



Histological effects of the interaction of some food additives on the kidney of pregnant rats

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Abstract

Many studies are still the subject of food additives to know their positive and negative effects, primarily as they are widely used globally. Therefore, this study aimed to identify the histological effects of sodium nitrite and monosodium glutamate on the histological structure of the kidney in pregnant rats. Twenty-four pregnant rats were used to achieve the aim of the study. The rats were classified into four groups, the first being the control group, the second treated with monosodium glutamate at 10 g/kg, the third injected with sodium nitrite at 115 mg/kg, and the fourth for interaction between the two substances and for the same concentrations. The results showed the occurrence of many lesions in the kidneys of experimental groups rats. The second group included interstitial tissue hyperplasia and necrosis of the glomeruli, infiltration of inflammatory cells, congestion of blood vessels, hydropic degeneration of some tubules, and necrosis of some of them. The third group included congestion, hemorrhage in the pulp area, degeneration of some urinary tubules, necrosis, and deformation of the glomerulus. However, degeneration of some tubules and necrosis were seen in the fourth group, such as glomerulus hyperplasia, reduction of Bowman's space, an increase in acidity of the cytoplasm of epithelial cells tubules, hyperplasia of the fibroblasts, and the desquamation of some tubules. The study concluded that these substances have harmful effects on the kidneys in pregnant rats, especially when they are overlapped, so they must be avoided during pregnancy to maintain kidney health.

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Introduction

Food is the reason for all organisms' life and one of the essential basics for obtaining a healthy body capable of carrying out its various vital activities and combating the risk of diseases that the body is exposed to and thus enjoying a robust immune system. With the advancement of technologies, the human diet differed significantly in recent decades, as this difference coincided with the emergence of processed foods that met with wide acceptance due to the use of food additives, both preservative, and flavoring, to improve some of the taste characteristics of food, preserve the nutritional value, reduce and prevent food spoilage (1). Food additives (FDs) are widely used for various purposes,

including Sweetening, Preservation, and Coloring. According to their function, FDs fall into five categories: Taste enhancers, antioxidants, preservatives, stabilizers and emulsifiers, and coloring agents (2). These additives are of either natural or synthetic origin (3). They are added to most fast food, especially those provided to children (3). Sodium nitrite 'NaNO₂' is used as an additive to food, as found in the early 1900s. It inhibits the growth of pathogenic microorganisms, gives flavor and color to the meat, and prevents the oxidation of fats that lead to rancidity. It also has the advantage of improving food safety, longevity and improving color and desired taste (4). Despite the benefits of NaNO₂ mentioned previously, it has been found that NaNO₂ poses a health risk when exposed to

it and is irritating to the eyes, lungs, and skin, and is toxic when consumed in high concentrations. It has been shown that nitrites and nitrates interact with various amines and amides to form Nitroso carcinogenic compounds. NaNO_2 has a vasodilating effect, which depends on the non-enzymatic reduction of nitrite to NO (5). On another side, Monosodium glutamate 'MSG' is one widely flavor-enhancing FDs. It helps improve taste and treat hypertension and hemoglobin deficiency. However, it has been found to have harmful effects such as toxicity, Chinese restaurant syndrome, and uncontrolled breathing during sleep. Through various experiments, these effects could be reduced by antioxidants such as quercetin and vitamin C (6). MSG also increases the appetite, and thus obesity occurs due to eating quantities of great food. Researchers from the University of North Carolina do a study among Chinese rural residents to examine the effects of MSG. They chose that region because most of its residents depend on their food to be prepared at home without being processed, but they still use MSG frequently. Thus, they are the most vulnerable to weight gain regardless of the total Calories and levels of physical activity (7). A study also revealed that MSG intake disrupted the energy balance by increasing food palatability, thus causing an imbalance in several hypothalamus areas, which caused metabolic and neuroendocrine changes to lead to obesity. Scientists believe that MSG causes brain injury by interfering with the hormone leptin (8).

It is common practice for pregnant women to eat a varied diet. They consume calories to ensure a healthy pregnancy and facilitate the fetus's growth and development (9). Hence, the study wanted to investigate the effect of two types of FDs, NaNO_2 and MSG, on the histological composition of the kidney of pregnant rats during the organogenesis stage of the pregnancy.

Materials and methods

Ethical approve

Ethical approval for this study was following the guidelines given by the Ethics Committee of the Canadian Council on Animal Care (CCAC) (certification # 2010-015).

The animals of the Study

The white rats, *Rattus norvegicus*, were used for this study, whose ages ranged between 10-12 weeks, and their average weight ranged between 225-240 gm. It was fed continuously. They were also bred under laboratory conditions (10).

The study doses

The concentrations of NaNO_2 and MSG were selected depending on LD_{50} for previous studies (11,12). A selected

dose 115 mg/kg of body weight for NaNO_2 and laboratory animals were injected with a 0.1ml intraperitoneal injection. A selected dose 10 g/ kg of body weight was for MSG, and the gavage did 0.5 ml of it.

The mating

After raising the rats to ensure that they have good health and are free from diseases, and to ensure the mating took place, a male and three females was placed in each cage at night. A vaginal smear was performed to verify the presence of sperm, which indicates the occurrence of pregnancy, and its presence was considered the day zero of pregnancy. Thus, the day following the first day of pregnancy (10).

Experimental design

For the objectives of the study, pregnant female rats were isolated in separate special plastic cages. The date of pregnancy is fixed on them. They were injected and dosed with specific doses of NaNO_2 , MSG every day starting from the 6th day of pregnancy (which marks the beginning of the organogenesis stage) until the 15th day. The rats were classified into four subgroups (6 rats), the first representing the control group. They were dosed with distilled water; the second group was injected with NaNO_2 ; the third group was given MSG; the fourth group was given the two substances together.

Histological sections preparation

After anesthesia, the rats were sacrificed, and after the rats were autopsied, the kidneys were removed. Histological sections of the kidney were then prepared for histological sections (13). The samples sections were colored with different stains: Mallory's Triple Stain (TS) (14), Delafield's Haematoxylin and Eosin stain (H+E) (15,16), Toluidin Blue Stain (TB), Alcian Blue Stain pH 2.5 (AB), and Periodic Acid - Schiff technique (PAS) (17,18). The sections loaded with DPX and the histological sections were photographed with a digital camera attached to an optical microscope.

Results

The current study results revealed histopathological lesions and histochemical changes of the kidneys of pregnant rats compared to the control group. The normal kidney tissue in pregnant rats consisted of the nephron, the kidney building unit. Each nephron consists of Bowman's capsule containing a network of capillaries called the glomerulus, the two proximal & distal convoluted tubules. The tubule's twisted parts are connected to the Henley loop, and the last part of the nephron is the collecting tubule (Figures 1-4).

When pregnant rats were treated with NaNO_2 115 mg/kg, histopathological lesions appeared upon microscopic examination of the kidneys, as interstitial

tissue hyperplasia and necrosis of the glomeruli were observed, infiltration of inflammatory cells, congestion of blood vessels, hydropic degeneration of some tubules, and necrosis of some of them (Figure 5). The epithelial tissue in some tubules is desquamated, and necrosis of some of them infiltrates inflammatory cells, disturbance in the diameters of the proximal and distal twisted tubules, and necrosis of the glomerulus (Figure 6). Also, dense carbohydrate mucus material was observed in the tubule cavities, infiltration of inflammatory cells and condensation of some nuclei of the urinary tubules (Figure 7), fibrosis of the blood vessel and glomerulus, necrosis and expansion of some tubules, congestion of blood vessels, hyperplasia of the glomerulus and hydropic degeneration of the urinary tubules, as well as glomerular atrophy and Bowman's capsule, was expanding (Figure 8).

When treating with MSG with 10 g/kg, congestion and hemorrhage in the pulp area were observed, infiltration of inflammatory cells, degeneration of some urinary tubules (Figure 9), congestion in the glomerulus and necrosis, congestion in the blood vessels, infiltration of inflammatory cells, deformation of the glomerulus with hydropic degeneration in some tubules and necrosis in others, and hyperplasia of fibroblasts in the interstitial material (Figure 10) the presence of mucous material at the tops of the epithelium of the urinary tubules, congestion of blood vessels (Figure 11), congestion in the blood vessels and glomeruli, degeneration in some tubules and a few fibrous deposits between the tubules (Figure 12).

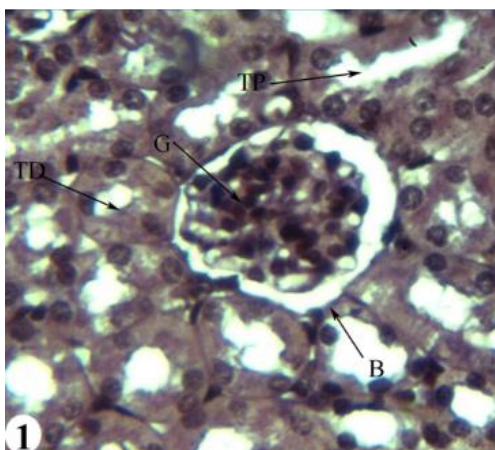


Figure 1: Normal structure of the kidney in the pregnant rat. PAS stain, 400X. (B) Bowman's capsule; (G) glomerulus; (TD) distal convoluted tubules; (TP) proximal convoluted tubules.

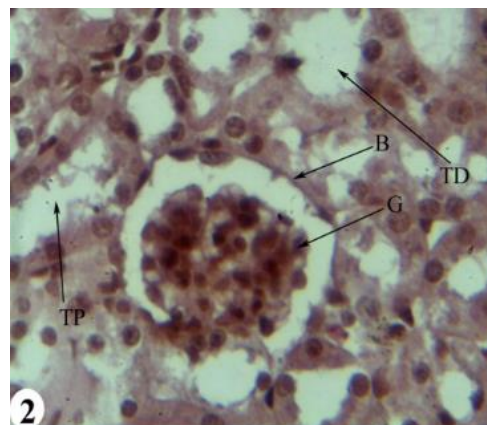


Figure 2: Normal structure of the kidney in the pregnant rat. H+E stain, 400X. (B) Bowman's capsule; (G) glomerulus; (TD) distal convoluted tubules; (TP) proximal convoluted tubules.

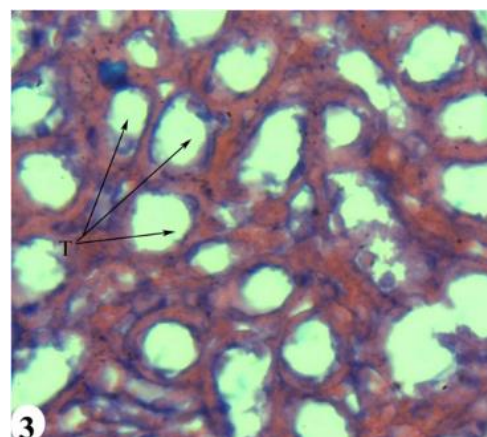


Figure 3: Normal structure of the kidney in the pregnant rat. AB stain, 400X (T) urinary tubules.

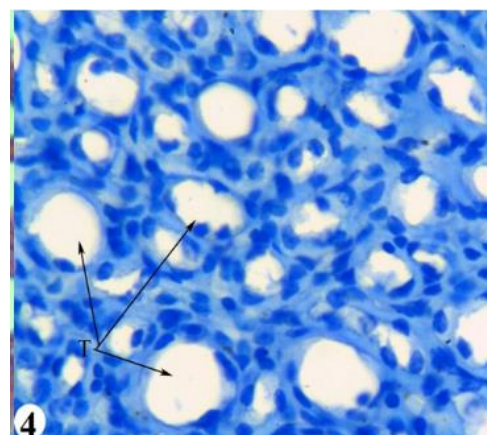


Figure 4: Normal structure of the kidney in the pregnant rat. TB stain, 400X. (T) urinary tubules.

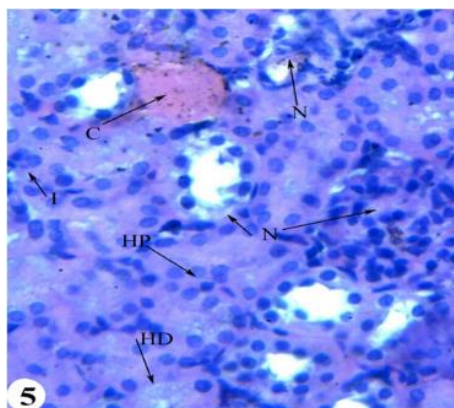


Figure 5: Histological structure of the kidney after being treated with NaNO₂. H+E stain, 400X. (I) Infiltration; (HP) hyperplasia; (HD) hydropic degeneration; (N) Necrosis; (C) congestion.

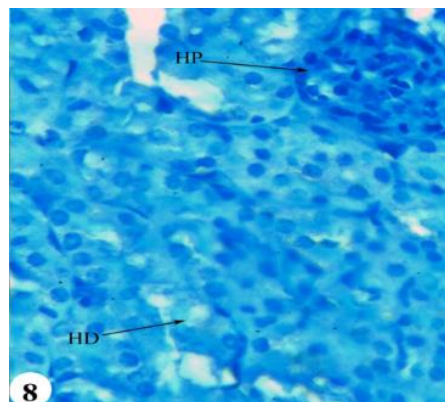


Figure 8: Histological structure of the kidney after being treated with NaNO₂. TB stain, 400X. (HP) hyperplasia; (HD) hydropic degeneration.

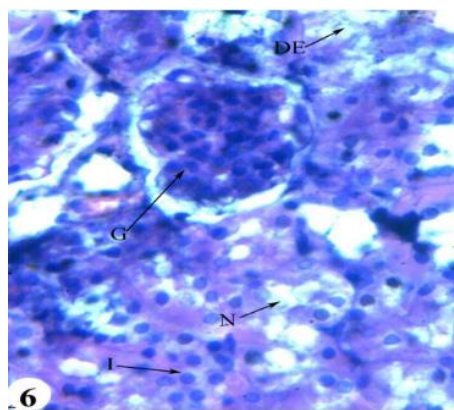


Figure 6: Histological structure of the kidney after being treated with NaNO₂. H+E stain, 400X. (G) glomerulus; (I) Infiltration; (N) Necrosis; (DE) desquamation.

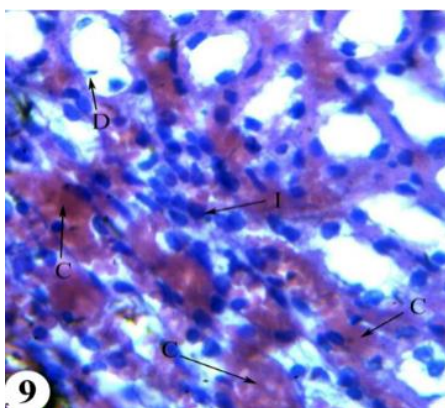


Figure 9: Histological structure of the kidney after treatment with MSG. H+E stain, 400X. (C) congestion; (D) degeneration; (I) Infiltration.

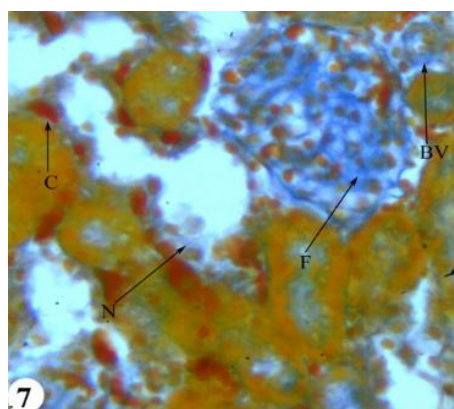


Figure 7: Histological structure of the kidney after being treated with NaNO₂. TS stain, 400X. (C) congestion; (BV) blood vessels; (F) fibrosis; (N) Necrosis.

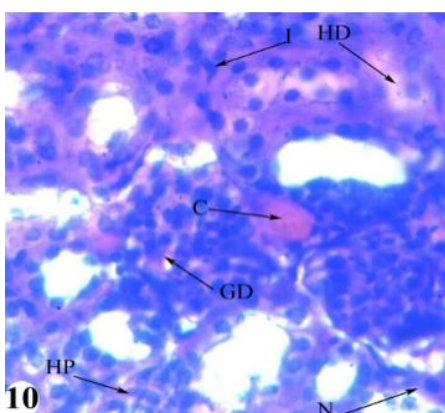


Figure 10: Histological structure of the kidney after treatment with MSG. H+E stain, 400X. (C) congestion; (N) Necrosis; (HP) hyperplasia; (HD) hydropic degeneration; (I) Infiltration; (GD) glomerulus deformation.

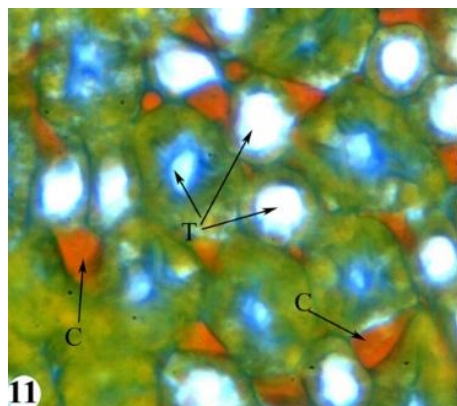


Figure 11: Histological structure of the kidney after treatment with MSG. TS stain, 400X. (C) congestion; (T) urinary tubules.

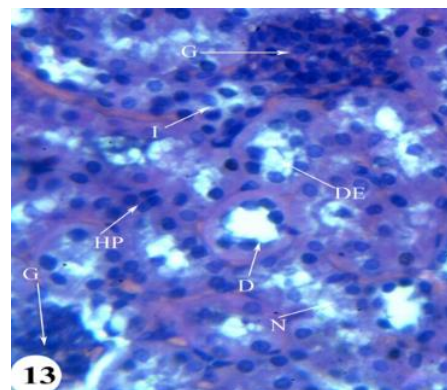


Figure 13: Histological structure of the kidney after being treated with NaNO₂ and MSG. H+E stain, 400X. (G) glomerulus; (I) Infiltration; (HP) hyperplasia; (D) degeneration; (N) Necrosis; (DE) desquamation.

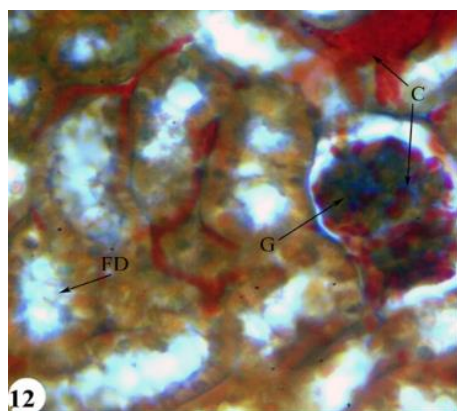


Figure 12: Histological structure of the kidney after treatment with MSG. TS stain, 400X. (C) congestion; (G) glomerulus; (FD) fibrous deposits.

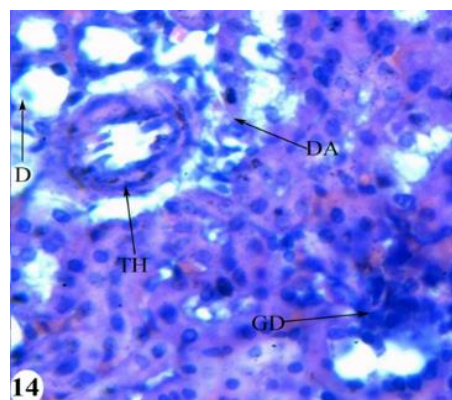


Figure 14: Histological structure of the kidney after being treated with NaNO₂ and MSG. H+E stain, 400X. (D) degeneration; (DA) damage; (TH) Thickness; (GD) glomerulus deformation.

As for treatment with the overlap of both substances together, histopathological and chemical histopathological effects were observed in the kidney of pregnant rats, as degeneration of some tubules and necrosis in some of them were seen, infiltration of inflammatory cells, hyperplasia of the glomerulus, reduction of Bowman's space, an increase in acidity of the cytoplasm of epithelial cells of the tubules, hyperplasia of the fibroblasts of the interstitial material and the desquamation of some tubules (Figure 13), damage to the glomerulus and the presence of its remnants, damage to some tubules and degeneration of some of them (Figure 14) and hydropic degeneration, hyperplasia and deformation of the glomeruli, reduction of Bowman's space, deposits of amyloid between the tubules (Figure 15). Perivascular fibrosis and congestion in the vessels, as well as interstitial hyperplasia (Figure 16).

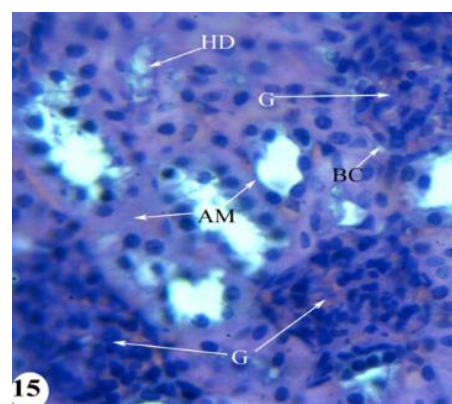


Figure 15: Histological structure of the kidney after being treated with NaNO₂ and MSG. H+E stain, 400X. (G) glomerulus; (HD) hydropic degeneration; (BC) Bowman's capsule; (AM) deposits of amyloid.

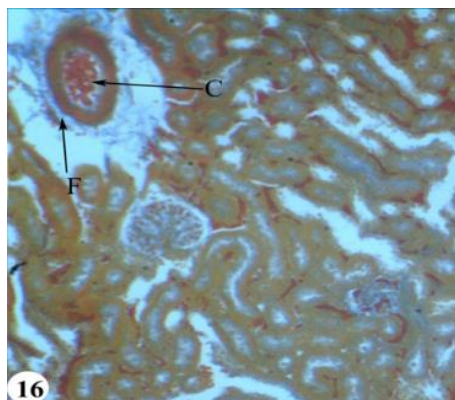


Figure 16: Histological structure of the kidney after being treated with NaNO₂ and MSG. TS stain, 400X. (C) congestion; (F) fibrosis.

Discussion

When pregnant rats were treated with NaNO₂ 115 mg/kg, many histopathological lesions appeared as interstitial tissue hyperplasia, necrosis, infiltration of inflammatory cells, congestion of blood vessels, and other histopathological changes. Moreover, when treating MSG with 10 g/kg, congestion, hemorrhage in the pulp area, infiltration of inflammatory cells, degeneration of some urinary tubules, and more histological effects were observed. It found that continuous administration of MSG increases the carboxylic acid cycle and thus stimulates ROS production. Thus, oxidative stress occurs in the kidneys of mice (19-21). MSG causes changes in the renal structure, such as hyperplasia of glomeruli cells and degeneration of their renal tubules, and infiltration of inflammatory cells in their renal cortex (22-25). Glomeruli shrinkage, degeneration of the urinary tubules, increased Bowman's space, congestion and rupture of blood vessels, hemorrhage, and necrosis, as well as the disappearance of glomeruli in some areas (26-28). also, damage to the kidneys' glomeruli, as MSG causes toxic effects on the renal cortex, shrinkage of the renal glomeruli, and thickness of the urinary tubules (29-32).

When treating the two substances together, histopathological effects were observed in the kidney of pregnant rats, as degeneration, necrosis, infiltration of inflammatory cells, hyperplasia of the glomerulus, and multiple histological lesions. Studies have shown that daily consumption of a mixture of MSG and NaNO₂ led to increased creatinine levels in the blood and urea. It is believed that the cause of the increase is closely related to some kidney functions, as the increased concentration of urea and creatinine in the blood causes disease, or kidney damage may be due to the high effectiveness of Xanthine oxide. Threshold changes and impaired tubular absorption, renal blood flow, and glomerular filtration rate occur

(3,33,34). The results are consistent with what was shown in a similar study by Ateya *et al.* (35-37). Administering a mixture of these two substances damaged the kidneys and caused damage to their function. It is responsible for removing toxins and foreign compounds in the body. The study by Helal *et al.* (2) that daily intake of the combination of two substances showed an increase in urea and creatinine concentration. There were noted that preservatives cause histopathological changes in the cell lining of convoluted tubules and renal corpuscles (38-40).

Conclusions

The current study found that food additives cause many tissue lesions in the kidney and that eating some food additives such as NaNO₂ and MSG with pregnant women's food above the permissible limit may lead to tissue damage in the fetus's kidney, which affects the health of the newborn in the future. Therefore, this study recommends the necessity of following health instructions by pregnant women to maintain their health first and their fetus second.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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حوامل، صنفت إلى أربع مجموعات، الأولى مجموعة السيطرة، والثانية عوملت بكلوتومات أحادي الصوديوم ١٠غم/كغم، والثالثة حقنت بنتريت الصوديوم ١١٥ ملغم/كغم، والرابعة للتداخل بين المادتين ولنفس التراكيز. بينت النتائج حدوث العديد من الأفات النسجية في الكلية جردان المجاميع التجريبية. ففي المجموعة الثانية، شملت تضخم النسيج الخلالي ونخر الكبيبات، وتسلسل الخلايا الالتهابية، واحتقان الأوعية الدموية، وتنكس استسقائي في بعض الأنابيب، ونخر بعضها. وفي المجموعة الثالثة، تضمنت احتقان، نزيف في منطقة اللب، تنكس بعض الأنابيب البولية، نخر، وتشوه في الكبيبات. بينما في المجموعة الرابعة، شوهد تنكس في بعض الأنابيب ونخر في بعضها، تضخم في الكبيبات، انخفاض مساحة بومان، زيادة حموضة سايتوبلازم الخلايا الظهارية للكبيبات، تضخم في الخلايا الليفية، وتؤسف بعض الأنابيب. واستنتجت الدراسة ان لهذه المواد اثار ضارة على الكلية في الجردان الحوامل خاصة عند التداخل بينهما. لذا يجب تجنب هذه المواد فترة الحمل للمحافظة على سلامة الكلية.

التأثيرات النسيجية لتداخل بعض المضافات الغذائية في كلية الجردان الحوامل

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الخلاصة

لا تزال المضافات الغذائية مثار للعديد من الدراسات للتعرف على اثارها الايجابية والسلبية خاصة وهي تستخدم على نطاق واسع على الصعيد العالمي. لذا هدفت هذه الدراسة التعرف على تأثير نترتيت الصوديوم وكلوتومات احادي الصوديوم على التركيب النسجي للجردان الحوامل. ولتحقيق هدف الدراسة، استخدمت أربع وعشرون من جردان