron transport chain, enzymatic activities, and organic acid consumption rates, enhancing protein toxicity and thus the production of reactive nitrogen and reactive oxygen species ROS. It may also affect the imbalance of mitochondrial permeability in different cases, causing shrinkage and swelling, and this is what was shown by the study of [12], Or the reason for this result may be the effect on the placenta due to the drug and the lack of blood supply, which plays an essential and effective role in the lack of nutrients and oxygen, resulting in malnutrition and a defect in the formation of tissues for the various organs in the body, including the studied organs (liver and kidneys) and stimulating the deterioration and degeneration of cells Others necrosis, infiltration of inflammatory cells, smashing of the central veins, enlargement of some cells, bleeding, congestion, atrophy, laceration, and self-destruction of cells [13], it is also possible to interpret the results of the current study that the drug has a vasodilator effect, and thus works to expand the vessels of the thin and structurally incomplete embryos, being in the stages of formation, leading to pumping them with quantities of blood that exceed their tolerance, Consequently, it is destroyed and the negative effects of this situation in terms of not delivering oxygen and nutrients to the body's systems, in addition to the occurrence of bleeding within the tissues and blood vessels, leading to the infiltration of inflammatory cells and the disturbance of the balance between antioxidants and oxidizing agents, resulting in a rise in the levels of the oxidative breakdown index (MDA) low indicators of antioxidants such as GSH and CAT in the liver or kidneys, which indicate the breakdown of fats and their oxidation in cell membranes, and the high level of free radicals that overcome antioxidants, which are weak in embryos, depleting their quantities within the tissues,

stimulating programmed cell death. during increasing gestational age [14].

Second: Studying the structural deformities of the fetuses during the gestation period (16, 20) days.

The results of the current study showed when the skeletons of fetuses of female rats were healed during pregnancy (16) days, the complete lack of ossification of the skeletons, and the study also showed the presence of various structural deformities at the stage of pregnancy (20) days and with two concentrations (10,20) mg / kg of lercanidipine, Among the most important of these deformities is the loss of some caudal vertebrae, the loss of some phalanx bones of the fore and hind limbs, deformations of some bones of the skull, and delayed ossification of cartilage, these deformities also increased with the increase in drug concentration, when compared to the translucent structures of normal fetuses of control groups and for the same gestational periods of 16 and 20 days, in view of the lack or absence of adequate studies on the effect of lercanidipine on the structural composition of fetuses of rats during different stages of pregnancy, it is possible to explain the result of this study to the effect of this drug on the process of formation of cartilage and bone through its effect on calcium concentrations in the blood, and because these ions are of great importance in the formation of cartilage and bone, as the disturbance of calcium ions and not allowing them to enter the cells leads to defects and deformities in the structure of the bones, and it is possible that they affected the cartilage inhibitors, as this leads to suppressing the expression of collagen II and proteoglycans, as the study showed the dependence of the functions of chondrocytes On the balance of intracellular ions such as calcium Ca++, potassium K+, and sodium Na+ [15], or the reason may be due to the lack of access to the materials necessary for the formation of bones in the embryos, either because of a defect in the passage through the placenta because of the drug, or because of the harmful effects of the drug on the mother, including anemia, which affects the health of the mother and thus the health and safety of the fetus, and the process of delaying the formation of cartilage in the fetus An essential role in the lack of cartilage ossification and thus a defect in the formation of bone for fetuses [16], or the drug may have a negative effect on the osteoblast cells, while the drug may affect the activation and differentiation of osteoclast cells, leading as a result to erosion and bone resorption, causing disturbances in bone formation during the different stages of embryonic formation, or the free radicals generated in the bodies of fetuses as a result of treating pregnant animals with the drug may play a role They play major roles in the ossification of the skeleton or the loss of some of its parts through their influence on cells and their damage.

Conclusion:

The results of the study also revealed the presence of various structural deformities in the skeleton of fetuses during the gestation period of 16 days represented by (not fully ossified the skeleton), the severity of these deformities also increased with the increase in the concentration of the drug and the duration of pregnancy,

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