ISSN 2063-5346

HISTOLOGICAL EFFECTS OF LERCANIDIPINE IN THE LIVERS AND KIDNEYS OF FEMALE RATS DURING PREGNANCY

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Article History: Received: 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

This revision was considered to explain the influence of oral administration of lercanidipine on the livers and kidneys of pregnant female rats during stages (16, 20) days of pregnancy, The current 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.The total number of rats used in this experiment is 45 male and female albino rats, about 15 of which were fertile male rats for the purpose of mating only And 30 adult female rats , and after mating between female and male rats and the occurrence of pregnancy and obtaining a sufficient number of pregnant rats, which were (30) pregnant rats. It was divided into 3 main groups, each of which contained 10 pregnant rats. The first group represented a control group and was dosed with physiological saline only, 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, The 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 the (20) day of gestation. Statistical analyzes recorded a significant decrease ($P < 0.05$) in the weights of livers and kidneys in groups of pregnant rats that were treated with lercanidipine at two concentrations of 10 mg/kg and 20 mg/kg during the gestation period of 16 and 20 days, respectively Compared with the weights of livers and kidneys of pregnant rats for control groups and for the same gestational periods (16 and 20) days, respectively The histological results of this study revealed that treatment of pregnant females with lercanidipine during pregnancy caused histopathological changes in the histological structure of the livers of pregnant rats Represented by (Central vein wall rupture, hepatocellular necrosis, enlarged sinusoids) during pregnancy 16 days While the changes were observed (Destruction of the wall of the central vein and changing its shape and width, enlargement of sinusoids, necrosis and degeneration of hepatic cells, necrosis of the hepatic tissue and infiltration of inflammatory cells) during the gestational stage 20 days of pregnancy, compared with the histological composition of the liver of pregnant rats in the control groups and during the same two periods of pregnancy above respectively The results of the microscopic examination of the histological structure of the kidneys of pregnant females during the gestation period of 16 days showed that these changes were (shrinkage of the renal glomerulus, widening of Bowman's space, necrosis of the cells of the inner lining of the urinary tubule) These pathological effects increased in the kidney tissues during pregnancy (20) days (renal tissue necrosis, hemorrhage, inflammatory cell infiltration, blood clotting) Comparison with the histological structure of pregnant females in the control groups for the two gestational periods (16 and 20) days, respectively, in which the histological structure was normal.

Keywords : gestational**,** histological**,** pressure**,** enzymes**.**

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DOI: 10.31838/ecb/2023.12.s1.085

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. This is called the circulatory system. The heart muscle contracts, pushing the blood and its contents into the aorta and then to the rest of the arteries (Ratan *et al*., 2022), 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 (Lustrek, 2019). 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[1, 2] 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 (Balwan and Kour, 2021). And it seems to us that 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, but it requires some deliberation when it comes to the pregnancy process [3, 4], as the situation becomes related to the mother and the fetus alike. Not to mention the chemical and physiological changes that a pregnant woman goes through with her fetus, this is when the health condition is stable, how about when the pregnancy process is accompanied by high blood pressure. Many drugs are available to treat high blood pressure, but it is difficult to choose the right drug that does not harm the mother and her fetus. No matter how effective the drugs are, they are not without negative effects and side effects on public health (Burnier, 2019). 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 (Chae et al., 2022). There are many chemicals, such as drugs or other environmental pollutants, that are dangerous teratogenic factors, and some diseases that the pregnant mother suffers from, such as diabetes, which occurs before or during pregnancy, which is known as gestational diabetes, are diseases that increase the possibility of congenital abnormalities of the fetus, or factors Internal due to genetic mutations or chromosomal abnormalities [4, 5] that stimulate these abnormalities during the different stages of pregnancy (AL-Essawi and ALJamali, 2019). Lercanidipine is one of the antihypertensive drugs of the family of calcium channel blockers, the third generation of dihydropyridines. 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. It reduces 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, and works to reduce the oxidation of low-density lipoprotein cholesterol in patients with high blood pressure and patients with type 2 diabetes (Altuner *et al*.,2020). 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 [7-9], so it is not recommended to take it during the lactation period (Cicero et al., 2020).

MATERIALS AND METHODS

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 free

way, 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 10-12]..

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 (Lambert, 2007), 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 [13-16].

Design of experiment

This study was designed to investigate the effect of oral administration of lercanidipine at two concentrations (10 and 20) mg/kg of body weight on the livers and kidneys of pregnant female rats during stages (16 and 20) days of pregnancy, respectively, in this experiment, (30) adult female rats and (15) fertile male rats were used, after the female rats were mated by male rats and the required number of pregnant women was reached, which were (30) pregnant female rats. They 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 gastric dosing with a dosing 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 of them were inoculated from each group during (20) day of pregnancy.

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

After anesthesia of pregnant female rats using diethyl ether, the animals were fixed on the dissection platform with staples for dissection during the two stages of pregnancy (16 and 20) days, respectively, And the abdominal cavity was opened for them, and the livers and kidneys were removed, which were washed with physiological saline and dried, and the livers and kidneys were weighed by means of a scale, then these organs were placed in formalin solution at a concentration of 10% for 24 hours for histological study.

Histological sections Preparation

The method of Suvarna (2013) was adopted for the purpose of preparing tissue sections of the livers and kidneys of pregnant female rats at 16 and 20 days of pregnancy.

Examination of histological sections

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

Photographer

Photographs of the histological sections of the organs of pregnant rats (kidneys and livers) 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.

Statistical analysis

The results of the experiment were analyzed statistically using the statistical program spss (2012), by the analysis of variance method (ANOVA-One way) according to the complete random design Arithmetic mean values (M±SE) using F-Teast and extract the least significant difference(L.S.D.) Below a significant level ($P<0.05$) to find significant differences between the mean weights of the study groups.

Results

The effect of lercanidipine on the weights of livers and kidneys, as well as the drug's histological effects on them in pregnant rats during the two gestation periods (16 and 20) days.

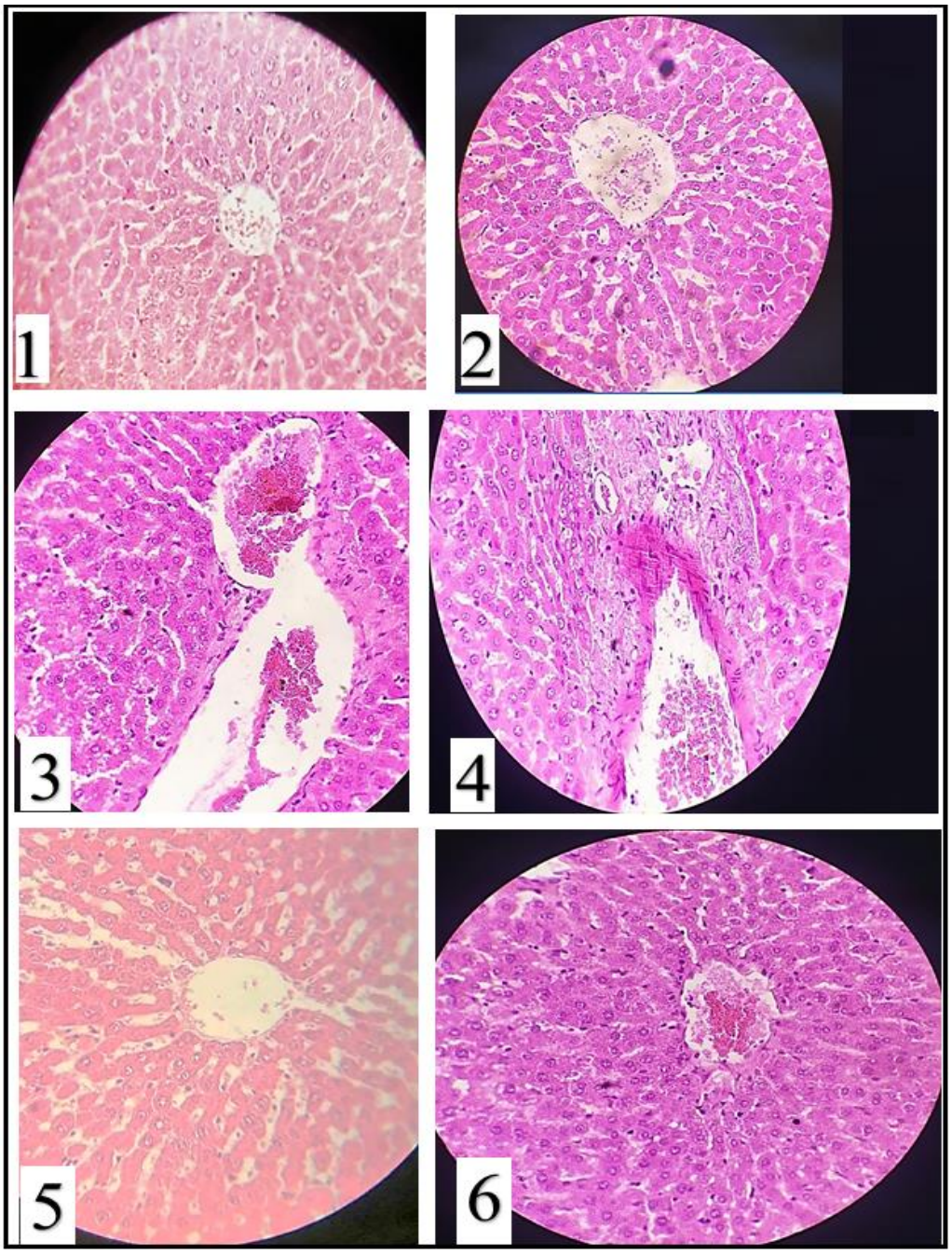
The current study showed a significant decrease ($P\leq0.05$) in the weights of the livers and kidneys of groups of pregnant rats treated with lercanidipine at two concentrations (10,20) mg/kg of body weight during the two pregnancy periods (16,20) days, this is done by comparing it with the weights of the organs mentioned above in pregnant rats from the control groups and for the same two gestational periods (16 and 20) days, respectively as shown in Tables (1 and 2), respectively the results of the microscopic examination also showed histopathological changes in the tissue sections represented by necrosis and degeneration of the hepatocytes, widening of the sinusoids, infiltration of inflammatory cells, expansion of the vein, its congestion and laceration, and the occurrence of bleeding in separate areas of the liver of pregnant rats treated with the drug at two concentrations of 10 mg / kg and 20 mg / kg in 16 days of Pregnancy and as shown in the pictures (2, 3, 4, 5), while these histological effects increased in the liver composition of pregnant females treated with the same concentrations of the drug above, by increasing the gestational age to 20 days, as in the pictures (6, 7, 8, 9), respectively, compared with the normal histological composition of pregnant rats in the control group and for the same pregnancy durations, as in the pictures (1) respectively, the results also showed changes in the kidney tissue in pregnant rats treated with lercanidipine at two concentrations of 10 and 20 mg/kg. These abnormal changes during 16 days of pregnancy were represented by shrinkage of the renal glomerulus, widening of Bowman's space, necrosis and laceration of the inner lining of the renal tubule, infiltration of inflammatory cells, And degeneration and hemorrhage in the renal tissue, as shown in pictures (11, 12, 13, 14), respectively, while the aforementioned histological changes were more severe in the tissue of the kidneys of pregnant rats that were treated with the drug at the same concentrations during the gestation period of 20 days and as shown in the pictures (15, 16, 17, 18), respectively, when compared with the histological structure of the kidneys of the control group animals that did not Any histological changes appear for the same pregnancy periods, respectively [17 ,18], as in the picture (10).

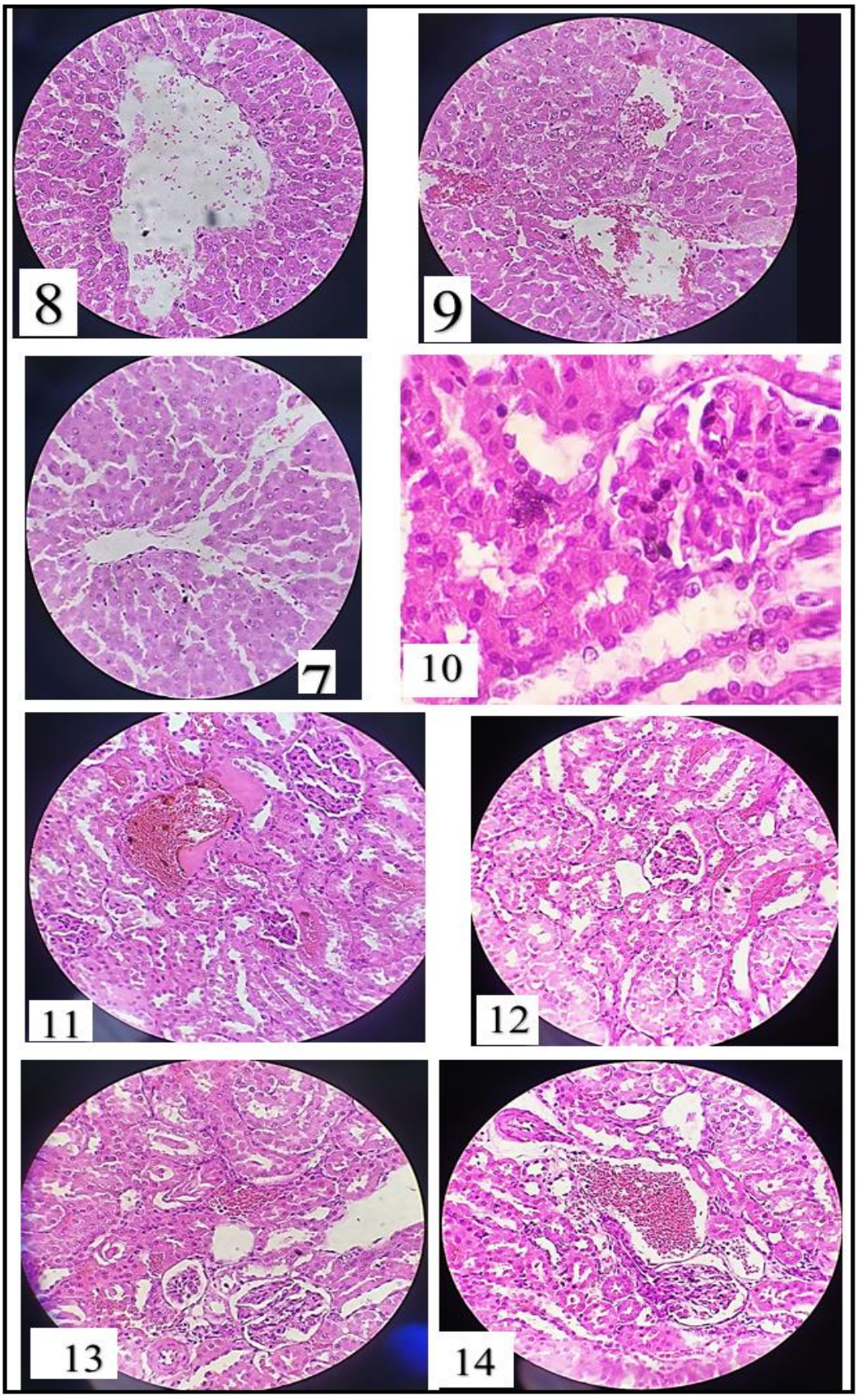
Table (1) The effect of lercanidipine on the weights of the livers and kidneys of pregnant rats during the gestation period of 16 days.

Weights (gm)		Transactions
Kidney	**Liver**	
1.41±0.05	8.59±0.03	control group
1.32±0.07 a	7.17±0.04 a	The group treated with lercanidipine at a concentration of 10 mg/kg
1.20±0.022 ab	6.09±0.03 ab	The group treated with lercanidipine at a concentration of 20 mg/kg
0.03	1.01	LSD (0.05)

Table (2): The effect of the treatments on the weights of the livers and kidneys of pregnant rats during the gestation period of 20 days .

Weights (gm)		Transactions
Kidney	**Liver**	
1.55±0.01	8.83±0.04	control group
1.21±0.03 a	6.48±0.03 a	The group treated with lercanidipine at a concentration of 10 mg/kg
1.00±0.02 ab	5.21±0.09 ab	The group treated with lercanidipine at a concentration of 20 mg/kg
0.11	0.88	LSD (0.05)

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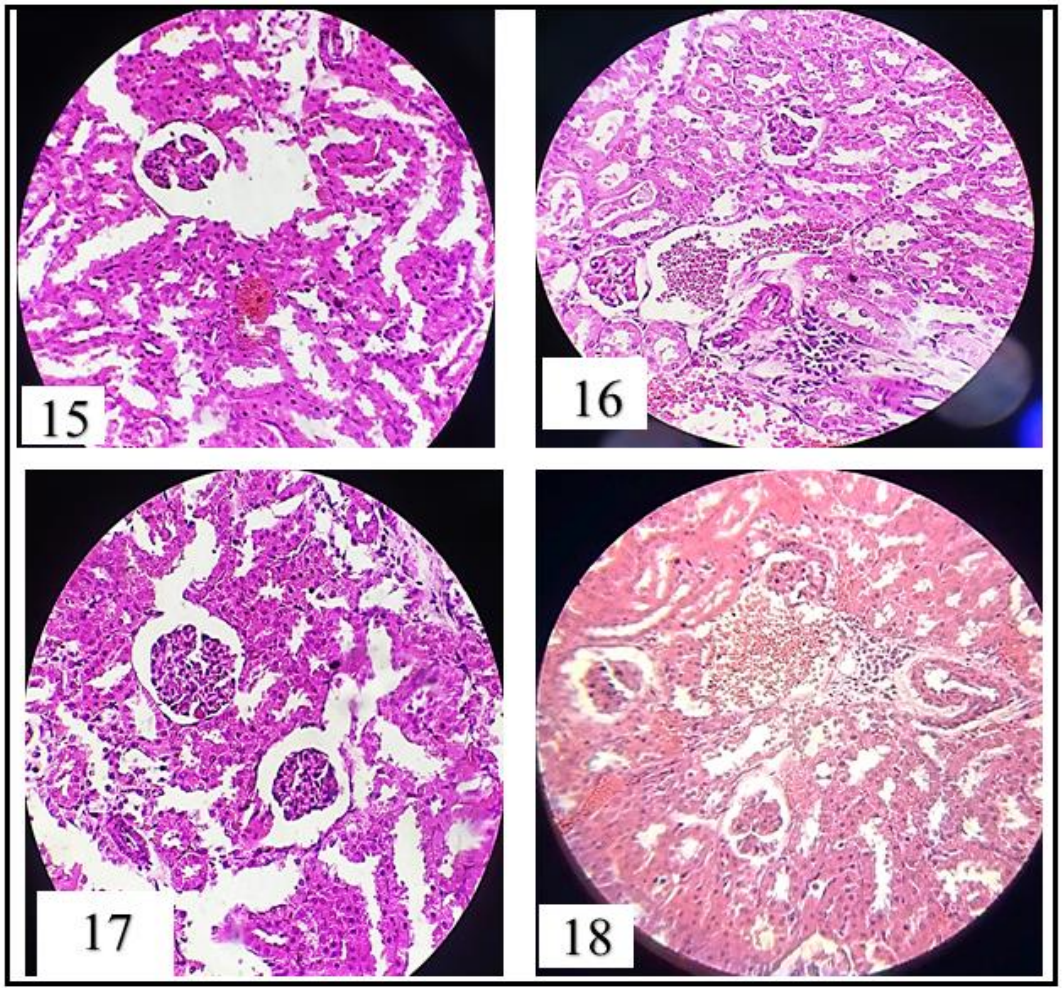

Discussion

The effect of lercanidipine on liver and kidney weights in pregnant rats at stages (16,20) days of pregnancy.

The statistical results showed a significant decrease in the weights of the livers and kidneys at the 16th and 20th days of pregnancy in groups of pregnant rats treated with lercanidipine at two concentrations (10 and 20) mg/kg when compared with the weights of these organs in pregnant rats from the control group and for the same stages of pregnancy (16 and 20) day in a row, due to the lack of studies on the effect of lercanidipine on the weights of the various organs in the body of female pregnant rats treated with this drug, including the livers and kidneys, so the results of the current study can be explained by the accumulation of the drug and its increased side effects in the body, and it may explain the cause of anemia, loss of appetite and lack of food intake, and this is what It was observed on animals during the study, and it is one of the side effects of the drug (Aljehani et al., 2022), or the reason for this may be the tissue changes that these organs suffered from due to the drug, such as necrosis and destruction of tissue or cells, which caused a decrease in their weights [19] , and this is what the current study showed.

The effect of lercanidipine on the histological structure of the livers and kidneys of pregnant rats at stages (16-20) days of pregnancy.

The results showed histological changes and damage during the histological examination of the studied organ sections of pregnant female rats treated with lercanidipine, and the occurrence of many changes such as necrosis and degeneration of cells, expansion and laceration of some central veins, bleeding and congestion within the tissues, infiltration of inflammatory cells, and expansion of sinusoids in the liver tissue and changes in the tissue of

the kidneys, such as shrinkage of the renal glomeruli and its destruction, the expansion of Bowman's space, the destruction of the urinary tubules, and other changes that increased in severity with increasing drug concentration and pregnancy duration compared with control groups, it is possible to explain these pathological changes, which agreed with the results of the study (Aggarwal and Kavanaugh 2014), as the drug causes inflammation, Blood vessels that stimulate the formation of antibodies and the formation of an immune complex and the deposition of immune complexes in the inflamed blood vessels and then damage to the vessels and the occurrence of bleeding and congestion within the veins, and the influx of large amounts of blood into the vessels with their dilation works to rupture the vessels [20] , as shown by the study of (Aljehani *et al*., 2022).

Calcium channel blockers lead to the formation of nitric oxides, leading to tissue destruction and inflammation, activation of neurohormones, and/or increased systemic calcification as a result of simultaneous calcium supplementation. Also, fluid accumulation due to edema formed outside the cells may affect cell osmosis and damage membranes and thus tissue damage (Steyl and Van Zyl-Smit, 2009). What makes the situation worse is that the drug is metabolized by cytochrome 3A4 enzyme (CYP3A4), which is one of the most important enzymes involved in the metabolism of xenobiotics and drug metabolism, as it controls the duration of drug existence and the speed of its breakdown. Excessive activity of these enzymes can lead to inhibition of drug efficacy. And if it is not active, the drug remains inside the body, causing drug poisoning. Since the center of this enzyme is in the liver, and with damage to liver cells, the drug may not be metabolized, leading to drug accumulation and hepatic and renal toxicity (Kerkhofs *et al*., 2018). Cytochrome B450 enzymes are also located inside the cell in the endoplasmic reticulum, because they have a role in processing and transporting proteins. Their enzymes perform the metabolism process responsible for drugs and environmental pollutants. In the mitochondria, the center of energy production and the process of metabolism and manufacturing of internal materials. Calcium for the manufacture of proteins by the endocrine network, and because of the drug's action in preventing the entry of calcium, may lead to a defect in the work of mitochondria, and the results of the study agree (Kim *et al*., 2021). The disturbance of calcium ions may negatively affect cell proteins, thereby enhancing protein toxicity, forming unfolded proteins, and inhibiting the function of the proteasome, which causes its accumulation, causing endoplasmic reticulum stress, and it agreed with a study that the drug lercanidipine enhances ER stress by the proteasome inhibitor (enhaced bortezomib Btz), and the loss of the capabilities of the mitochondrial membrane, which impairs the metabolic functions of the mitochondria and the lack of necessary proteins necessary for the formation of enzymes, eventually causing the breakdown of cell work and the formation of reactive oxygen species ROS and the generation of free radicals that caused oxidative stress and cell death called paraptosis or cell death caused by the expansion of mitochondria [21] or the network Endoplasma (Zhao et al., 2019).

Conclusion:

From the results of this study, we can conclude that oral administration of lercanidipine to pregnant rats from the first day of pregnancy reduced the weights of both livers and kidneys and caused pathological and negative effects in the histological structure of the livers and kidneys of pregnant rats that were treated with lercanidipine from the first day of pregnancy, with two concentrations. 10 and 20 mg/kg of body weight for 16 and 20 days of pregnancy, respectively. These pathological effects increased with increasing drug concentration and pregnancy duration.

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