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Original Article

Detect the LD50 of the preservative sodium nitrite (E250) in female rats.

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Abstract

The present study was conducted to detect the LD50 (median lethal dose) and toxicological effects of a preservative, sodium nitrite (E250), administered orally in female rats. We performed this experiment at the Animal House Facility, College of Education for Girls, University of Kufa, from October 11 to December 16, 2022. Adult female albino rats (Rattus rattus), around 13 weeks of age and weighing between 200 g, were used. On the other hand, the study has precisely estimated the LD50 and toxic dose of sodium nitrite. Twenty-eight female rats were divided into seven groups, with four animals in each group. The variant doses of sodium nitrite groups were administered orally at 20, 30, 45, 70, 100, 130 and160 mg/kg body weight using a gastric gavage tube once a day. The differences in the acute oral toxicity of 70, 100, 130, and 160 mg/kg body weight were significant for female rats dying within the last period after dosing between 24 and 48 hours (the lethal concentrations). A 45 mg/kg body weight dose was the LD50 recorded in female rats, with mortality of half the rats within 48–72 hours. Nevertheless, this dosage eventually killed those remaining rats after a minimum of seven days. Nonetheless, both 30 and 20 mg/kg body weight doses were non-lethal and non-toxic. Therefore, the use of doses under 20 mg/kg (10 and 15 mg/kg body weight) was considered safe for the preservative in products. The study is for the disease of sodium nitrite (E250) in Rattus rattus female LD50 45mg/Kg.

Keywords: Sodium nitrite, E250, median lethal dose (LD50), Rattus rattus, toxicology, preservative.

1. Introduction

Food additives, including chemical preservatives, can pose health risks when used in excessive quantities. Consequently, the United States Food and Drug Administration (FDA) has established stringent regulations for the approval of various synthetic food additives. Additionally, the World Health Organization (WHO) and the European Food Safety Authority (EFSA) have collaborated to develop a program for the evaluation and designation of food additives [1].

Aims of the Study

The present study aims to determine the median lethal dose (LD50) and assess the toxicity of the preservative sodium nitrite ($NaNO_2$, E250) in female albino rats (Rattus rattus)

2. Literature Review

2.1. Preservatives in Food Systems

Preservatives are organic or inorganic compounds added to foods in order to prevent microbial growth and undesirable chemical changes that can extend the shelf life of a food product [2]. One of the most commonly used preservatives is sodium nitrite ($NaNO_2$; E250), which has been predominantly used to prevent lipid oxidation in meat preservation [3]. Sodium nitrite (E250): The Joint Expert Committee on Food Additives again re-evaluated sodium nitrite and set an ADI from 0.06 to 0.07 mg/kg body weight

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per day for almost all population groups Methemoglobinemia, a condition in which nitrite binds with hemoglobin to form methemoglobin, and blocks the binding site of oxygen on it, thus preventing oxygen uptake by blood is one of the most important toxicological effects of sodium nitrite in the blood. Symptoms include cyanosis of the lips and peripheries, as well as diarrhea, which is common in infants, while methemoglobin levels > 70% in blood can be fatal due to anoxia. This syndrome is also well recognized, known as "blue baby" (frequently), [3]. The brain, which uses more oxygen than any other organ in the body, is especially sensitive to a lack of oxygen and can begin to die within five minutes after oxygen supply has been cut. This can then cause problems such as lack of attention, stability imbalance, and lack of mobility function [4]. In addition, nitrite used for preservation or colorant has been reported to be linked with the occurrence of cancer, such as gastric and esophageal cancer, colorectal cancer, liver cancer, bladder TCCs, and thyroid carcinoma [5]. For example, long-term intake of sodium nitrite has been shown to cause adverse effects on the male reproductive system via a consequential decrease in testosterone and follicle-stimulating hormone (FSH) levels. Such changes lead to lower numbers of viable spermatozoa, higher proportions of dead and morphologically abnormal sperm, subsequently decreasing the fertility potential in males [6]. However, sodium nitrite is capable of causing renal dysfunction by increasing levels of blood urea and creatinine, suppression in proximal tubule reabsorption, and triggering glomerular hyperfiltration [7]. The studies demonstrated decreased levels of antioxidants caused by sodium nitrite exposure, inhibiting mechanisms that lead to suppression of steroid hormone production in the ovaries and adrenal glands, with lower enzymatic antioxidant activity on pituitary tissues as well as on ovarian hormone synthesis. Unfortunately, this results in ovarian follicle loss and impaired female fertility. Studies by [8] revealed that the treatment with 75 mg/kg sodium nitrite in males resulted in an increase of free radical formation, lipid peroxidation, and creatinine as well as urea levels, which may lead to kidney and liver tissue damage, subsequently can cause hepatic dysfunction (2020).

2.2. Additives Food

Food additives are chemical substances of natural or synthetic origin, which are added to foodstuffs specifically for their technical and/or organoleptic (Sensory) effect in the finished product. According to the report [9], food additives are categorized according to their function in food. (2020), into the following categories:

a) Coloring agents
b) Acidity regulators and anti-caking agents
c) Flavoring agents
d) Thickeners, stabilizers, and emulsifiers
e) Firming agents and foaming agents

Preservatives

What are Preservatives? A preservative is a chemical component that is added to food products for the prevention of spoilage from microbial growth, enzymatic activity, or oxidation. They are important in the food, pharmaceutical, and cosmetic industries through the prevention of bacterial growth and bio-enrichment [10].

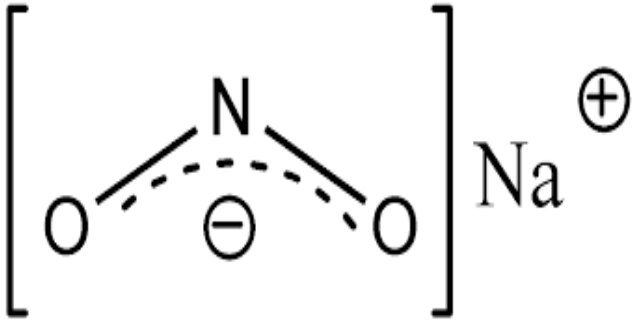
Types of Preservatives

a) **Natural Preservatives:** For ex-salt, spices, sugar, oils, etc., used in the production of jams, juices, and pickles [11]

b) **Synthetic Preservative:** These are used to reduce the chances of chemical and biological spoilage of food, although there is an amount that needs to be monitored, as these preservatives are known to cause cancer if they exceed a safe level. Based on both chemical composition and mechanism of action, they can further be classified as:

- **Organic Preservatives:** These are sorbic acid, benzoic acid, and acetic acid.

- **Inorganic Preservatives:** like nitrates (NO_3^-) and nitrites which is (NO_2^-). International designator E250: Chemical structure of sodium nitrite. Sodium nitrite is one type of inorganic preservative. Daily dosing of sodium nitrite is safe in humans and accepted internationally at a dose of approximately 10 mg/kg body weight [12].



Chemical Composition of Sodium Nitrite [12]

Table 1: Chemical and Physical Properties of Sodium Nitrite ($NaNO_2$)

Property	Description
Chemical Formula	$NaNO_2$
Molecular Weight	68.9953 g/mol
Melting Point	271 °C
Density	2.17 g/cm³
Boiling Point	115 °C (decomposes)
Classification	Inorganic compound, corrosion inhibitor
Solubility	Soluble in water and ethanol
IUPAC Name	Sodium nitrite
Appearance	White to yellowish crystals
Alternative Names	E250
Solubility in Water	82 g/100 mL water at 20 °C
Taste	Slightly salty
Decomposition Temperature	320 °C (608 °F)

2.3. Sources of Sodium Nitrite

Drinking Water: About 25% of salivary nitrate is metabolized to nitrite by oral microflora, especially the bacteria located in the dorsal surface of the tongue. [13] found that the intestine absorbs this nitrite to be used later. Nitrate to nitrite reduction in the oral cavity is probably the major source of human exposure to nitrite, especially from dietary sources [14].

Plants and Soil: Source of nitrates — green leafy vegetables, root vegetables. The use of fertilizers leads to an increase in Nitrogen and the consumption of organic material results in a change in form, changing it into N, which is used by plants to form nitrate, and this result for many nitration of many vegetables [15].

2.4. Antioxidants

Sodium Nitrite Source and Application in Regulation level Sodium nitrite at 20mg/kg decreased the lipid oxidation of meat product [16]. A study reported reducing the concentration to 50 mg/kg and still achieving effectiveness as an antioxidant in beef and poultry. Sodium nitrite, in combination with salts, is added to processed meats as a safety enhancer since it inhibits the growth of pathogenic microorganisms. Concentrations of nitrite up to 45–119 mg/kg have been shown to stabilize color in meats, with an ideal concentration for maximal color stability between 10 and 20 mg/kg [17]. Sodium nitrite is also employed in the commercial food sector as a colour fixative in confectionery, dairy goods, jams, preserved meats, gelatin, dry soft drink mixes, and canned foods at recommended levels ranging from 5–20 mg/kg to give a red-bound coloration to meats [18]. This compound is used in food and beyond food applications (pharmaceutical, cosmetic), including aqueous pharmaceutical solution, tablets (0.1 g or 110 mg sodium nitrite), capsules (110 or 30mg NaNO2), toothpaste, cosmetics, as well as industrial uses — ink production [19]. Despite the foregoing effects, CNV is also a vasodilator; when used in human and veterinary medicine, it has been utilized to treat various vascular conditions as well [20]. For this purpose, intramuscular injections of sodium nitrite (1–10 mg/kg) have been proven to be effective at mitigating the adverse effects related to exposure to chlorine gas as well as in treating cyanide and carbon monoxide poisoning, especially when dealing with life-threatening circumstances [21]. International Regulations for Sodium Nitrite

The percentage of nitrite and nitrate must meet a given order of magnitude in processed meats, which are used to stabilize the color and prevent the growth of bacteria. The Joint Expert Committee on Food Additives (JECFA) in the Food and Agriculture Organization of the United Nations/World Health Organization [22] has established an acceptable daily intake as 0.06–0.07 mg/kg body weight for a 60-kilogram (130 lb-) adult, equal to 4.2 mg/day. The maximum allowable level of sodium nitrite that can be added to processed meats and fish is 200 mg/kg in the United States [23].

3. Materials and Methods

3.1. Animals Laboratory

Albino female adult rats (Rattus rattus albinus Sprague-Dawley) aged between 10 and 13 weeks, weighing between 200 and 220 g, were used in the experiment. Rats were obtained from the Animal House Facility of the College of Sciences at the University of Kufa, and their non-pregnancy, disease-free status, and that they had not been previously used in any experimental designs were further confirmed. Animals were provided with normal feed and drinking water through bottles. The animals were kept for 1 week in the laboratory under standard conditions before the initiation of experiments. Experimental rats: The female rats were housed in special dosing cages, and the rat sprayers wrote down the start date of the experiment and their name. The cages were provided with water and feed, and dosing was carried out in a rat-proof oral gavage tool shown in the figure.

Gavage Dosing Procedure

3.2. Design of experiments

The intention of this experiment is designed to estimate the median lethal dose (LD50) of sodium nitrite ($NaNO_2$) in female rats at day 1. The study was conducted on 28 adult female albino rats (Rattus rattus Sprague-Dawley strain). Animals and treatment. Seven female Wistar rats were divided into seven groups with four animals each. Rats received a 0.1 mL sodium nitrite through a metal stomach tube, orally once daily [24] as follow

1. **Group 1:** [4] female rats dosed by gavage with sodium nitrite at a concentration of 160 mg/kg body weight**.**
2. **Groups 2:** Four female rats received via oral intubation with Sodium nitrite at a concentration of 130 mg/kg B W**,**
3. **Group 3** (four female rats were injected with 100 mg/kg body weight of sodium nitrite**.**
4. **Group 4:** Four female rats were administered the preservative sodium nitrite at a concentration of 70 mg/kg body weight**.**
5. Group 5: Four female rats were administered a preservative of sodium nitrite (45 mg/kg body weight**.**
6. Group 6: Four normal female rats were administered the preservative sodium nitrite at a dose of 30 mg/kg body weight**.**
7. Group 7. 4 female rats — preservative = sodium nitrite at a dose level of 20 mg/kg body weight**.**

Results

All female rats injected with sodium nitrite at doses of 160, 130, 100, and 70 mg/kg body weight died within the first day of treatment. Those levels were classed as toxic and mortal to female rats. On the other hand, half of the rats treated with 45 mg/kg body weight died within 48–72 hours, and all others died within the next 7–10 days. Therefore, this dose (45 mg/kg) was the LD50 of sodium nitrite for female rats. In light of this finding, lower doses (30 and 20 mg/kg body weight) were also applied, and the results showed that these concentrations are not lethal to female rats, since all treated animals survived. Consequently, further female rat dosing studies were carried out using doses below the LD50 and specifically lower than 20 mg/kg body weight**.**

Table 2: Determination of the Median Lethal Dose (LD50) and Toxicity of Sodium Nitrite in Female Rats

Concentration (Dose)	Treated Rats	Number of Rats Deceased Within 24 Hours Post-Dosing	Number of Rats Deceased After 24 Hours Post-Dosing	Effects on Female Rats
160 mg/kg body weight	4	4	0	Lethal
130 mg/kg body weight	4	4	0	Lethal
100 mg/kg body weight	4	4	0	Lethal
70 mg/kg body weight	4	3	1	Lethal
45 mg/kg body weight	4	2	2	Lethal
30 mg/kg body weight	4	0	1	Toxic
20 mg/kg body weight	4	0	0	Toxic

4. Discussions

In this experiment, all female rats treated with sodium nitrite concentrations of 160, 130, 100, or 70 mg/kg body weight on the 1st day died within a day after exposure. The toxic or lethal doses (concentration) for female rats were therefore recorded. Female rats killed with 45 mg/kg body weight showed that in half of the animals, death occurred after 48-72 hours, and all others died within 7–10 days. As a result, 45 mg/kg was regarded as the median lethal dose (LD50) of sodium nitrite for female rats. Considering the above result, lower doses of 30 and 20 mg/kg body weight were applied, and such concentrations were not lethal, as all female rats treated with an equivalent dosage survived. These observed effects could be associated with sodium nitrite interacting with the nitric oxide (NO) present in the organism, causing vasodilatation and vascular smooth muscle relaxation, resulting in a fall off of oxygen delivery leading to severe tissue injury at organs such as the kidneys and liver, respectively, followed by death [25]. Other research also links sodium nitrite to cerebral hypoxia and brain tissue damage, thanks to the way it prevents your bloodstream from getting oxygen. Mild hypoxia results in symptoms that include ataxia and short-term memory loss, whereas severe or prolonged hypocapnia can lead to coma, seizures, and cerebral death. This is consistent with the results of [26], who found that a single dose of 60 and 100 mg.kg body weight caused mortality in male and female albino rats, while repeated doses of 10 to 30 mg.kg body weight did not cause any death. So, too, did the present study demonstrate that doses of 20–30 mg/kg body weight (given to female rats) were not lethal.

Conflict of interest: NIL

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