جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ديـالـــى
كلية الطب البيـطري

تقييم الجرعة النصفية القاتلة لنترات الصوديوم فموياً في ذكور الفئران

بحث مقدم الى كلية الطب البيطري من قبل الطالبة

أزهار هاشم هندي

وذلك كجزء من متطلبات الحصول على شهادة البكالوريوس في الطب والجراحة البيطرية

بإشراف

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1435 هـ **2014 م**

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Determination of The Medium Lethal Dose (LD_{50}) of Sodium Nitrite Orally in Male Mice

Research Presented to the college of Veterinary Medicine
by

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As a partial fulfillments of obtaining the Bachelor's degree in veterinary medicine and surgery (BVMS.)

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2014 A.D **1435 A.H**

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Summary

This experiment was carried out to evaluate the medium lethal dose (LD_{50}) of sodium nitrite orally by using the Up and Down method (Dixon 1980-method) , when used by oral administration to fifteen healthy male white mice the range of body weight are between (19 - 29 gm) . After a period of adaptation which lasts for a week , The oral dosing begin to single male mice daily after overnight fasting , and prepared sodium nitrite solution for certain concentration was carried out by using insulin syringes with modified stainless steel needle. Doses of median lethal dose (LD_{50}) group of sodium nitrite were (120 , 140 , 160 , 180 , 200) mg/kg of body weigh . A dose of (120) mg/kg. of body weight of the mice was the initial dose and increase gradually at percent (20 %) , with noted the clinical signs that appear on dosage mice .

The calculated median lethal dose (LD_{50}) of sodium nitrite was 187.62 mg/kg. of body weight , and the clinical signs that showed after administration of sodium nitrite are included (tachypnea, dyspnea , cyanosis , anxiety , fatigue, weakness) .

Certification

It is hereby to certify that this project (**Determination of The Medium Lethal Dose (LD_{50}) of Sodium Nitrite Orally in Male Mice**) was prepared by **Azhar Hashim Hendi** under my supervision at the College of Veterinary Medicine / Diyala University as a partial fulfillment of the requirements for Bachelor Degree in Veterinary Medicine and Surgery .

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1. Introduction

Nitrates and nitrites are chemicals used in fertilizers, in rodenticides (to kill rodents), and as food preservatives [(1)]. Nitrates and nitrites come in various forms, but when dried are typically a white or crystalline powder. Nitrate (NO3-) and nitrite (NO2-) are also naturally-occurring compounds that are a metabolic product of microbial digestion of wastes containing nitrogen, for example, animal feces or nitrogen-based fertilizers [(2)]. Sodium and potassium nitrates are used as fumigants in canisters, which are placed underground in rodent dens and holes, and then ignited to explode and release gases that kill the rodents [(3)]. Sodium nitrite is a food additive that is used as a preservative [(4)].

Exposure to nitrates and nitrites at levels above health-based risk values has been reported to have adverse health effects on infants and children. The health effect of most concern to the U.S. EPA for children is the "blue baby syndrome" (methemoglobinemia) seen most often in infants exposed to nitrate from drinking water used to make formula [(5)]. The nitrate binds to hemoglobin (the compound which carries oxygen in blood to tissues in the body), and results in chemically-altered hemoglobin (methemoglobin) that impairs oxygen delivery to tissues, resulting in the blue color of the skin [(6)]. The blue coloration can be seen in the lips, nose, and ears in early stages of blue baby syndrome, and extend to peripheral tissues in more severe cases. Exposure to higher levels of nitrates or nitrites has been associated with increased incidence of cancer in adults, and possible increased incidence of brain tumors, leukemia, and nasopharyngeal (nose and throat) tumors in children in some studies [(7)] .

1.1. Physical and Chemical Properties of Sodium Nitrite [8] :

Table (1) : Show the Physical and Chemical Properties of Sodium Nitrite

Synonym(s)	Chile salpeter; niter; nitric acid sodium salt ;salpeter; soda niter.
Registered trade name(s)	Nitric acid, sodium salt
Chemical formula	NaNO3
Chemical structure	NaNO3
Color	Colourless
Physical state	trigonal (rhombohedral) crystals
Appearance	White powder or colorless crystals with sweet smell
Density, g/cm3	2.26
Molecular formula	NaNO3
Molecular weight	84.9947 g/mol
Melting point °C	306
Boiling point °C	380
Odor	Odorless
Solubility in water	**(92 g/100 g at 25 °C; 180 g/100 g at 100 °C);**
Solubility	Soluble in ammonia; soluble in ethanol and methanol slightly soluble in acetone and glycerol; practically insoluble in dimethyl ether.

1.2. History, Sources, Uses, of Sodium Nitrite :

The largest accumulations of naturally occurring sodium nitrate are found in Chile and Peru, where nitrate salts are bound within mineral deposits called caliche ore [9]. For more than a century, the world supply of the compound was mined almost exclusively from the Atacama desert in northern Chile until, at the turn of the 20th century, German chemists Fritz Haber and Carl Bosch developed a process for producing ammonia from the atmosphere on an industrial scale with the onset of World War I, Germany began converting ammonia from this process into a synthetic Chilean saltpeter which was as practical as the natural compound in production of gunpowder and other munitions. By the 1940s, this conversion process resulted in a dramatic decline in demand for sodium nitrate procured from natural sources[10,11].

Nitrites are a normal part of human diet, found in most vegetables [12,13]. Spinach and lettuce can have as high as 2500 mg/kg, curly kale (302.0 mg/kg) and green cauliflower (61.0 mg/kg), to a low of 13 mg/kg for asparagus. Nitrite levels in 34 vegetable samples, including different varieties of cabbage, lettuce, spinach, parsley and turnips ranged between 1.1 and 57 mg/kg, e.g. white cauliflower (3.49 mg/kg) and green cauliflower (1.47 mg/kg) [13,14]. Boiling vegetables lowers nitrate but not nitrite [13]. Fresh meat contains 0.4-0.5 mg/kg nitrite and 4–7 mg/kg of nitrate (10–30 mg/kg nitrate in cured meats) [12] .The presence of nitrite in animal tissue is a consequence of metabolism of nitric oxide, an important neurotransmitter [15]. Nitric oxide can be created de novo from nitric oxide synthase utilizing arginine or from ingested nitrate or nitrite [16].

The industries include glass, enamel, porcelain, explosives and charcoal briquettes manufacturing. Some grades of NaNO3 contain anticaking agent. Molten salt mixtures containing NaNO3 are employed as heat-treatment baths, as heat transfer fluids and as sensible heat storage media [17].

Sodium nitrate application has been suggested in aquaculture ponds with several environmental and economical benefits such as its suitability as nitrogen source, and that it does not produce acidity by nitrification as an ammonium fertilizer [18]. Conversely, adding nitrate to seawater may increase pH because of its dissolution process in seawater [19].

1.3. Sources of exposure

Nitrate and nitrite are permitted additives in selected foods only. Maximum permitted levels are specified in Standard 1.3.1 of the Australia New Zealand Food Standards Code [20].

Nitrates added to meats serve as antioxidants, develop flavor, and stabilize the red color in meats but must be converted to nitrite to exert these actions. Sodium nitrite is used as a colorant, flavor enhancer, and antimicrobial agent in cured and processed meats. Nitrate and nitrite use in meat products, including bacon, bologna, corned beef, hot dogs, luncheon meats, sausages, and canned and cured meat and hams is subject to limits put forth in Food and Drug Administration (FDA) and US Department of Agriculture (USDA) regulations[21,22].

Excessive levels of nitrate can be reduced to nitrite which couples with oxyhaemoglobin resulting in formation of MetHb. The extent of MetHb formation in infants and young children depends on several variables including nitrate and nitrite exposure from diet and water, gastro-intestinal infections, diarrhea, acidosis and exposure to a number of drugs [(23)].The acceptable daily intake for nitrate is set to 3.7 mg/kg body weight, which is 250 mg for an adult. Ironically, one glass of fresh beetroot juice [(24)] .

1.4. Pharmacokinetics of nitrite

1.4.1. Absorption and distribution of nitrite

Ingested nitrate is readily and completely absorbed from the upper small intestine. Nitrite may be absorbed directly from both the stomach and the upper small intestine. Part of the ingested nitrite reacts with gastric contents prior to absorption. At least 25% of the ingested nitrate is transported into the saliva, where the concentration is approximately 10 times greater than that in plasma as a result of bioconcentration. About 20% of the nitrate in saliva is converted to nitrite by commensal bacteria on the surface of the tongue. Individuals with gastroenteritis have a higher conversion rate [(25)]. There is evidence that the use of antibacterial mouthwashes may reduce this conversion [(26)] .

Nitrate is rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and re-enter the stomach. Bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract, but not normally in the stomach; exceptions are reported in humans with low gastric acidity, such as artificially fed infants, certain

patients in whom hydrochloric acid secretion is slower than normal or patients using antacids [27]. In rats, active secretion and reduction of nitrate in saliva are virtually absent [28]. Absorbed nitrite is rapidly oxidized to nitrate in the blood. Nitrite in the bloodstream is involved in the oxidation of hemoglobin (Hb) to methaemoglobin (metHb): the Fe2+ present in the haem group is oxidized to its Fe3+ form, and the remaining nitrite binds firmly to this oxidized haem. The Fe3+ form does not allow oxygen transport, owing to the strong binding of oxygen [29].

1.4.2. Metabolism and elimination of nitrite

After binding to plasma and erythrocytes, nitrite when present at normal levels is metabolized to nitrate in animals which is then metabolized to nitric oxide, which has a wide range of physiological functions. After transport to the stomach, the acidic conditions will rapidly transform nitrite to nitrous acid, which in turn spontaneously decomposes to nitrogen oxides including nitric oxide. Exogenous intake of nitrite/nitrate leads to nitric oxide in the upper intestine. These levels are up to 10,000 times higher compared with levels resulting from endogenous production of nitric oxide from L-arginine by nitric oxide synthases [30].

Under normal physiological conditions, nitrate is readily excreted in the urine [25]. Thus does not accumulate in tissues. Although much of the nitrate is eventually excreted in the urine, up to 25% is actively taken up by the salivary glands and is concentrated up to 20-fold in saliva [31].

However, nitrate has a slower rate of excretion and the major part of the primary urinary nitrate (ca 80 %) is pumped back to the blood by an active transport mechanism. This salvaging of nitrate from the urine, in addition to the known recycling of nitrate from saliva and also from the intestines (after biliary excretion) further suggests that the body is acting to conserve a substance of physiological importance [(25)].

In humans, mean clearance and inter-compartmental clearance for nitrite were 0.948 and 0.67 L/min respectively, with a terminal half-life of 42 minutes [(32)].

1.5. Toxicity of nitrite:

Nitrate contamination of drinking water may increase cancer risk, because nitrate is endogenously reduced to nitrite and subsequent nitrosation reactions give rise to N-nitroso compounds; these compounds are highly carcinogenic and can act systemically [(33)].

Nitrate and nitrite exposure has been associated with negative health effects through the consumption of cured and processed meats [(34)].

Sodium nitrate is used commonly as an important antimicrobial agent in meat because it inhibits the growth of *Clostridium botulinum* and toxin production in crude meats, a deadly food borne illness and fixing colour in red meat poultry and fish products [(35)].

Consumption of red and processed meats is associated with an increased risk of certain types of cancer and chronic obstructive pulmonary disease [36].

Maternal (during pregnancy) or child consumption of nitrite-containing meats may be associated with increased incidence of brain tumors in children [37].

Nitrate and/or its N-nitroso metabolites can traverse the placenta and affect the fetus in utero [38]. These effects include CNS anomalies such as neural tube defects [39].

Other health effects following fetal exposure to elevated levels of nitrates in drinking water included intrauterine growth retardation [40].Increased incidence of Sudden Infant Death Syndrome (SIDS) [41] cardiac defects [42], and increased risk of nervous system defects [43].

Reduced oxygenation of hemoglobin (methemoglobinemia) has been reported after exposure to nitrate- and nitrite-contaminated drinking water; also called the "blue baby syndrome" because of the cyanotic (oxygen-deficient) symptoms that result from the reduced oxygenation of the blood [44] .Adverse reproductive outcomes of nitrates in drinking water have been reviewed [45].

High nitrate concentrations in drinking water caused decline in sperm count and motility in mice. This was accompanied with alteration in testicular 17-beta hydroxysteroid dehydrogenase and gamma glutamyl transpeptidase [46].

2. Materials and Methods :-

2.1. Materials

Table (2) :- Show the material uses in this experiment - :

Item	Company	Origin
Sodium Nitrite	Riedel-de Haen AG	Germany
Insulin Syringes	Huaian City Hengchun Medical Product Co., Ltd	China
Modified Stainless Steel Needle (22) G	Abu Dhabi Medical Devices Company	Emirates

2.2. Up and Down Method :

This test calls for dosing individual animals in sequence singly at 24 hours intervals, with the initial dose set at (the toxicologist best estimate of LD_{50}) following each death or moribund state, the dose is lowered, following each survival, it is increased according to pre specified dose progression factor. If death follows an initial direction of increasing doses of 10-20% or a survival follows an initial direction of decreasing dose with the same ratio. These additional animals are tested by the Up and Down following the dose adjustment pattern.

The LD_{50} is calculated using the following equation .

LD50 =XF +Kd

XF = last dose administered

K = value from Dixon table in appendix (1)

d = difference between dose levels.

3. Results and Discussion

Determination of acute toxic median lethal dose (LD_{50}) in male albino mice was done by up and down method according to Dixon (1980)[47], the value of LD_{50} was 187.62 mg/kg B.W, and it was calculated according to the following equation result: Table ( ) .

$$\mathbf{LD_{50} = xf + kd}$$

$$\mathbf{= 180 + (0.381) \times 20 = 187.62 \ mg/kg \ B.W}$$

LD50 = Medium Lethal Dose .

xf = Last dose used in the experiment .

d = Difference between doses .

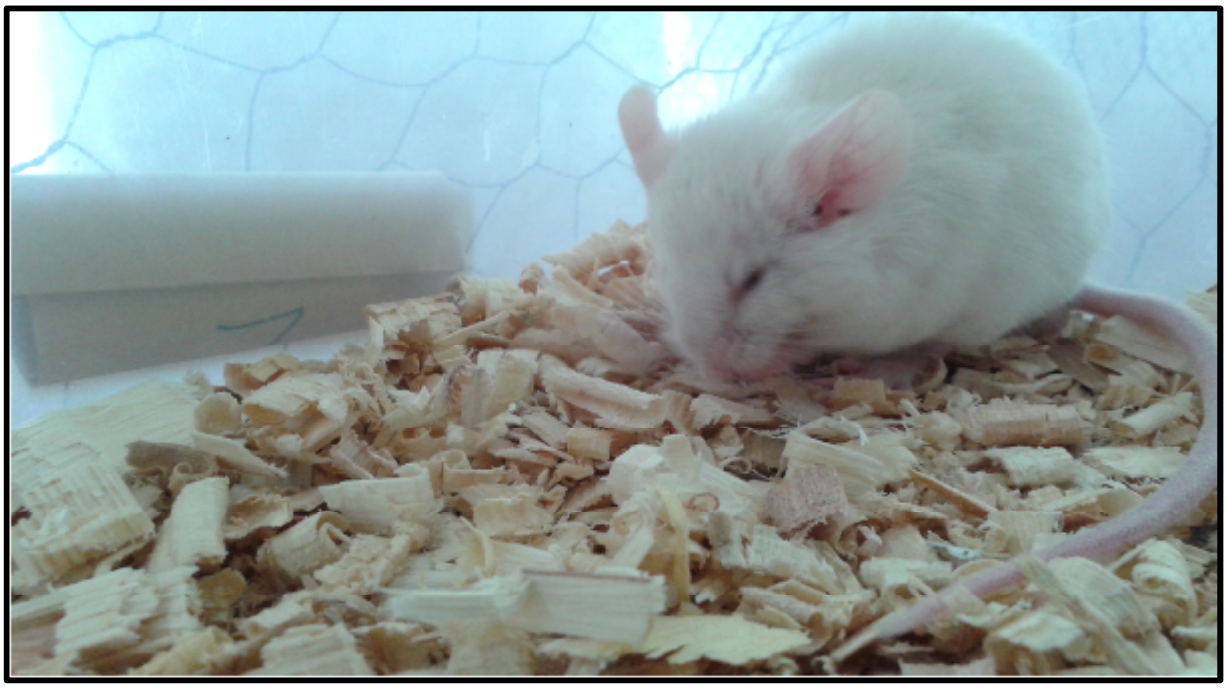
k = Factor of change .

Table (3) : Calculation of LD_{50} for Sodium Nitrite by Up and Down Method-Orally (Dixon ,1980) [47] .

Range of doses	Decrease or increase in dose	Death or survival of animal after 24 hours.	Value of (K) table	Last used dose (xf)	Value of LD_{50}
120 – 200 mg /kg B.W	20 mg /kg B.W	OOOOXXOO	0.381	180 mg /kg	187.62 mg/kg B.W

O: survival , X : death

The oral LD_{50} of Sodium Nitrite considered as very toxic as its toxicity rate is (4), appendix (1) . The symptoms indicated (tachypnea, dyspnea , cyanosis , anxiety , fatigue, weakness) .



Picture show the fatigue after administration of sodium nitrite

Picture show the cyanosis after administration of sodium nitrite

Table (4): shows the data of orally administration to sodium nitrite to male mice :

Mice	Weight (g)	Dose (mg/Kg. B.W)	Results (O or X)
1	19	120	O
2	21	140	O
3	22	160	O
4	19	180	O
5	24	200	X
6	19	180	X
7	22	160	O
8	29	180	O

O: survival , X : death

LD_{50} values of 85–220 mg of sodium nitrite per kilogram of body weight have been reported for mice and rats [(48)] . While the world health organization (WHO) has indicated a toxic dose of (0.4 -200) mg/kg body weight and a lethal dose of 33 – 250 mg / kg body weight for nitrites taken orally [(49)] . symptoms of poisoning include cyanosis which becomes pronounced when the methemoglobinaemia affects approximately 10 % of red blood cells .

When over 20 % of the erythrocytes are effected , other symptoms appear , including headaches , dizziness , panting , tachycardia and general weakness . when 60 % of cells are effected serious disorders , such as loss of consciousness can arise , while at over 70 % untreated poisoning rapidly becomes fatal [50] .

4. Conclusions and Recommendations :

From the previous result it is concluded that sodium nitrite considered as very toxic in small amounts therefore recommendation must be careful from fertilizers, rodenticides and foods that additive to it sodium nitrite as a preservative specially meats that added sodium nitrite as a colorant, flavor enhancer, and antimicrobial agent in cured and processed meats.

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الخلاصة

أجريت هذه التجربة لأجل تقييم الجرعة النصفية القاتلة (LD_{50}) للنترات الصوديوم عن طريق الفم باستخدام طريقة Up and Down method ، التي استخدمت عن طريق الاعطاء الفموي لخمسة عشر من ذكور الفئران البيض السليمة بمعدل وزن جسم ما بين (19 - 29 غرام) . وبعد فترة التكيف التي تستمر لمدة أسبوع ، بدأ التجريع الفموي للفئران بشكل منفرد ويومياً بعد تصويم الحيوان لليلة كاملة ومن ثم تحضير محلول نترات الصوديوم بتراكيز معينة واستخدامت محاقن الأنسولين مع تعديل إبرة الفولاذ المقاوم للصدأ . وكانت مجموعة الجرعة النصفية القاتلة (LD_{50}) المستخدمة لنتريت الصوديوم هي (120، 140، 160، 180، 200) ملغم / كغم من وزن الجسم . وكانت الجرعة (120) ملغم / كغم. من وزن الجسم للفئران هي الجرعة الأولية المستخدمة و تزداد تدريجيا بنسبة (20 ٪) ، مع ملاحظة العلامات السريرية التي تظهر على الفئران المجرعة .

كانت الجرعة النصفية القاتلة (LD_{50}) لنترات الصوديوم هي 187.62 ملغم / كغم. من وزن الجسم، و العلامات السريرية التي ظهرت بعد أعطاء نتريت الصوديوم تضمنت (تسرع في التنفس ، وضيق التنفس ، أزرقاق ، عدم الارتياح ، تعب ، ضعف) .

Appendix

Appendix 1

Table –Measurement of LD50

Second part of serial	+K : Tests serial				
	O	OO	OOO	OOOO	
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX
XOOX	-0.878	-0.861	-0.860	-0.860	OXXO
XOXO	0.701	0.737	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.186	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	-0.305	-0.169	-0.144	-0.142	OOXO
XXXO	1.288	1.500	1.544	1.549	OOOX
XXXX	0.555	0.897	0.985	1.007	OOOO
	X	XX	XXX	XXXX	
	-K : Tests serial				Second part of serial