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Histopathological changes on the pregnant rat's lung induced by sodium nitrite and monosodium glutamate

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Abstract

Food additives and preservatives are widely used globally, which, despite their many benefits, have great harm if they are used without health restrictions or control, as they cause many health problems and tissue lesions. Therefore, the present study aimed to investigate the histopathological effects on the lung of pregnant rats of two types of these substances: Monosodium glutamate (MSG) and Sodium nitrite ($NaNO_2$). Twenty-four pregnant rats used for this study, and they were divided into four groups equally. The control group was dosed with distilled water from the sixth day to the fifteenth day of pregnancy. The second was dosed with MSG at a 12g /kg concentration for the same period in the first group. The third injected with a concentration of 120 mg/kg of $NaNO_2$ for the same period. The fourth was dosed with MSG and $NaNO_2$ together, with the same concentrations and the above period. The results showed that the second group's lungs showed many histopathological changes, including strong infiltration of inflammatory cells, congestion of blood vessels, necrosis of bronchioles and alveolar septa, and emphysema of some alveoli. In the third group, changes included hyperplasia of the fibroblasts, hemorrhage in the alveoli, desquamation and necrosis in bronchioles, peri-bronchial fibrosis, blood vessel congestion. The fourth group showed infiltration of inflammatory cells, necrosis in multiple lung areas, emphysema, fibrosis in some alveoli, and hypoplasia of the muscle fibers around the blood vessels. The study concluded that MSG and $NaNO_2$ caused much tissue damage in the lungs of pregnant rats.

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Introduction

Food additives are defined as "natural or synthetic chemicals that add to food and affect its qualities and used in the food industry widely to increase the life of the products and the characteristic as well as to enhance certain properties in foods including preservation, coloring, and sweetening" and with this has negative effects, as some of them have been banned due to their toxic effects (1). One of the most widely used food additives globally is Monosodium Glutamate MSG, a white crystalline powder (2). It is a flavor enhancer, and is a commercially prepared food. MSG is used to prepare canned and dried soups, dried foods, cured meats, fruits, and vegetables, as they consume large quantities of this substance, approximately 67.5 million kg/year (2). It is added to food products that protein-rich as in certain types of cheese, red meat and fish or vegetables such as tomatoes, and broccoli or in mushrooms, and is the fifth basic taste as well as sweet, sour, salty, and bitter (3). Previous studies indicated the harmful toxic effect of MSG on humans and laboratory animals, when ingested in large quantities, clinical symptoms, such as numbness, sweating, and headache, were reported. Other studies confirm that taking MSG causes aggravating disease conditions such as ventricular arrhythmia, abdominal pain, and neuropathy. Receptors of the glutamate and glutamate themselves play an important role in many neurological and eye diseases, such as trauma, ischemia, diabetic retinopathy, bruising, and glaucoma (4). On the other hand, there are special food preservative

additives in many products Meat, most notably nitrates and nitrites, contributes to the final product's flavor and inhibits the bacteria *Clostridium botulinum* that produces toxins that cause poisoning. It effectively controls the rancidity caused by stimulating lipid oxidation to ensure excellent storage and as a color stabilizer to maintain processed meats' fresh taste and appearance (5). Sodium nitrite $NaNO_2$ is a powder of crystalline that has yellowish-white color that slightly has unique synthetic and pharmacological properties, that used as medicines agent in human as well as veterinary, as a vasodilator and antagonist to protect against hypoxia, and as an antimicrobial agent and preservative to prevent microbial contamination and is sold only in a mixture with table salt NaCl known as E250 or as a salt substitute (6). Studies have assumed that $NaNO_2$ is one of the potential risk factors for colorectal cancer. It increases esophageal cancer in the case of exposure for long-term to it. Whereas in other studies have shown, in body tissues, there were no carcinogenic influence of $NaNO_2$ alone, but while associated with antioxidants, the promoters act in primary tissues (7). Therefore, this study came to identify the effect of MSG as a substance enhancer for flavor and $NaNO_2$ as a preservative, each separately and their interactions together on the histological structure of the lung in pregnant rats in the period of organogenesis and at specific concentrations determined according to the average lethal dose LD_{50}.

Materials and methods

Study animals

Albino rat *Rattus norvegicus* aged 11-12 weeks was conducted for this study, whose average weight ranged from 225-235 g. They were placed in the laboratory under special conditions of the light period, temperature, and ventilation, and they were given water and food daily continuously (8).

Doses of the study

Dose concentrations were selected based on the median lethal dose 50 (LD_{50}) (9), for MSG (10), and $NaNO_2$ (11), which are: 12 g/kg body weight for MSG, and the dose administered in 0.5 ml of distilled water through mouth by gavage needle, and 120 mg/kg body weight for $NaNO_2$, in 0.2 ml of distilled water by injection intraperitoneal.

The mating

After being examined, the rats were selected to ensure that they were free of any disease and in good health. Females ready for fertilization were placed at a rate "one male with three females" in each cage during the night hours, and fertilization was confirmed mating, and pregnancy confirmed by making a vaginal swab to investigate the presence of sperm, as the day that was observed was counted as the day zero of pregnancy and the next day is the first day of pregnancy (12).

Experimental design

The pregnant females were isolated in separate plastic cages, as the date of fertilization was placed on them, and were dosed and injected with MSG, $NaNO_2$ daily from the sixth day of pregnancy, the stage of organogenesis, to the fifteenth day of pregnancy. The rats were divided into four subgroups, six rats in each group, the control group and dosed with distilled water; the second group was dosed with MSG; the third group was injected with $NaNO_2$; while the fourth group was dosed with MSG and $NaNO_2$, respectively.

Histological preparation

The rats were sacrificed by anesthesia, and rats were dissected for the control group, and experimental groups with chloroform were left until the rat stopped moving, then the lung was removed (13). The histological sections were prepared by passing a series of steps, according to Suvarna *et al*. (13), fixation with 10% neutral formalin for 36 hours (14). They were washing with running water for 30 minutes (15). Dehydration with an ascending chain of ethyl alcohol at concentrations of 50, 70, 90, and 100% for 30 minutes/concentration except for the last concentration for two hours (16). Clearing with xylene for 30 minutes, Infiltration of samples and embedding with paraffin wax (13). Trimming and sectioning through a rotary microtome with a thickness of 6 micrometers (15). Deparaffinization of the tissue sections was done through a Coplin jar containing hot xylol in the oven.

Staining process

The sections of the lung colored with the following stains: Delafield's Haematoxylin and Eosin stain "HE" (14). Mallory's Triple stain "TS" (15). Alcian Blue stain pH2.5 "AB", and Toluidin Blue stain "TB" (16). Mounting The sections were loaded with (DPX), then the histological sections were examined and photographed with a combined optical microscope using a digital camera (17).

Results

The results showed that lung tissue in the control group consisted of primary, secondary and tertiary bronchioles, terminal bronchioles, respiratory bronchioles, alveolar ducts, alveoli, and interalveolar septa (Figure 1a and 1b). The rest of the groups showed the occurrence of pathological and chemical histopathological lesions of the lung of pregnant rats compared to the control group. In the second group treated with MSG, strong infiltration was observed in inflammatory cells, hypertrophy in the blood vessel endothelial cell, necrosis in the bronchioles and septa between the alveoli, and hemorrhage (Figure 2a). There is congestion in the blood vessel, hemorrhage, hyperplasia of the septa between the alveoli and emphysema (Figure 2b). Also, desquamation in bronchioles, emphysema in some alveoli, fibroid deposits in the septa between the alveoli,

necrosis of some bronchioles (Figure 2c). Fibrosis around bronchioles in the septa between the alveoli and around the blood vessels, extensive fibroid deposits between blood vessels and bronchioles, and vascular congestion (Figure 2d) also observed moderate reaction with AB stain (Figure 2e).

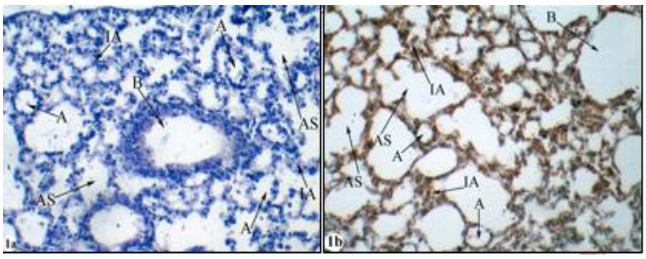

Figure 1: Normal histological structure of the lung in the pregnant rat. (a): TB stain, 400X. (b): TS stain, 400X.

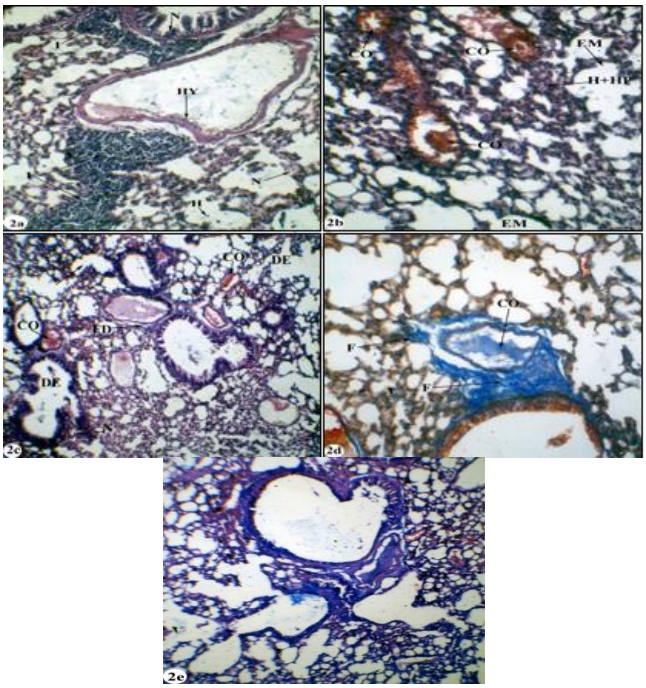

Figure 2: Histological structure of the lung in the pregnant rat treated with MSG. (a): HE stain, 400X. (b): HE stain, 400X. (c): HE stain, 400X. (d): TS stain, 400X. (e). AB stain, 400X. Abbreviations: (A) alveoli; (AS) alveolar sac; (B) bronchioles; (CO) Congestion; (DE) desquamation; (EM) Emphysema; (F) Fibrosis; (FD) Fibroid Deposits; (IA) Inter alveolar tissue; (I) Infiltration; (H) Hemorrhage; (HP) hyperplasia; (HY) hypertrophy in the blood vessel endothelial cell; (N) Necrosis.

In the third group, treated with $NaNO_2$, hemorrhage was seen in the alveoli, between the alveoli, and in the bronchioles (Figure 3a), hyperplasia fibroblasts, the vessel wall, and the septa between the alveoli, necrosis of some septa (Figure 3b). Also, necrosis and desquamation of the trachea, hemorrhage, and infiltration were observed in inflammatory cells (Figure 3c). fibrosis around the bronchioles, blood vessels, and between the alveoli (Figure 3d). Necrosis of the bronchioles and septa between the alveoli. Strong infiltration in inflammatory cells and hyperplasia of some alveolar septa (Figure 3e and 3f).

In the fourth group, treatment with the interaction of both MSG and $NaNO_2$, histopathological and histochemical effects of the lung of pregnant rats were observed, as strong infiltration was observed in inflammatory cells, hyperplasia of some of the tissue of the bronchioles wall, necrosis of some bronchioles and their lining, in the septa between the alveoli and of the wall blood vessels, and fibroid deposits in the interalveolar (Figure 4a). Also, hyperplasia of the muscle fibers around the blood vessels and in the fibroblasts, blood vessel congestion and infiltration of inflammatory cells (Figure 4b), strong infiltration of inflammatory cells, vasodilation, emphysema of some alveoli, hyperplasia of the septa between the alveoli, fibroid deposits in Septa, vascular congestion (Figure 4c), peri-bronchial fibrosis, and alveolitis (Figure 4d) and emphysema (Figure 4e).

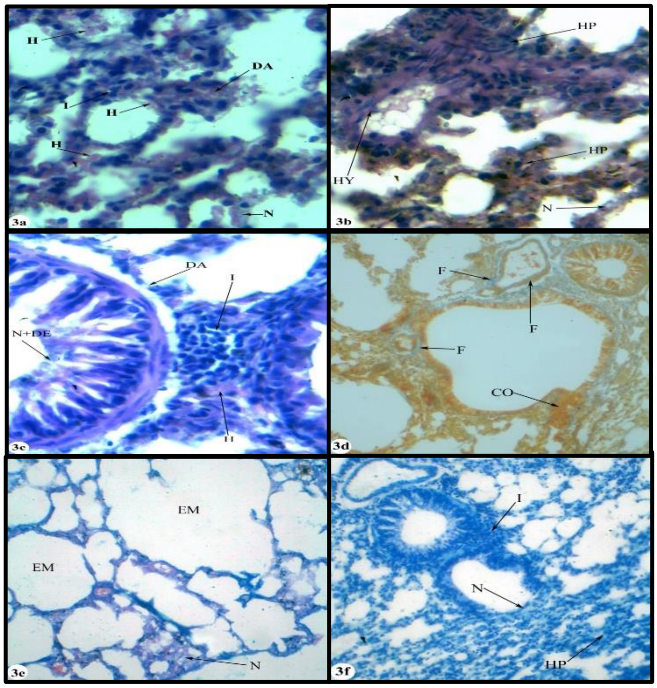

Figure 3: Histological structure of the lung in the pregnant rat treated with $NaNO_2$. (a): HE stain 400X. (b): HE stain 400X. (c): HE stain, 400X. (d): TS stain, 400X. (e): AB stain, 400X. (f): TB stain, 400X. Abbreviations: (CO) Congestion; (DA) damage; (DE) desquamation; (EM) Emphysema; (F) Fibrosis; (FD) Fibroid Deposits; (I) Infiltration; (H) Hemorrhage; (HP) hyperplasia ;(HY) hypertrophy in the blood vessel endothelial cell; (N) Necrosis.

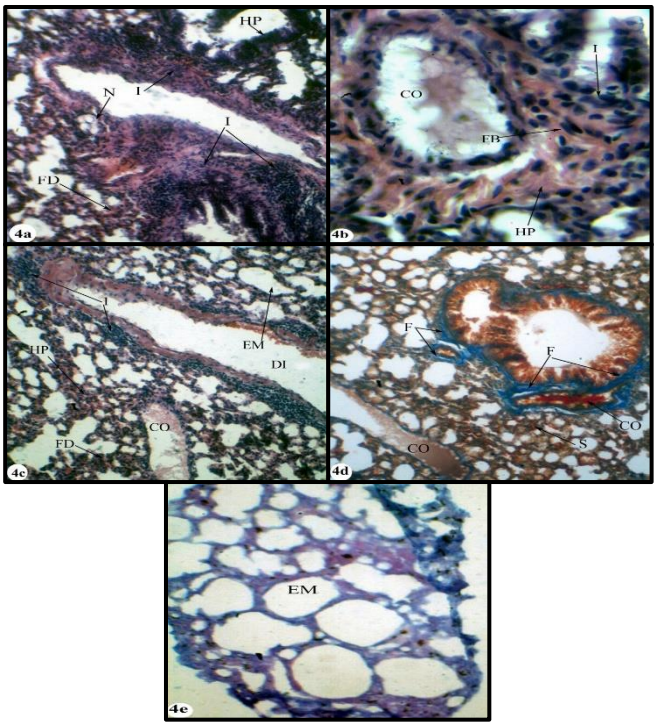

Figure 4: Histological structure of the lung in the pregnant rat treated with MSG and $NaNO_2$. (a): HE stain, 400X. (b): HE stain, 400X. (c): HE stain, 400X. (d): TS stain, 400X. (e): AB stain, 400X. Abbreviations: (CO) Congestion; (DA) damage; (DE) desquamation; (DI) Dilatation; (EM) Emphysema; (F) Fibrosis; (FB) fibroblasts; (FD) Fibroid Deposits; (I) Infiltration; (H) Hemorrhage; (HP) hyperplasia; (HY) hypertrophy in the blood vessel endothelial cell; (N) Necrosis; (S) Slipped.

Discussion

The exposure to MSG, many histopathological changes observed including, strong infiltration in inflammatory cells, hypertrophy of endothelial cell, necrosis, hemorrhage, congestion, hyperplasia and emphysema. Also, desquamation in bronchioles, fibroid deposits, Fibrosis around bronchioles, and moderate reaction with AB stain. Multiple studies have reported (18,19) on the histopathological effect in different body organs such as the lung, liver, and kidneys on the harmful effect of MSG (19). In the study of Hatziefthimiou *et al.* (18) regarding the effect of MSG on the lung, it was found that it could be a trigger for asthma attacks and that intense exposure to glutamate causes toxicity to the lung because the assumptions for activating the excitatory amino acid receptors are mainly of the type N-methyl-D-aspartate NMDA contributes to a variety of pathologies of lung lesions such as acute lesions, pulmonary edema, obstructive tissue injury, airway hyper responsiveness, and inflammation.

In another study conducted Sakr (19) on mice after treating their mothers during pregnancy with MSG, it led to the collapse of the alveoli due to thickening of the airways and narrowing in the pulmonary airways, and a large increase in collagen inside the septa was seen. Also, a study was conducted on the effect of MSG on the fetus's lung after exposure of the pregnant. Changes were observed in the lung of the offspring as the primitive bronchial cavity appeared (20). The cells lined up with each other with a marked disintegration between them and thickened in the nucleus, necrosis of cells, and the epithelium's lack of growth in different parts of the bronchioles (18). Immuno reactive increased positively due to cell nuclear antigens' proliferation in epithelium and stroma of the bronchial (20).

Also, treated with $NaNO_2$, the histological lesions included hemorrhage in the and between the alveoli, hyperplasia of fibroblasts, necrosis of some septa, desquamation of the trachea, and infiltration were observed in inflammatory cells. fibrosis around the bronchioles, blood vessels, and between the alveoli. $NaNO_2$ has very harmful effects due to the increased oxidation it can have harmful to different organs (21). Reactive nitrogen species have many toxicities, including those produced by exposure to nitrite, including Hepatotoxicity, Nephrotoxicity, disorganization of inflammatory responses, and tissue injury (22). Exposure to nitrite, which is a type of reactive nitrogen of the most Causes leading to cancer through Its interaction with different tissues of the body and the stimulation of fats Peroxide, enzyme inactivation, DNA lesions, and various organ damage (21).

Regarding the lung, it founded that experimental animals, when inhaled with vapors of fluids containing $NaNO_2$, cause oxidative stress and lung injury, as well as an increase in neutrophilic white blood cells and an expansion of the barriers between the alveoli in the lungs, and the damage increased with repeated inhalation (22). Bronchiolar wall necrosis and pulmonary edema due to pulmonary artery blood pressure were observed by Ferguson *et al.* (23). Lung histopathological changes were also observed in other studies, including bronchiectasis, infiltration of mononuclear cells around the bronchioles and parenchyma, hyperplasia of the epithelium the bronchi, and desquamation, and pneumocytes cell enlargement was observed in the septa between the alveoli (24).

In the fourth group, treatment with the interaction of both MSG and $NaNO_2$, histopathological and histochemical effects of the lung of pregnant rats that were observed, strong infiltration, hyperplasia, necrosis and fibroid deposits in the interalveolar. Also, hyperplasia of the muscle fibers around the blood vessels and in the fibroblasts, blood vessel congestion, vasodilation, and emphysema. These results confirm the occurrence of synergism between MSG and $NaNO_2$, which caused extensive damage to lung tissue more than the effect of each of them separately due to the free radicals produced by these substances (25). Histopathological changes in lung

tissue similar to these results have emerged when these two substances are used together in adult mice by AlThanoon (25), confirming the two substances' synergy to cause histopathological lesions. It was found that there is a change of liver function due to MSG and / or $NaNO_2$, through liver damage caused by Chemical absorption of these compounds. This may be evidence of reduced function of the liver, which in turn leads to enhanced liver function Fluids in tissue spaces.

Conclusions

Food additives, especially MSG and $NaNO_2$, have harmful effects on various body organs, including the lung's tissue structure, when added above the permissible limit. Therefore, the World Health Organization recommendations must be followed when using these materials, especially for pregnant women. Also, this study concluded that the $NaNO_2$ was more harmful than MSG in causing histopathological lesions, and therefore caution must be taken when using this substance in preserving foods.

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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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التغيرات المرضية النسيجية في رئة الجرذان الحوامل الناجمة عن نتريت الصوديوم وكلوتامات أحادي الصوديوم

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الخلاصة

تُستخدم المضافات الغذائية والمواد الحافظة على نطاق واسع على مستوى العالم، والتي بالرغم من فوائدها العديدة، إلا أن لها ضررا كبيرا إذا تم استخدامها دون قيود أو ضوابط صحية، لأنها تسبب العديد من المشاكل الصحية والآفات النسجية. لذلك هدفت الدراسة الحالية إلى التعرف على التأثيرات المرضية النسيجية على رئة الجرذان الحوامل لنوعين من هذه المواد: كلوتامات أحادي الصوديوم ونتريت الصوديوم. تم استخدام أربعة وعشرين فأر حامل في هذه الدراسة، وتم تقسيمها إلى أربع مجموعات بالتساوي. تم تجريع مجموعة السيطرة بالماء المقطر من اليوم السادس إلى اليوم الخامس عشر من الحمل. وتم تجريع المجموعة الثانية بكلوتامات أحادي الصوديوم بتركيز ١٢ غم/كغم لنفس الفترة. وتم حقن المجموعة الثالثة بتركيز ١٢٠ ملغم/كغم من نتريت الصوديوم لنفس الفترة. وتم تجريع وحقن المجموعة الرابعة بكلوتامات أحادي الصوديوم ونتريت الصوديوم معا، بنفس التركيزات والفترة المذكورة أعلاه. أظهرت النتائج أن رئة المجموعة الثانية أظهرت العديد من التغيرات المرضية النسيجية، بما في ذلك ارتشاح قوي للخلايا الالتهابية، واحتقان الأوعية الدموية، ونخر في القصيبات والحواجز السنخية، وانتفاخ في بعض الحويصلات. في المجموعة الثالثة، شملت التغييرات تضخم الخلايا الليفية، ونزيف في الحويصلات الهوائية، وتقشر ونخر في القصيبات، والتليف حول القصبات، واحتقان الأوعية الدموية. المجموعة الرابعة أظهرت ارتشاح الخلايا الالتهابية والنخر في مناطق متعددة من الرئة وانتفاخ والتليف في بعض الحويصلات الهوائية ونقص تنسج ألياف العضلات حول الأوعية الدموية. واستنتجت الدراسة إلى أن كلوتامات أحادي الصوديوم ونتريت الصوديوم تسببان في تلف أنسجة الرئة للجرذان الحوامل.