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Study of some histological effects of the preservative sodium nitrite in the fetuses and pregnant rat females

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***Abstract*---**This study was carried out in the animal house of the College of Science at the University of Kufa from 15/11/2021 to 15/5/2022for the purpose of studying the harmful histological effects of the preservative sodium nitrite in some organs (livers, kidneys and brains) of fetuses during gestation (16,20 days) and during the three stages of gestation (7,16,20) days respectively of pregnant female rats that were treated with sodium nitrite,45 pregnant rat female of *Rattns rattus* type (Sprague Dawley) whose averages weights were (230) g and ages were (12) weeks, pregnant rats were divided into 3 main groups and each group contained 15 pregnant females: the first group was treated with physiological salt solution and was as a control group, while the second group was dosed sodium nitrite at a concentration of 10 mg/kg of body weight, while the third group was dosed sodium nitrite at a concentration of 15 mg/kg of body weight, each of the main groups was divided into 3 secondary groups, each of which included 5 pregnant rats, the first, second and third secondary groups from each main groups were dissected during (7,16,20) days of gestation, respectively, pregnant female rats were dosed in all groups from the first day of gestation with a single dose daily by gastric dosing device, these pregnant female rats were dissected in the three stages of gestation (7, 16, 20) days respectively, and the livers, kidneys and brains of pregnant rats and fetuses were removed to study histological changes in them. The results of the current study showed that sodium nitrite caused pathological histological changes in the livers, kidneys and brains of pregnant female rats during the gestation stages (7,16,20) days respectively, as well as the livers, kidneys and brains of the fetuses during the two gestation periods

(16,20) days respectively, and these effects increased abnormal histological changes with an increase in sodium nitrite concentration and an increase in gestation period compared with the histological structure of these organs of pregnant rats and fetuses in the control groups and for the same gestational periods of pregnant female rats (7,16,20) and of fetuses (16,20) respectively. Conclusion: The conclusion of this current study was that the preservative sodium nitrite with two different concentrations caused harmful pathological effects in the histological structure of the livers, kidneys and brains of pregnant female rats during the different stages of gestation (7,16,20) days, as well as in the histological structure of these organs in the fetuses during the two stages gestation (16,20) days and these histopathological effects increased by increasing the concentration of sodium nitrite and the duration of gestation.

***Kaywords*---**Albino rats, Preservative, Sodium nitrite, Histological effects, pregnant female.

Introduction

Food additives have been defined as any natural or industrial chemical that is not usually consumed as food in itself and is not usually used as a main component of food (Linke *et al.*, 2018), and one of the important types of food additives is preservatives which are chemicals that are added to products food to prevent decomposition by microbial growth, enzymatic activity and oxidation and is very important in the food, drug and cosmetic industry to prevent bacterial growth and to improve the nutritional value, texture and taste of foods (Al-dhaher, 2008), and one of these preservatives is sodium nitrite (NaNO2), which is an inorganic sodium salt and its international code is 250E (Moorcroft *et al.*, 2001).

Nitrates and nitrites are found within the ecological, food, industrial and physiological system and humans are constantly exposed to sodium nitrite through food and drinking water and they also occur naturally in the body and therefore form part of the diet system of human, these substances are used in many food products to prevent the growth of yeasts and molds as a substance preservative in meat, fish and others, and after its discovery it became possible to get rid of adding large amounts of salt or other preservatives to preserve meat for a certain period, as the use of these materials made meat products of high quality products with a longer shelf life (Anand *et al* .,2013).

The use of nitrites and nitrates in processed meats in order to preserve flavor, stabilize color and as an antimicrobial preservative and must be within limited concentrations and any excess of these concentrations leads to harmful effects on consumers, as the entry of sodium nitrite in large quantities into the body has negative effects on health, nitrates and nitrite sodium enter the body through food and drinking water, the nitrates in saliva are converted into nitrites and then nitrites are absorbed by the blood through the intestinal membrane or through the stomach, once absorbed, it is rapidly distributed in the plasma and bound to red blood cells.

When the concentration of nitrite in the body increases, it is converted into nitrite oxide (NO) causing various damages to the body such as methemohemoglobin in the blood (Lundberg *et al.*, 2008; Gichrist *et al.*, 2010), and when the factors that form nitrous N2O3 derived from nitrite interact with secondary and tertiary amines and amides in the stomach resulting N-nitros compounds (NOCs) causing cancerous diseases and extensive damage to the eye, such as damage of the outer part of the photoreceptor cells and fibers of the optic nerve, sodium nitrite in high concentrations leads to encephalitis and brain cancer and causes double breaks of DNA and the union of sister chromatids, in addition to different effects on different body systems (Ozen, *et al.*, 2014).

Amis of the study

The aim of this current study was to study the possible negative histopathological effects of the preservative sodium nitrite in some organs represented by blackpad, kidneys and brains in pregnant female rats during the three gestation stages (20, 16, 7 days) as well as in fetuses during the two gestation period (16,20) days respectively.

Materials and Methods

(45) adult albino white rats of the type *Rattns rattus* (Sprague Dawley) were used in this study whose average weights were 230 g and their ages were 12 weeks, as well as (45) fertile albino white male rats were used whose average weights were 210 g and their ages were 10 weeks, , which were used for mating with female rats for gestation, Male and female rats were mated by placing one female rat with one male rat in mating cages throughout the night without food and water, and in the morning the mated female rats were examined to watch the mating plug in the vaginas of female rats or in the mating cages.

Sodium nitrite preparation

The German-origin sodium nitrite NaNO2 preservative was used in the current study which was supplied from Sekama Office of Chemical Supplies / Baghdad (Riedel-Haen/Germany), pregnant female rats were administered orally after dissolving them with 1 ml of distilled water for each concentration.

Experience design

The experiment of current study was designed to study the histopathological effects of the preservative sodium nitrite in the livers, kidneys and brains of pregnant female rats during the three gestation stages (7,16,20) days, as well as the changes in the histological structure of the livers, kidneys and brains of fetuses during the two gestation periods (16,20) days respectively , 45 white female rats of the type *Rattus rattus* (Sprague Dawley) were used in this experiment which were married with 45 fertile white male rats of the same type, after obtaining the required number of pregnant female rats (45), these animals were divided into 3 main groups, each group contained a 15 pregnant female rat:
The first group was as a control group, which was treated with physiological salt solution (0.9% normal saline) only, while the second group was treated with

sodium nitrite at a concentration of 10 mg / kg of body weight, while the third group was treated with sodium nitrite at a concentration of 15 mg / kg of body weight, each 5 animals of pregnant female rats from each of the three main groups were dissceted during different stages of gestation (7, 16, 20) days respectively after administered them orally with a single dose daily from the first day of gestation.

Anatomy of pregnant rats during (7, 16, 20)days of gestation

Pregnant female rats were dissected during (7, 16, 20) days of gestation after being anesthetized with diethyl ether, then the animals were fixed in a dissection dish and the abdominal cavity was opened to them, livers, kidneys and brains were extracted from the body of pregnant female rats and placed in a 10% formalin solution for a period of 48 hours for the purpose of preparing histological sections.

Fetal anatomy during (16, 20)days of gestation

After dissection of pregnant female rats during (16, 20) days of gestation, the uterine horns were extracted from their bodies and opened with sharp scissors and the fetuses that were anesthetized with diethyl ether, then these fetuses were fixed in a dissection dish and the abdominal cavity was opened of them, livers, kidneys and brains were extracted from the fetuses and placed in 10% formalin solution for 48 hours for histological study.

Preparation of histological sections

Histological sections were prepared for the livers, kidneys and brains of pregnant female rats during the gestation stages (7,16,20) days, as well as the livers, kidneys and brains of the fetuses during the two stages of gestation (20,16) days respectively depending on the method of Suvarna *et al* (2013).

Examination and imaging of tissue sections

Histological sections of pregnant female rats during the different stages of gestation and fetuses during the two stages of gestation were examined and photographed using an optical microscope (Compound-Olympus) which is equipped with a camera using a color film and at the magnification power (40X) as for all study groups.

Results and Discussion

1- Histological study of pregnant female rats during gestation stages (7,16,20) days.

1- The livers

The histological sections of the liver of the pregnant female rats in the control groups did not show any histopathological changes during the three stages of gestation (20,16,7) days respectively and as shown in the figure (1), while the

histological sections of the liver of the pregnant female rats treated with Sodium nitrite at concentrations (10) mg / kg pathological effects during the three stages of gestation (20, 16, 7) respectively such as destruction of the wall of the central vein, necrosis and degeneration of hepatocytes, dilation of sinusoids, destruction of hepatic tissue, hemorrhage, infiltration of inflammatory cells and as shown In the figures(2,4,6) respectively, these negative effects were more severe in the liver tissue of pregnant female rats treated at a concentration of 15 mg/kg, and their intensity increased with increasing stage of gestation as in the figures (3,5,7) respectively

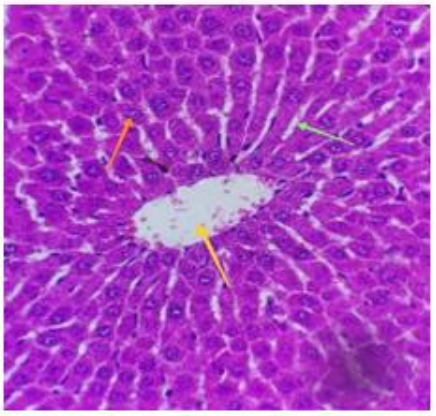

Figure (1) A cross section of the liver of a pregnant female rat from the control group in which it is noted: Central vein Hepatocytes Sinusoids Hepatocytes(Hematoxylin-Eosin-400X Stain) .

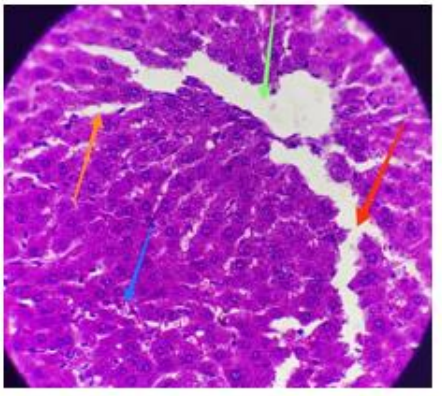

Figure (2) A cross section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg/kg for a period of 7 days gestation, in which it is noted: Destruction of the hepatic vein wall Necrosis of hepatocytes Destruction of hepatocyte Expansion of sinusoids (Hematoxylin-Eosin -400X Stain) .

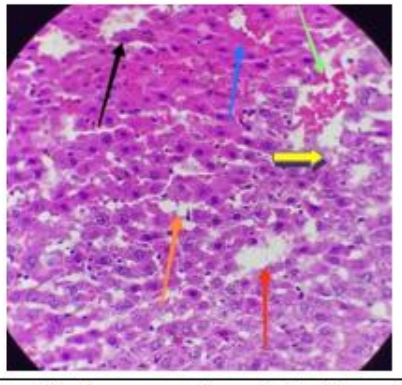

Figure (3) A cross-section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a period of 7 days gestation, in which it is noted: Destruction of the hepatic vein Necrosis of hepatocy Destruction of hepatocytes Destruction of hepatic tissue Hemorrhage in the liver tissue (Hematoxylin-Eosin -400X Stain).

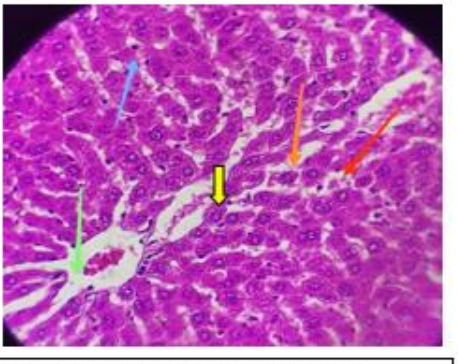

Figure (4) A cross-section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a gestation period of 16 days, in which it is noted: Destruction of the hepatic vein wall Necrosis of hepatocytes Destruction of hepatocytes and the occurrence of bleeding in theme Expansion of sinusoids Destruction of the liver tissue Hemorrhage in the liver tissue (Hematoxylin-Eosin -400X Stain) .

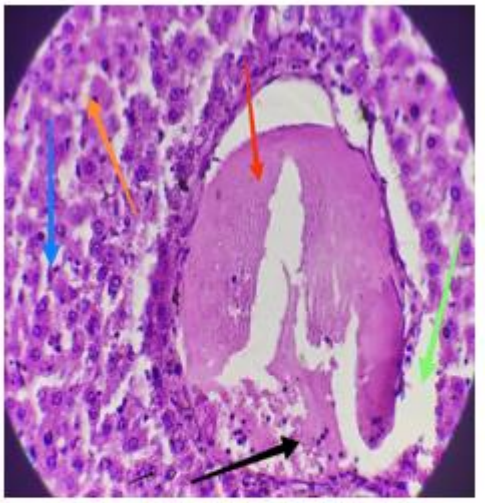

Figure (5) A cross-section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a gestation period of 16 days, in which it is noted: Destruction of the central vein wall Hemorrhage in the central vein Necrosis of hepatocytes, Expansion of sinusoids Destruction of hepatic tissue (Hematoxylin-Eosin-400X Stain).

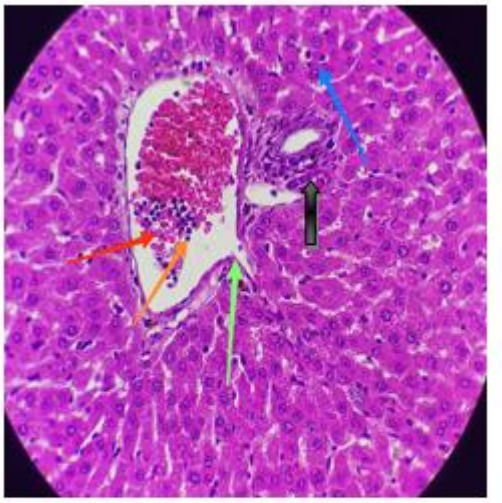

Figure (6) A cross-section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg/kg for 20 days in which it is noted: Increased destruction of the central vein wall Severe hemorrhage in the central Necrosis of hepatocytes Destruction of hepatoc Infiltration of inflammatory cells (Hematoxylin-Eosin-400X Stain).

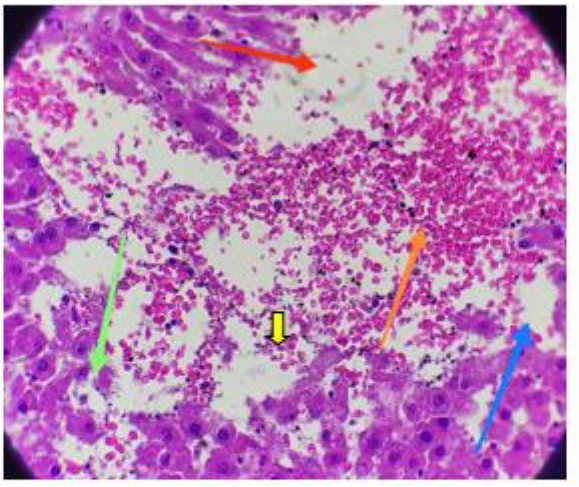

Figure (7) A cross-section of the liver of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for another 20 days in which it is noted: Destroy of the central vein and severe bleeding occurred in it severe necrosis of the liver tissue Destruction of hepatocyte infiltration of inflammatory cells severe expansion of the sinusoids Severe damage of the liver tissue (Hematoxylin-Eosin-400X Stain).

2- *The kidneys*

The results of the current study showed in the histological sections of the kidneys of pregnant rats in groups treated with sodium nitrite at a concentration of 10 mg/kg during the various stages of gestation (7,16,20) days respectively as several histopathological changes represented by shrinkage and necrosis of the renal glomerulus and the occurrence of bleeding in it , widening of Bowman's space, destruction of the wall of Bowman's capsule, separation of the urinary tubules from the basement membrane, shedding of the inner layer lining the urinary tubules, hemorrhage and destruction of the urinary tissue as in the figures (9,11,13) respectively,and these effects increased in the livers of pregnant rats in groups treated with sodium nitrite at a concentration of 15 mg/kg, and increased with an increase in gestation during the different stages of gestation(20,16,7) days respectively as shown in the figures (10,12,14) respectively compared with the histological structure of the kidneys of pregnant rats in the control group whose histological sections did not show any changes other than normal during the stage of gestation as in the figure (8).

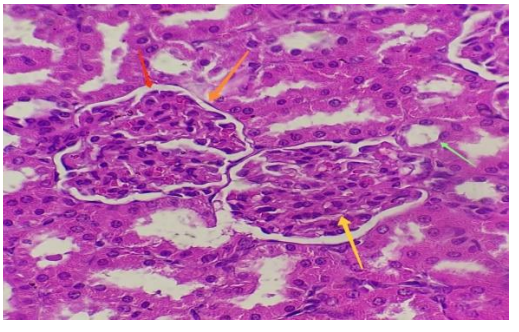

Figure (8) A cross-section of the kidney of a pregnant female rat from the control group, in which it is noted: Renal glomerulus Urinary tubules Bowman's space Bowman's capsule wall (Hematoxylin-Eosin-400X Stain).

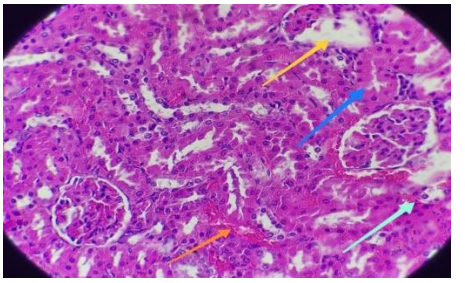

Figure (9) A cross-section of the kidney of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 7 days gestation, in which it is noted: Necrosis of the renal glomerulus, Necrosis of the wall of the urinary tubule Hemorrhage in the urinary tissue (Hematoxylin-Eosin-400X Stain).

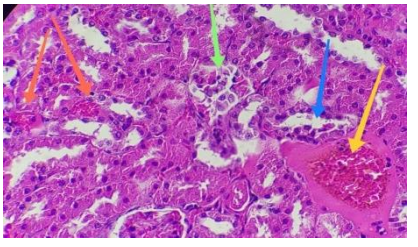

Figure (11) A cross-section of the kidney of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 16 days gestation, in which it is noted: disintegration and hemorrhage of the renal glomerulus Necrosis and shrinkage of the renal glomerulus Necrosis of the wall of the urinary tubule Hemorrhage into urinary tissue(Hematoxylin-Eosin-400X Stain).

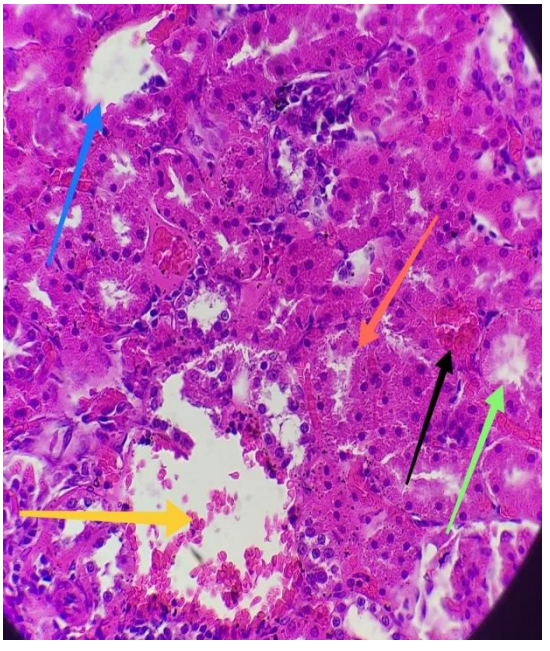

Figure (12) A cross-section of the kidney of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg / kg for a period of 16 days gestation, in which it is noted: Necrosis and shrinkage of the renal glomerulus Necrosis of the wall of the urinary tubule Damage the wall of the urinary tubule Hemorrhage in the urinary tissue (Hematoxylin-Eosin-400X Stain).

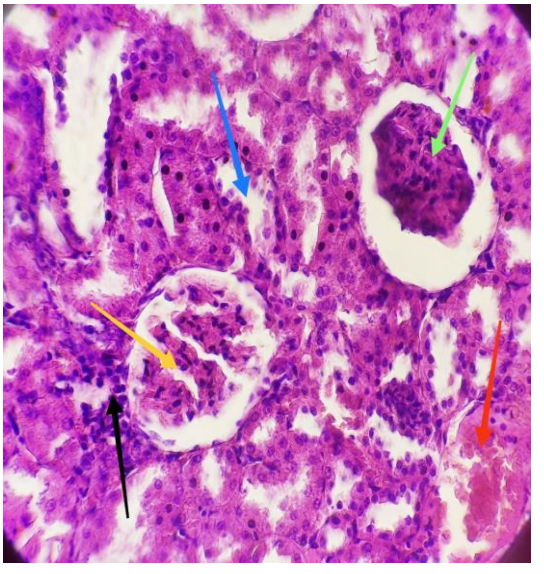

Figure (13) A cross section of the kidney of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 20 days gestation, in which it is noted:

Necrosis of the renal glomerulus severe shrinkage of the renal glomerulus and the occurrence of bleeding in it necrosis in the wall of the urinary tubule bleeding of the urinary tubule, Infiltration of inflammatory cells Severe bleeding in the urinary tissue (Hemotoxylin-eosin -400X stain).

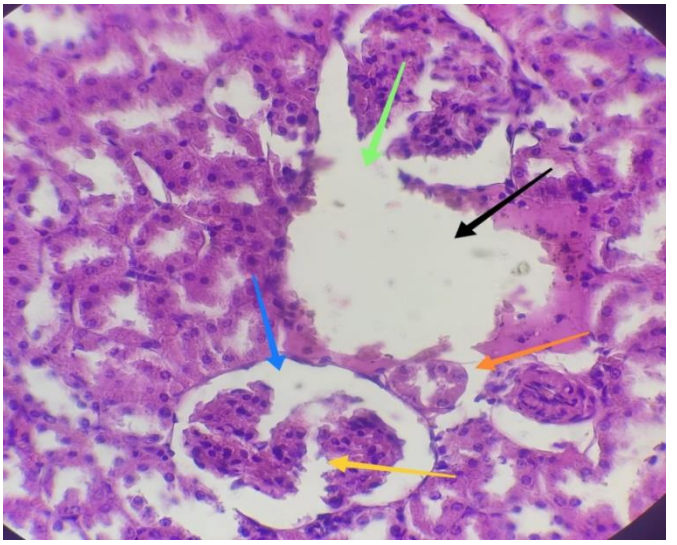

Figure (14) A cross section of the kidney of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a period of 20 days gestation, in which it is noted: Severe Necrosis of the renal glomerulus Expansiopn of Bowman's space Separation of the inner lining of the urinary tubule from the urinary tissue Necrosis of the wall of the urinary tubule Severe damage in the urinary tissue (Hemotoxylin-eosin 400X stain).

3- The brains

The results of microscopic examination of the tissue sections of the brains of pregnant rats treated with sodium nitrite at a concentration of 10 mg / kg revealed harmful histological effects on the histological structure of the brain in the three stages of gestation (20,16,7) days respectively as the histological sections of the brain showed that necrosis astrocyte ,shrinkage of microglia, shrinkage of oligodendrocytes, thickening of neurons, degeneration of neurons, infiltration of inflammatory cells, hemorrhage in nervous tissue, rupture and destruction of nervous tissue as shown in the figures (16,18,20) respectively ,and in the groups of pregnant rats treated with a concentration of 15 mg/kg of sodium nitrite these histopathological effects were more severe than the groups treated with a concentration of 10 mg/kg of the same stages of gestation(7,16,20)days respectively as in the figure(17,19,21)respectively when compared with the histological structure of the brains of pregnant rats of the control group of the same stages of gestation as shown in the figure (15).

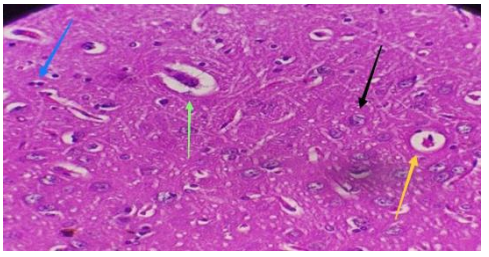

Figure (15) A cross-section in the brain of a pregnant female rat from the control group, in which it is noted: Astrocytes Microglia oligodendrocytes Neurons (Hematoxylin-Eosin-400X Stain).

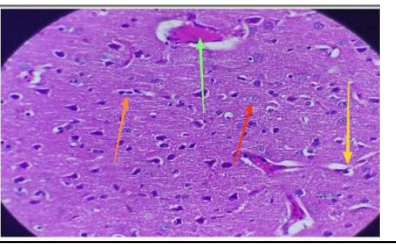

Figure (16) A cross-section in the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg/kg for a period of 7 days gestation, in which it is noted: Thickening of nuclei of astrocytes Necrosis and hemorrhage in microglia Necrosis of oligodendrocytes Necrosis of neurons (Hematoxylin-Eosin-400X Stain).

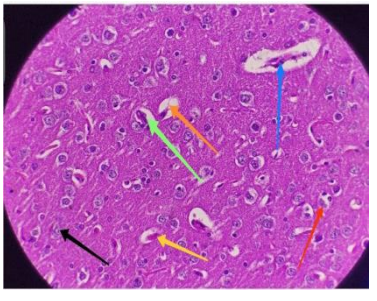

Figure (17) a cross-section of the gray matter of the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a period of 7 days, in which it is observed: Necrosis of astrocytes Thickening of the nuclei of microglia cells Necrosis of the nuclei of oligodendrocytes Thickening of the nuclei of (Hymotoxylin-eosin 400X stain).

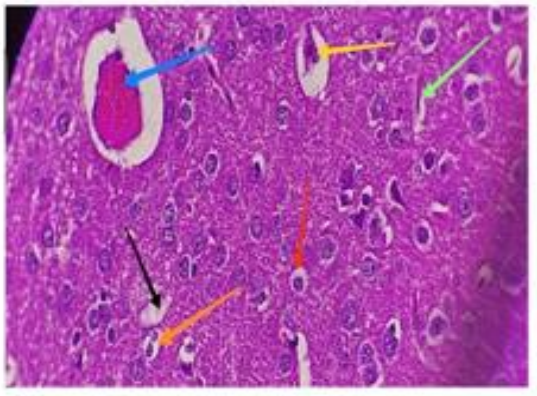

Figure (18) A cross-section in the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg/kg for a period of 16 days gestation, in which it is noted: Necrosis of astrocytes Necrosis of microglia cells Necrosis of oligodendrocyte Necrosis of neuron Nervous tissue rupture Damage of nervous tissue (Hematoxylin-Eosin-400X Stain).

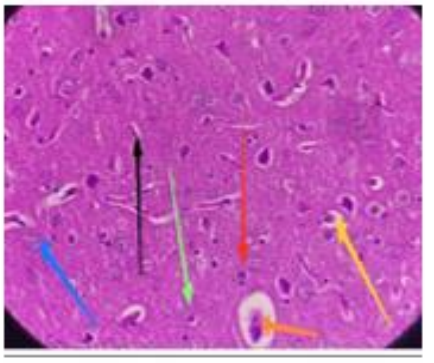

Figure (19) A cross-section in the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a period of 16 days gestation, in which it is noted: Necrosis of astrocytes Necrosis of microglia cells Necrosis of oligodendrocytes Necrosis of neurons Nervous tissue rupture Damage of nervous tissue (Hematoxylin-Eosin-400X Stain).

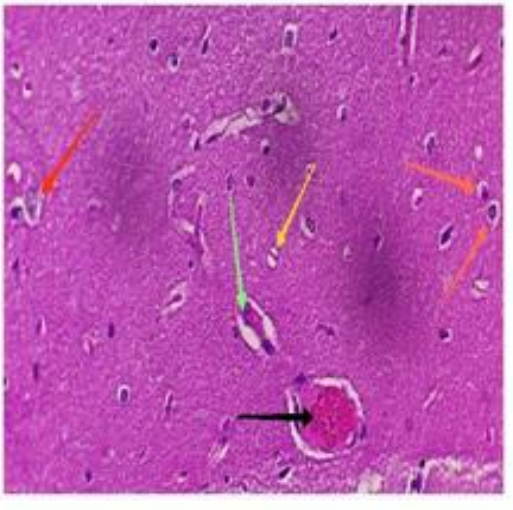

Figure (20) A cross-section in the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 10 mg/kg for a period of 20 days gestation, in which it is noted: Severe shrinkage in astrocytes Thickening of the nuclei of microglia cells Necrosis of oligodendrocytes Thickening of the nuclei of neurons Damage and hemorrhage in the nervous tissue (Hematoxylin-Eosin-400X Stain).

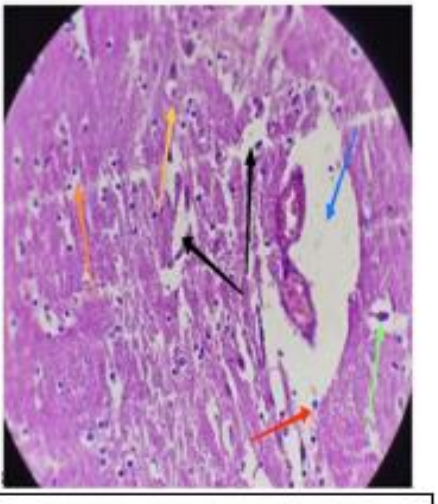

Figure (21) A cross-section in the brain of a pregnant female rat from the group treated with sodium nitrite at a concentration of 15 mg/kg for a period of 20 days gestation, in which it is noted: Degeneration and hemorrhage of astrocyte Shrinking and wall damage of microglia cells Severe necrosis of oligodendrocytes Damage of neurons and their nuclei are ejected Damage of nervous tissue (Hematoxylin-Eosin-400X Stain).

2- Histological study of feteues of pregnant female rats during two gestation stages (16,20) days.

1-The livers

Histological sections of the livers of the fetuses of the control groups did not show any abnormal histological changes during the two stages of gestation (20,16) days as shown in the figure (22), while the microscopic examination of the livers of the fetuses whose mothers were treated with the concentration (10) mg / kg during the two stages of gestation revealed (16, 20) days respectively various histopathological changes such as the destruction of the wall of the central vein, necrosis of hepatocytes, expansion of sinusoids, necrosis of the liver tissue, infiltration of inflammatory cells, hemorrhage and destruction of the liver tissue as in the figures (23,25) respectively and the severity of the damage in the histological structure of the liver increased with an increase in the concentration of sodium nitrite 15 mg / kg during the two stages of gestation (20, 16) days respectively as shown in figures (24,26) respectively.

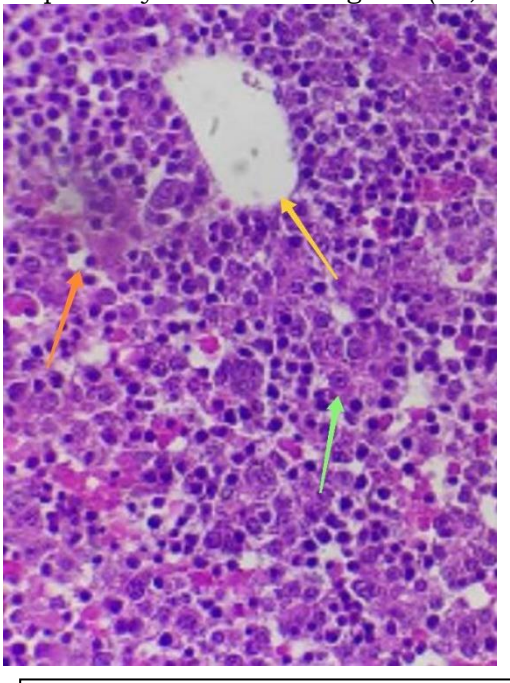

Figure (22) A cross-section in the liver of a fetus rat from the control group, in which it is noted: Central vein Hepatocytes , Sinusoids (Hematoxylin-Eosin-400X Stain).

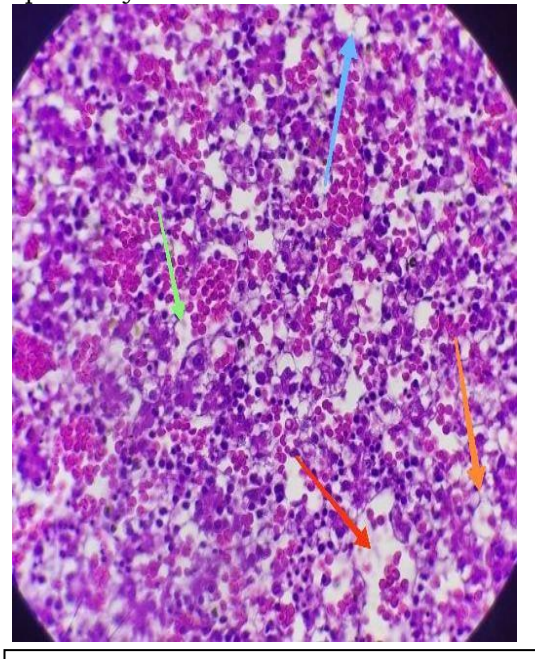

Figure (23) A cross-section in the liver of a fetus rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 16 days gestation, in which it is noted: Damage of the wall of the central vein and occurrence of bleeding in it Damage of the hepatocytes Necrosis of the hepatocytes Damage of the hepat tissue and occurrence of bleeding in it (Hematoxylin-Eosin-400X Stain).

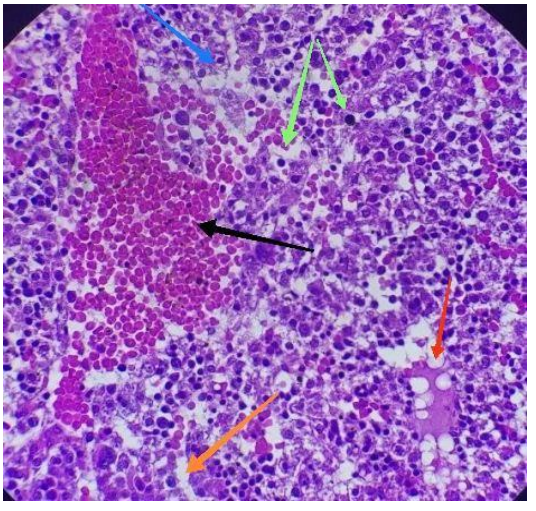

Figure (24) A cross-section in the liver of a fetus rat from the group treated with sodium nitrite at a concentration of 15 mg / kg for a period of 16 days gestation, in which it is noted: Necrosis of hepatocytes Damage of hepatocytes Expansion of sinusoids Severe hemorrhage in the hepatic tissue Necrosis of the hepatic tissue and occurrence of bleeding in it (Hematoxylin-Eosin-400X Stain).

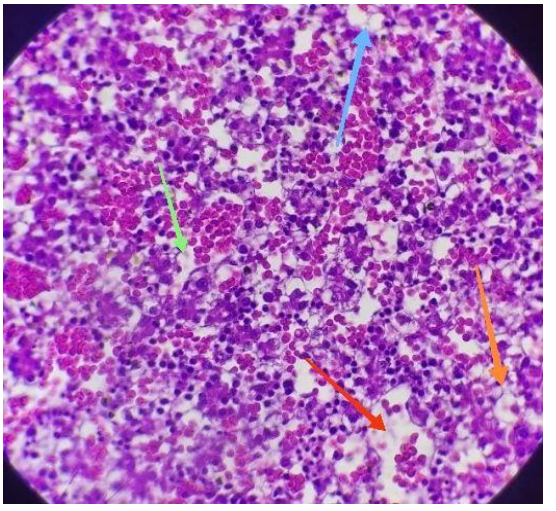

Figure (25) A cross-section in the liver of a fetus rat from the group treated with sodium nitrate at a concentration of 10 mg / kg for a period of 20 days gestation, in which it is noted: Necrosis of hepatocytes Damage of the wall of the central vein and occurrence of bleeding in it Damage of hepatocytes Damage of the hepat tissue and occurrence of bleeding in it (Hematoxylin-Eosin-400X Stain)

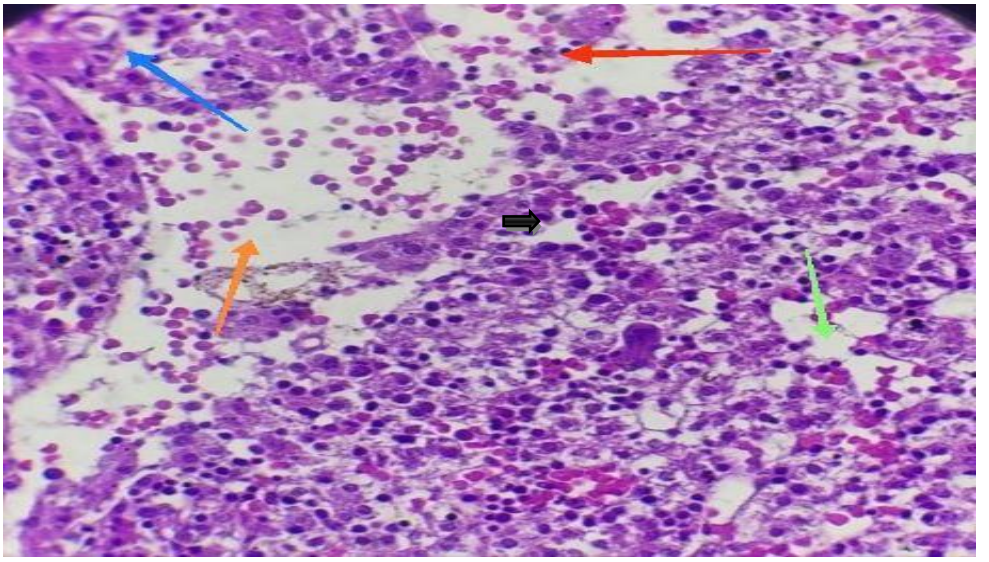

Figure (26) A cross-section in the liver of a fetus rat from the group treated with sodium nitrate at a concentration of 15 mg / kg for a period of 20 days gestation, in which it is noted: Damage of the wall of the central vein and occurrence of bleeding in it Necrosis of hepatocytes Severe damage of hepatocytes Severe hemorrhage in the hepatic tissue Damage of the hepatic tissue and occurrence of bleeding in it (Hematoxylin-Eosin-400X Stain).

The kidneys

From the results of the histological examination in the control groups of the kidney tissue of the fetuses,it was observed that no pathological changes were observed during the two stages of gestation (20,16) days as shown in the figure (27) while the histological sections of the kidneys of the fetuses whose mothers were treated with sodium nitrite at two concentrations (15,10) mg /kg showed during the 16th day of gestation adverse negative changes in the form of shrinkage and necrosis of the renal glomerulus, destruction of the wall of Bowman's capsule, necrosis of renal cells, hemorrhage in the renal tissue, necrosis and hemorrhage in the urinary tubule, necrosis of the cells of the inner lining of the urinary tubules, detachment The urinary tubule from the basement membrane was destroyed in the renal tissue as in the figures (28,29)respectively and the severity of these effects was increased for the liver tissue of the fetuses on the 20th day of gestation whose mothers were treated with the same concentrations of the previous two mentioned previously as in the figures (30,31)respectively .

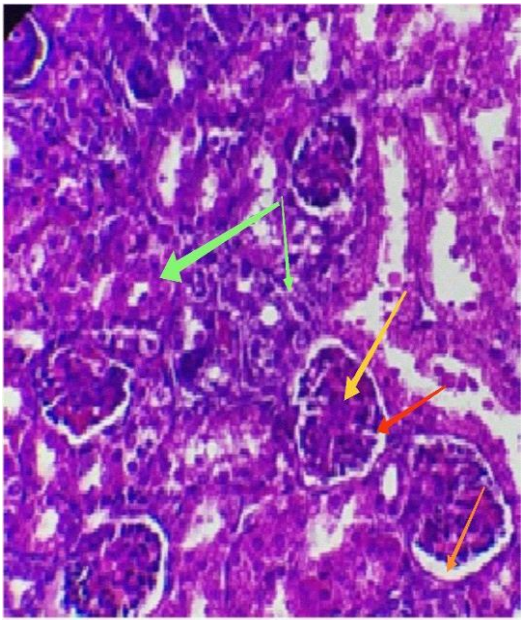

Figure (27) A cross-section in the kidney of a fetus rat from the control group, in which it is noted: Renal glomerulus Urinary tubules Bowman's space Bowman's capsule wall (Hematoxylin-Eosin-400X Stain).

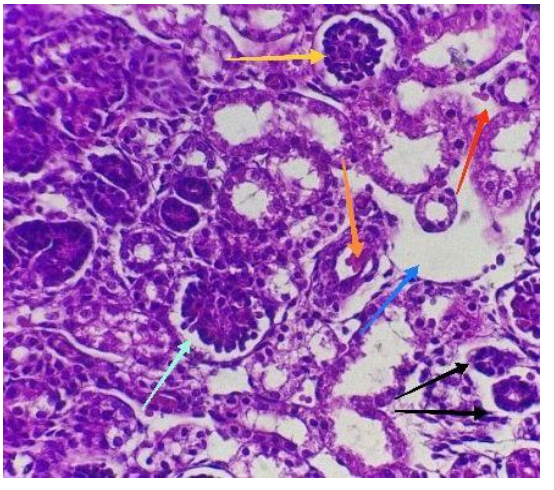

Figure (28) A cross-section in the kidney of a fetus rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 16 days gestation, in which it is noted: Shrinkage of the renal glomerulus Expansion of Bowman's space Damage the wall of the urinary tubule and the occurrence of bleeding in it Damage of the urinary tubule Damage of the urinary tissue detachment of the tubule from the urinary it (Hematoxylin-Eosin-400X Stain).

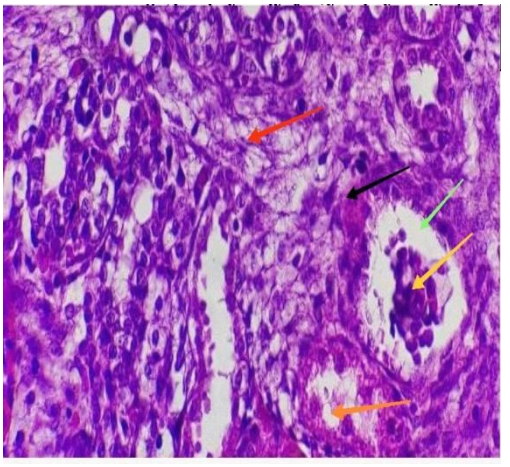

Figure (29) A cross-section in the kidney of a fetus rat from the group treated with sodium nitrite at a concentration of 15 mg / kg for a period of 16 days gestation, in which it is noted: Severe shrinkage of the renal glomerulus Severe expansion of Bowman's space Damage to the inner lining of the urinary tubule Damage of the urinary tubule Damage of the urinary urinary (Hematoxylin-Eosin-400X Stain).

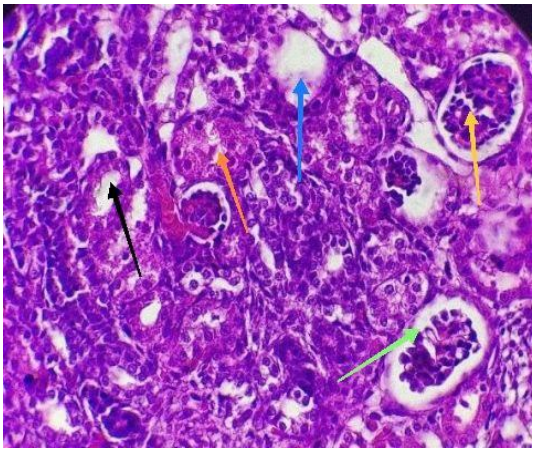

Figure (31) A cross-section in the kidney of a fetus rat from the group treated with sodium nitrite at a concentration of 15 mg / kg for a period of 20 days gestation, in which it is noted: Necrosis of the renal glomerulus Severe shrinkage of the renal glomerulus Severe damage of the inner lining of the urinary tubule Detachment of the tubule from the urinary tissue bleeding in urinary tubule (Hematoxylin-Eosin-400X Stain).

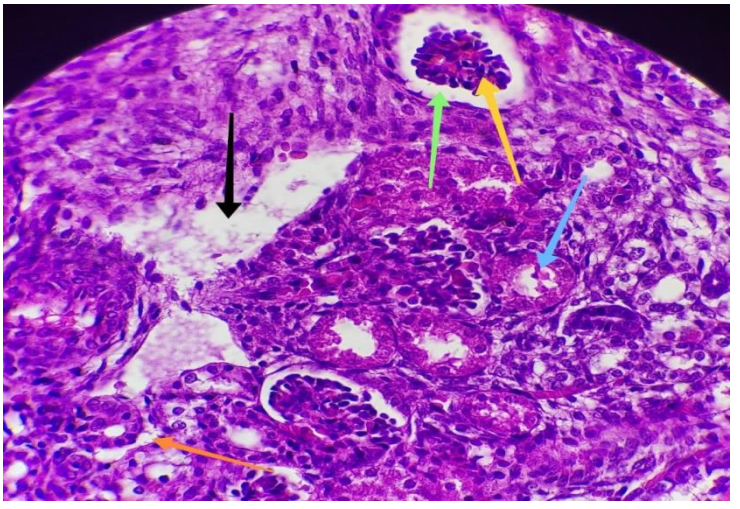

Figure (30) A cross-section in the kidney of a fetus rat from the group treated with sodium nitrite at a concentration of 10 mg / kg for a period of 20 days gestation, in which it is noted: Severe shrinkage of the the renal glomerulus Expansion of Bowman's space slough off the inner lining of the urinary tube and the occurrence of bleeding in it Separation of the urinary tubules from the urinary tissue Hemorrhage in urinary tissue (Hematoxylin-Eosin-400X Stain).

3-The brians

Histological study of sections of the brain of fetuses whose mothers were treated during the two stages (20 , 16) days of gestation with preservative sodium nitrite and at a concentration of 10 mg / kg showed various negative structural changes such as thickening of astrocytes, contraction of oligodendrocytes, thickening of neurons, thickening of microglia, degeneration and hemorrhage in the nervous tissue as can be seen in the figures (33,35) and these histological effects increased when using a concentration of 15 mg/kg of sodium nitrite and for the same two stages of gestationrespectively as shown in the figures (34,36) respectively compared with the normal structure of the brain in the control groups during the two stages as in figure (32).

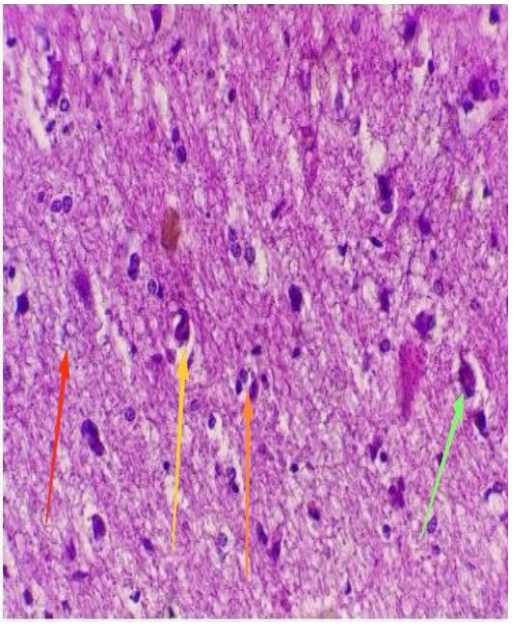

Figure (32) A cross-section in the brian of a fetus rat from the control group, in which it is noted: Astrocytes, Microglia cells Oligodendrocytes Neurons (Hematoxylin-Eosin-400X Stain).

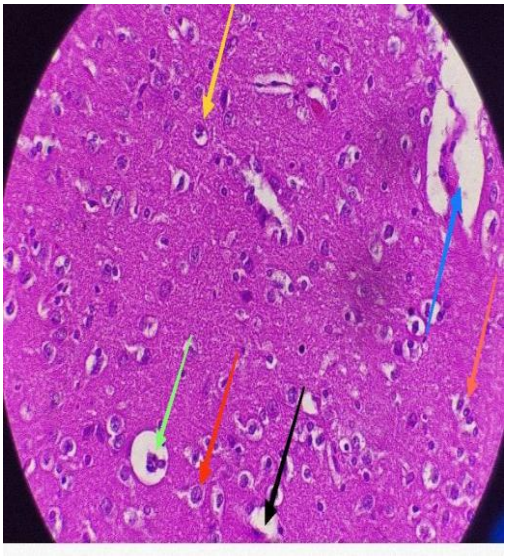

Figure (33) A cross-section in the brian of a fetus rat from the group treated with sodium nitrate at a concentration of 10 mg / kg for a period of 16 days gestation, in which it is noted: Necrosis of astrocytes Shrinkage of microglia cells Shrinkage of oligodendrocytes Thickening of the nuclei of neurons Degeneration of nervous tissue Damage of nervous tissue (Hematoxylin-Eosin-400X Stain).

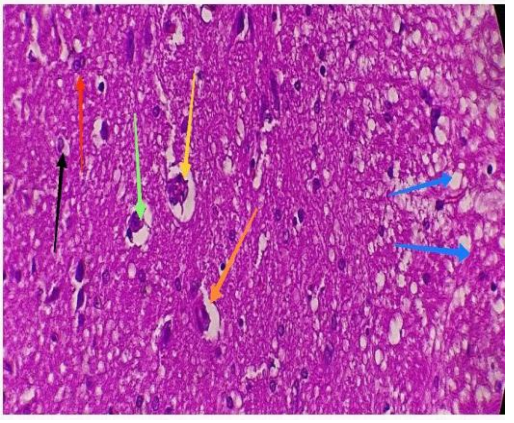

Figure (34) A cross-section in the brian of a fetus rat from the group treated with sodium nitrate at a concentration of 15 mg / kg for a period of 16 days gestation, in which it is noted: Necrosis of astrocytes Thickening of the nuclei of microglia cells Shrinkage of microglia cells Thickening of the nuclei of oligodendrocytes Thickening of the nuclei of neurons Degeneration of nervous tissue (Hematoxylin-Eosin-400X Stain).

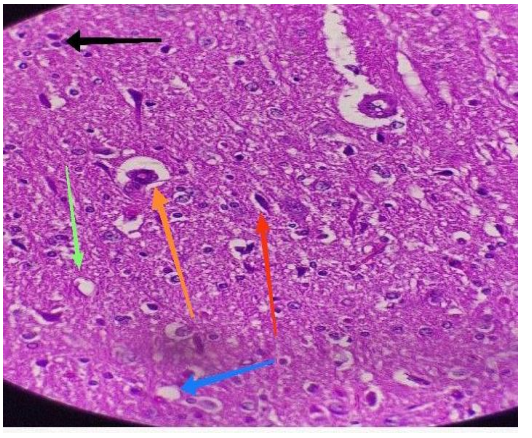

Figure (35) A cross-section in the brian of a fetus rat from the group treated with sodium nitrate at a concentration of 10 mg / kg for a period of 20 days gestation, in which it is noted: Severe thickening of the nuclei of astrocytes Severe shrinkage of astrocytes Thickening of the nuclei of microglia cells Severe shrinkage of microglia cells shrinkage of oligodendrocytes Degeneration of neurons (Hematoxylin-Eosin-400X Stain).

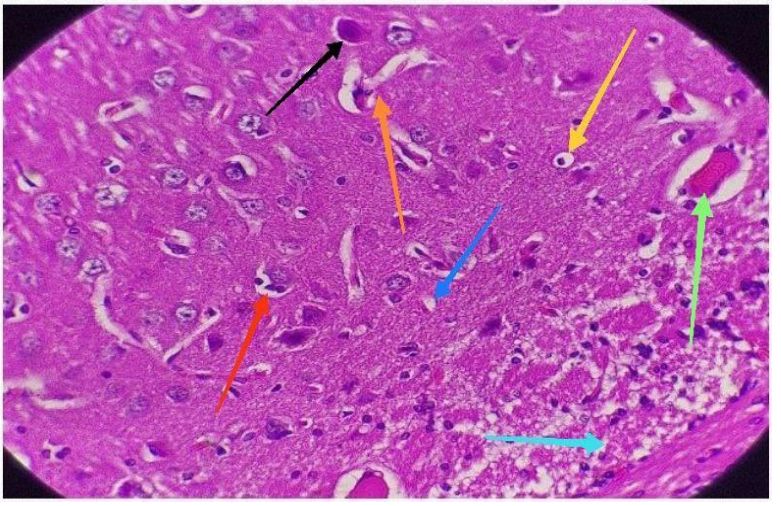

Figure (36) A cross-section in the brian of a fetus rat from the group treated with sodium nitrate at a concentration of 15 mg / kg for a period of 20 days gestation, in which it is noted Severe thickening and bleeding of the nu of astrocytes Severe shrinka of astrocytes Thickening of the nuclei of microglia cells evere shrinkage of microglia cells shrinkage of oligodendrocytes Degeneration of neurons nicrosis in neurons (Hematoxylin-Eosin-400X Stain).

The reason of these histopathological changes in the livers, kidneys and brains of pregnant female rats during the different stages of gestation and fetuses during the two periods of gestation may be due to the rapid absorption of this substance by the digestive system and its entry into the tissues of the body and it turns into nitrite oxide which causes necrosis in various tissues in the body including cells of livers, kidneys , brain cells and others, some studies have shown that disruption of the cell membrane affects its permeability causing enzymes to leak out of the cell and affecting the liver tissue and causing the death of cells in the liver and thus decreasing in the number of cells and the occurrence of bleeding in it (Prater *et al.*, 2006), another study showed that the presence of high concentrations of nitrite causes the synthesis of nitric oxide and an increase in the activity of nitrotyrosine in the liver which causes an increase in the rate of free radicals and induces oxidative stress which leads to damage of cell membranes and necrosis in hepatocytes and consequently the expansion of the sinusoids and infiltration of inflammatory cells and damage of the wall of the central vein in the liver and the occurrence of bleeding in it (Sierra et al, 2018), the results of the current histological study agreed with the results of the study of El-Nabarawy *et al* (2020) in which sodium nitrite was dosed orally at a concentration of (75,60,40,20) mg/kg to adult white rats causing pathological effects in the livers of animals such as necrosis and degeneration of hepatocytes consequently, the liver tissue was destroyed, some studies showed that sodium nitrite affected the kidneys causing a decrease in renal tubular reabsorption, glomerular filtration rate and blood flow to the kidneys as a result of damage to the renal glomeruli and renal tubules causing a defect in the functions of the kidneys (Sosedova, 2019).

and the results of the this study agreed with the results of the study of Wahyuningsih *et al* (2020) which indicated that oral administration of sodium nitrite of rats led to the destruction of the renal tubules, atrophy of the glomeruli, damage of the renal tissue and infiltration of inflammatory cells in the kidney tissue, in addition to a high creatinine and urea levels and a decrease in antioxidants, as some studies revealed that treatment with sodium nitrite caused extensive damage of purkinje cells of the cerebellum and the degeneration and shrinkage of brain cells stimulating brain inflammation and weak energy and these changes are due to the formation of excessive free radicals and poor balance between the interaction of oxidative stress / antioxidants which eventually lead to the stimulation of apoptosis of cells and this causes damage of the tissues(Zhengs *et al.*, 2003), as some studies pointed to sodium nitrite is a major factor in the damage of lipid bilayers in cell membranes by the lipid peroxidation process generating oxidative stress which stimulates damage to cell membranes which stimulates cell degeneration and tissue destruction in the body which causes high levels of malon didehyde which is an indicator of oxidative damage in tissues but at the same time the levels of endogenous antioxidants decrease as a result of their interaction with free radicals to rid the body of their damaging damages and this is what was revealed the current study(Chow *et al.*, 2002), in addition to that sodium nitrite affects the formation and fetal growth of different organs during the different stages of gestation causing their lack of development and incomplete growth such as livers, kidneys, and brains, or these pathological effects of tissues in the various organs of the fetus may be due to the fact that sodium nitrite negatively affected the uterine tissue of pregnant rats and the formation and development of the placenta as well as on its tissues causing a defect in the work of the placenta in the transfer of nutrients and oxygen to the fetus during the early stages of gestation and increased during the advanced stages of it.

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