Study of Some Fetal Parameters and Skeletal Distortions in Fetuses and Neonates of Pregnant Albino Rats Treated with the Preservative Sodium Nitrite

Zainab Majed Mohamad AL-Rubaie[1], Dalal Abdel-Hussein Kadhem AL–Essawi[2]
[1,2] Department of Biology, Faculty of Education for Girls, University of Kufa, AL-Najaf AL-Ashrif Iraq
Email: Zianbmajad@gmail.com

Abstract

This experiment was carried out in the animal house of the College of Science at the University of Kufa from 15/11/2021 to 15/5/2022 for the purpose of studying the harmful effects of the preservative sodium nitrite on some fetal parameters during 16 days and 20 day of pregnancy and skeletal distortions in fetuses for a pregnancy two periods of 16 days and 20 days and neonates after the birth respectively of pregnant female rats that were treated orally with sodium nitrite from the first day of pregnancy by a rats administeration device and the administeration was daily as a single dose , (45) pregnant female albino rats of the type Rattns rattus (Sprague Dawley)with weights were between (200 – 230) g and ages between (11-12) weeks. 45 pregnant rats were divided into3 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 given sodium nitrite at a concentration of 10 mg/kg of body weight, while the third group was given sodium nitrite at a concentration of 15 mg/kg from body weight, each group of the main groups was divided into 3 sub-groups each of which included 5 pregnant rats, pregnant female rats were administered in all groups from the first day of pregnancy with a single dose daily by gastric administeration device, the pregnant rats were dissected on the 16th and 20th days of pregnancy and the numbers of total, live, absorbed and dead fetuses were calculated, the bodies of the fetuses were also cleared for 16 days and 20 days of pregnancy and the neonates after birth to show their skeletons. The results of the study showed that the number of total and live fetuses decreased significantly (P≤ 0.05), while the numbers of absorbed and dead fetuses increased significantly (P≤ 0.05)in the groups of pregnant female rats treated with sodium nitrite at two concentrations (10, 15 mg/kg body weight) respectively during pregnancy for 16 days and 20 days compared with the numbers of total, live, absorbed and dead fetuses in the control group during 16 days and 20 days of pregnancy and there were significant differences (P≤ 0.05)in the number of total, live, absorbed and dead fetuses between the two groups treated with sodium nitrite when comparing between the two groups. The results also showed the occurrence of various skeletal deformations in fetuses such as loss of caudal vertebrae, deformations of skull bones and loss of a number of phalanges in fetuses for 16 and 20 days respectively , the neonates also suffered from atrophy in the skeleton causing short body length, loss of some caudal vertebrae and deformities in the pelvic bones, skull, and phalanges of phalanges of the upper and lower extremities in groups of pregnant rats treated with sodium nitrite at a concentration of 10 mg / kg of body weight and these skeletal abnormalities were increased in fetuses during 16 days and 20 days of pregnancy and neonates after birth of pregnant rats treated with sodium nitrite at a concentration of 15 mg/kg body weight compared to the skeletons of fetuses during (16 ,20) days of pregnancy and neonates after birth respectively. Conclusion: We can conclude from the current study that the preservative sodium nitrite stimulated harmful effects on fetal parameters during pregnancy for a period of 16 days and 20 days by increasing the number of absorbed and dead fetuses and reducing the number of total and live fetuses, as well as these preservative stimulated various structural abnormalities in fetuses during pregnancy for 16 days and 20 days and in neonates after the birth and these pathological effects were increased by the increase in the concentration of sodium nitrite and the duration of the administration.

1. 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, 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 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 [1], and one of these preservatives is sodium nitrite ($NaNO_2$), which is an inorganic sodium salt and its international code is 250E [2].

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

doi.org/10.31838/hiv22.02.317 Received: 14.06.22, Revised: 15.06.22, Accepted: 20.08.22

materials made meat products of high quality products with a longer shelf life [3], female rats [4].

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.

The research experience design.

The current study experiment was designed for the purpose of studying the negative effects on some fetal and birth skeletal abnormalities and parameters in pregnant white female rats treated with the preservative sodium nitrite during the pregnancy two stage s(16,20) days and after birth, (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 species and after obtaining 45 pregnant female rats which were divided into three main groups and each group contained 15 pregnant female rats:

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, the first five of pregnant female rats from each of the three main groups were dissected during (16,20) days of pregnancy respectively , while the remaining five of pregnant rats were left to the birth after administered them orally by the administeration device of rats from the first day of pregnancy.

Anatomy of pregnant rats during (16, 20) days of pregnancy

Pregnant female rats were dissected on the 16th and 20th days of pregnancy after being anesthetized with diethyl ether, then the animals were fixed in a dissection dish and the abdominal cavity was opened of them, the fetuses in the uterine horns were examined to identify the number of live and dead fetuses, then the uterine horns were extracted from the bodies of pregnant rats and opened with sharp scissors to calculate the total number of fetuses and numbersof fetuses in each horn, as well as the number of the developed and absorbes fetuses.

Study of the skeletons of fetuses and neonates

Preperation of Potassium hydroxide Solution.

A Potassium hydroxide solution was prepared by mixing 1 g of potassium hydroxide in 100 ml of distilled water.

-Preparation of alizarinred-s-stain Solution.

Alizarin red stain solution was prepared by adding 2 gm of stain powder to 200 ml of distilled water, and then adding 50 ml acetone and 50 ml of xylene.

Clearing the bodies of fetuses and neonates

The bodies of fetuses for a pregnancy period of 16 days , 20 days and neonates after birth were cleared according to the method of McLeod [5].

Examination of bone skeleton

The skeletons of fetuses during (16,20) days of pregnancy and neonates after birth were examined using a disecting microscope at the magnification power of (5x) on all study groups.

Bone Skeletal imaging

The skeletons of the fetuses during 16 days and 20 days of pregnancy and the neonates after birth were photographed using a camera of the same type and using a color film (Konica) for this purpose.

2. -Statistical Analysis

The results of the study were statistically analyzed using the Package Social Sciences Statistical (SPSS) system version (21), the values represented the mean and the standard error using the F-test (F-Test), and the Least Significant Difference (L.S.D) under a significant level P≤ 0.05was used to find the significant differences of the fetal parameters in this study.

3. Results and Discussion

Effect of sodium nitrite on the numbers of total, live, absorbed and dead fetuses during 20 days of pregnancy.

The statistical results indicated that there was a significant increase P(P≤ 0.05)in the numbers of absorbed and dead fetuses, while there was a significant decrease (P≤ 0.05)in the numbers of total and live fetuses in the groups of pregnant female rats treated with sodium nitrite at two concentrations (10, 15) mg/kg of body weight respectively during the two pregnancy period of 16 days 20 days respectively compared with the numbers of total, live, absorbed and dead fetuses in the control group during (16,20) days of pregnancy as shown in tables (1,2) respectively , the same table also shows that there are significant differences (P≤ 0.05)5in the numbers of total of total, live, absorbed and dead fetuses between the two groups treated with sodium nitrite when compared between them during the two pregnancy period of 16 days 20 days respectively, the reason for this result may be due to the fact that the preservative sodium nitrite has affected the implantation processes , growth and development of fetuses causing their adsorption and death and this is due to the role of sodium nitrite in the synthesis of free radicals in tissues causing cell destruction in uterine tissues of pregnant rats by oxidizing lipids in cell membranes and the destruction of intracellular molecules such as proteins, nucleic acids and others leading to decrease in the blood supply to the fetuses thus, the lack of nutrients and oxygen to them, in addition to this chemical substance stimulated the formation of free radicals in the embryos themselves as a result of their crossing from the placenta to the fetuses during the early stages of pregnancy causing oxidative stress in the tissues of the fetuses which caused a decrease in the implantation of fetuses and the adsorption of some implanted fetuses and their lack of development, in addition to the death of some advanced fetuses and their failure to reach advanced stages of fetal development

leading to a decrease in the number of total and live fetuses, while the number of absorbed and dead fetuses increased of treated female rats [6], or the reason for this may be attributed to the fact that sodium nitrite caused a decrease in the growth and formation of red blood cells when giving sodium nitrite in drinking water to pregnant rats and thus the lack of access of basic nutrients and other life necessities to the fetuses causing toxic effects on implantation and fetal development processes of the fetuses which increases the number of absorbed and dead fetuses, or this result can be due to that the preservative sodium nitrite caused methemoglobinemia which led to a reduction in the transfer of oxygen in the blood and other necessary substances from mothers to fetuses during pregnancy leading to cyanosis of fetuses or the so-called blue fetal syndrome due to hypoxia of the blood especially in high concentrations and this was shown in this study which dosed their mothers at a concentration of 15 mg / kg of sodium nitrite [7], or this result may be due to that giving the sodium nitrite at a concentration of (50, 25, 5, 5)mg / kg to pregnant female rats stimulated decreased fetal blood oxygenation which led to the lack of implantation of fetuses and the absorption of some of them and the lack of growth and development of fetuses and consequently their death and the lack of live developed fetuses [6].

Table (1): The effect of sodium nitrite on the numbers of total, live, absorbed and dead fetuses during 16 days of pregnancy.

Groups	Fetal parameters			
	Total fetuses' numbers	Live fetuses' numbers	Absorbed fetuses' numbers	Dead fetuses' numbers
Contral group (Normal saline 0.9%)	8.56± 0.001	8.50 ± 0.008	0.03 ± 0.001	0.03 ± 0.001
Treated group with sodium nitrite at a concentration of 10 mg/kg body weight	7.46 ± 0.005 a	2.05 ± 0.002 a	2.18 ± 0.003 a	3.31 ± 0.009 a
Treated group with sodium nitrite at a concentration of 15 mg/kg body weight	ab6.20 ± 0.006	2.00 ± 0.001 ab	2.08 ± 0.004 ab	2.20 ± 0.002 ab
Least Significant Difference (L.S.D)	0.011	0.133	0.031	0.010
Probability level	P≤ 0.05	P≤ 0.05	P≤ 0.05	P≤ 0.05

Values: Represent mean ± standard error Mean ± SE).
A: Represents a significant difference from the control group.
B: Represents a significant difference between the treated groups.

Table (2): The effect of sodium nitrite on the numbers of total, live, absorbed and dead fetuses during 20 days of pregnancy.

Fetal parameters				Groups
Dead fetuses' numbers	Absorbed fetuses' numbers	Live fetuses' numbers	Total fetuses' numbers	
0.13 ± 0.001	0.20 ± 0.001	8.53 ± 0.006	8.86± 0.005	Contral group (Normal saline 0.9%)
2.05 ± 0.015 a	3.00 ± 0.003 a	2.01 ± 0.051 a	7.06 ± 0.040 a	Treated group with sodium nitrite at a concentration of 10 mg/kg body weight
2.00 ± 0.012 ab	3.01 ± 0.007 ab	1.50 ± 0.033 ab	Ab 6.46 ± 0.032	Treated group with sodium nitrite at a concentration of 15 mg/kg body weight
0.0114	0.0119	0.258	0.0344	Least Significant Difference (L.S.D)
P≤ 0.05	P≤ 0.05	P≤ 0.05	P≤ 0.05	Probability level

Values: Represent mean ± standard error (Mean ± SE).
a: Represents a significant difference from the control group.
b: Represents a significant difference between the treated groups.

Study of skeletal distortions of fetuses during 20 days of pregnancy and neonates after birth.

No malformations were observed in the skeletons of the fetuses during (16, 20)days of pregnancy and the neonates after birth of pregnant female rats in the control groups and as shown in figures (1,5,9) respectively, but the results of the current study revealed that the skeletons of the fetuses during(16, 20) days of pregnancy and the neonates after the birth whose mothers were treated with two concentrations of (10 and 15) mg/kg body weight of sodium nitrite, as the skeletons of the fetuses showed various deformities such as loss of caudal vertebrae, deformation of the skull bones, loss of a number of phalanges of the limbs and deformities of the pelvic and shoulder bones in groups pregnant rats treated with sodium nitrite at a concentration of 10 mg / kg body weight as shown in the figures (2,3,6,7) respectively,and these malformations increased in the fetal skeletons in pregnant rats that were treated with a concentration of 15 mg / kg body weight of sodium nitrite, as shown in the figures (4,8), and the neonates after birth showed many skeletal abnormalities such as short length, atrophy in the skeleton, loss of caudal vertebrae and phalanges of the upper and lower extremities and malformations of the pelvic and shoulder bones as shown in the figures (10,11), and these structural changes of skeleton in neonates whose mothers were treated with a concentration of 15 mg / kg of sodium nitrite were more severe than the previous concentration and as shown in the figures (12,13) compared with fetuses for a period of (16, 20)days pregnancy and postpartum neonates in control groups,

and these results can be interpreted for the effect of sodium nitrite on on tissues of pregnant rats, causing harmful effects and stimulating lack of delivery of nutrients through the placenta necessary for the formation of bone for fetuses And the births later, or it may be due to a delay in the formation of cartilage in the fetus, which affects the formation of bone, The reason for this result may be due to genetic reasons as a result of mutations, through the breaking of DNA strands and a change in the process of transcription and gene expression leading to the deletion of the double-strand breaks between DNA and the union of sister chromatids, and this causes the lack of cartilage formation, which affects the formation of the skeleton of the fetus During pregnancy, these abnormalities appear in newborns after birth [8], or this result may be due to the fact that the preservative sodium nitrite as any chemical substance stimulates the generation of free radicals when it enters the body causing oxidative stress in the tissues of pregnant rats which destroys the mother's tissues which leads to a failure to provide the fetuses with the basic necessary materials to build the skeleton of the fetus, in addition to this preservative when passes to the fetus through the placenta, it creates free oxygen radicals in the fetal tissues which stimulates the oxidation of lipids in the cell membranes and damages the molecules inside the fetal cells destroying the developed tissues such as the skeleton and thus deforming them in the fetus during pregnancy which continues its effect until after birth in the neonates and these abnormalities increase with the increase in the concentration and duration of the dose as a result of the excessive oxidative stress that stimulates the destruction of the tissues of fetuses and neonates [9].

Figure (1): A normal skeleton of a clear rat fetus from a control group for a period of 16 days pregnancy (Alizarin red stain -5X)

Figure (2): Skeleton of a clear rat fetus from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body for a period of 16 days pregnancy, notes in it: 1- Deformation of the skull bones 2- Loss of the lower r extremity phalanges 3- Loss some of the caudal vertebrae (Alizarin red Stain -5X).

Figure (3): Skeleton of a clear rat fetus from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body for a period of 16 days pregnancy, notes in it: 1- Deformation of the skull bones 2- Deformation of the pelvic bones 3- Loss of the lower extremity phalanges 4- Loss of the caudal vertebrae .(Alizarin red Stain -5X).

Figure (4): Skeleton of a clear rat fetus from group treated with the preservative sodium nitrite at a concentration of 15 mg / kg of the body for a period of 16 days pregnancy, notes in it: 1- Deformation of the skull bones 2- Loss of the upper extremity phalanges 3- Deformation of the pelvic bones 4- Loss of the lower r extremity phalanges 5- Loss of the caudal vertebrae .(Alizarin red Stain -5X)

Figure (5): A normal skeleton of a clear rat fetus from a control group for a period of 20 days pregnancy (Alizarin red Stain -5X)

Figure (6): Skeleton of a clear rat fetus from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body for a period of 20 days pregnancy, notes in it: 1- Deformation of the jaw bones 2- Loss of some vertebrae 3- Loss of the upper extremity phalanges 4- Loss of the lower extremity phalanges 5- Loss of the caudal vertebrae .(Alizarin red Stain -5X)

Figure (7): Skeleton of a clear rat fetus from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body for a period of 20 days pregnancy, notes in it: 1- Deformation of the skull bones 2- Loss of the upper extremity phalanges 3-Loss of the caudal vertebrae 4-Loss of the lower extremity phalanges.(Alizarin red Stain -5X)

Figure (8): Skeleton of a clear rat fetus from group treated with the preserative sodium nitrite at a concentration of 15 mg / kg of the body for a period of 20 days pregnancy, notes in it: 1- Deformation of the skull bones 2- Loss of some vertebrae 3- Loss of the upper extremity phalanges 4- Loss of the lower extremity phalanges 5- Loss of the caudal vertebrae .(Alizarin red Stain -5X).

Figure (9): A normal skeleton of a clear rat newborn after birth from a control group (Alizarin red Stain -5X)

Figure (10): Skeleton of a clear rat newborn after birth from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body, notes in it: 1- Deformation of the skull bones 2- Loss of the upper extremity phalanges 3- Deformation of the pelvic bones 4- Loss of the lower r extremity phalanges 5- Loss of the caudal vertebrae .(Alizarin red Stain -5X).

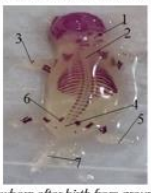

Figure (11): Skeleton of a clear rat newborn after birth from group treated with the preservative sodium nitrite at a concentration of 10 mg / kg of the body, notes in it: 1- Not being a skull 2- Deformation of the jaws bones 3- Atrophy of some upper vertebrae of backbones 4- Loss of some lower vertebrae of backbones 5- Loss of Pelvic bones 6- Lack of bones of the lower extremity7- No caudal vertebrae 8- Lack of bones of the upper extremity (Alizarin red Stain -5X).

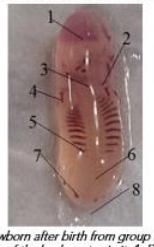

Figure (12): Skeleton of a clear rat newborn after birth from group treated with the preservative sodium nitrite at a concentration of 15 mg / kg of the body, notes in it: 1- Deformation of the skull bones 2- Loss of upper some vertebrae 3- Loss of the upper extremity phalanges 4- Loss of some lower vertebrae of backbones 5- Loss of the lower r extremity phalanges 6-Deformation of the pelvic bones 7- Loss of the caudal vertebrae (Alizarin red Stain -5X).

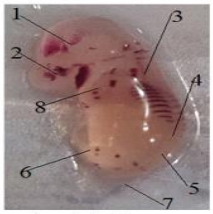

Figure (13): Skeleton of a clear rat newborn after birth from group treated with the preservative sodium nitrite at a concentration of 15 mg / kg of the body, notes in it: 1- Deformation& atrophy of the skull bones 2- Atrophy of the Shoulder bone atrophy 3-Atrophy &deviation of the some upper vertebrae of backbones 4- Atrophy of the upper extremity bones 5- Atrophy of some ribs 6- Absence of pelvic bones 7- Atrophy of the upper extremity bones8- Absence of caudal vertebrae (Alizarin red Stain -5X)

References

1. Al-dhaher ZA. The antibacterial activity of aqueous extract of cinnamon and clove against Staphylococcus aureus. Al-Nahrain Journal of Science. 2008;11(2):131-5. https://www.anjs.edu.iq/index.php/anjs/article/view/1371
2. Moorcroft MJ, Davis J, Compton RG. Detection and determination of nitrate and nitrite: a review. Talanta. 2001;54(5):785-803. https://doi.org/10.1016/S0039-9140(01)00323-X
3. Anand S, Sati N. Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. International journal of pharmaceutical sciences and research. 2013;4(7):2496. Available from: https://citeseerx.ist.psu.edu/messages/downloadsexceeded.html
4. Schroder J. Reproduction and breeding techniques for laboratory animals. Canadian Journal of Comparative Medicine. 1971;35(4):345. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1319595/
5. McLeod MJ. Differential staining of cartilage and bone in whole mouse fetuses by alcian blue and alizarin red S. Teratology. 1980;22(3):299-301. https://doi.org/10.1002/tera.1420220306
6. Manassaram DM, Backer LC, Moll DM. A review of nitrates in drinking water: maternal exposure and adverse reproductive and developmental outcomes. Environmental Health Perspectives. 2006;114(3):320-7. https://doi.org/10.1289/ehp.8407
7. Ward MH, Jones RR, Brender JD, De Kok TM, Weyer PJ, Nolan BT, Villanueva CM, Van Breda SG. Drinking water nitrate and human health: an updated review. International journal of environmental research and public health. 2018;15(7):1557. https://doi.org/10.3390/ijerph15071557
8. Özen H, Kamber U, Karaman M, Gül S, Atakişi E, Özcan K, Atakişi O. Histopathologic, biochemical and genotoxic investigations on chronic sodium nitrite toxicity in mice. Experimental and Toxicologic Pathology. 2014;66(8):367-75. https://doi.org/10.1016/j.etp.2014.05.003
9. Zheng S, Zuo Z. Isoflurane preconditioning reduces purkinje cell death in an in vitro model of rat cerebellar ischemia. Neuroscience. 2003;118(1):99-106. https://doi.org/10.1016/S0306-4522(02)00767-4