The antagonism effect of sodium nitrate by ascorbic acid (vitamin C) on neurobehavioral of mice

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Abstract

Evaluates the neurobehavioral effects were resulted from dosing of sodium nitrate in mice. Mice were divided into 5 equal groups, the first group; control group was fed from concentrated feed (Barley, Wheat, Soybeans, Corn and Bran), the second group was added 0.2% sodium nitrate and the third group was added 0.2% sodium nitrate with 0.4% ascorbic acid, fourth group was added sodium nitrate 0.4% alone and the fifth group was added 0.4% sodium nitrate with 0.8% of ascorbic acid for five weeks. Sodium nitrate did not produce clear signs of toxicity, but a significant decrease in motor activity and standing on the hind legs (rearing) was observed in the open-field activity test, where the lowest level was reached in the fourth week of treatment, and these declines returned gradually to reach the control group level values at the end of the study period. Sodium nitrate was significantly delayed at the time of the negative geotaxis test at a 45 ° while returning to the control level in the fifth week, also showed that there was a significant increase in body weight compared to pre-treatment value. In this study 0.8% of ascorbic acid with 0.4% sodium nitrate in group 5 showed differed significantly with 0.4% sodium nitrate only in group 4, that means the ascorbic acid give a beneficial result when used for remedy of nitrate toxicity.

***Keyword*:** Ascorbic acid, Mice, Neurobehavioral, Sodium nitrate
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التأثير المضاد لنترات الصوديوم بواسطة حامض الأسكوربيك (فيتامين ج) على السلوك العصبي للفئران

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الخلاصة

قيمت التأثيرات السلوكية العصبية لنترات الصوديوم والمستخدمة في علف الفئران البيضاء. قسمت الفئران إلى ٥ مجاميع متساوية، أعطيت المجموعة الأولى مجموعة السيطرة، علف مركز حاوي على الشعير والقمح وفول الصويا والذرة والنخالة وغذيت المجاميع الأخرى بنفس علف المجموعة الأولى، أضيف الى المجموعة الثانية ٠,٢% من نترات الصوديوم والمجموعة الثالثة ٠,٢% من نترات الصوديوم مع ٠,٤% من الأسكوربيك أسيد وللمجموعة الرابعة ٠,٤% من نترات الصوديوم في المجموعة الخامسة ٠,٤% من نترات الصوديوم مع ٠,٨% الأسكوربيك أسيد ولمدة ٥ أسابيع. أظهرت الدراسة أن نترات الصوديوم لم تظهر علامات واضحة للتسمم ولكن لوحظ انخفاض معنوي في النشاط الحركي داخل المكان المفتوح، ووصلت إلى أدني مستوى في الأسبوع الرابع من المعاملة ثم عادت هذه الانخفاضات الى الارتفاع تدريجيا للوصول إلى مستوى مجموعة السيطرة في نهاية مدة الدراسة. نترات الصوديوم أخرت بدرجة معنوية في وقت إنجاز اختبار الأرضي السالب بزاوية ٤٥ درجة في حين رجع للوصول إلى مستوى السيطرة في الأسبوع الخامس، وعانت الفئران المعالجة في هذه الدراسة من الزيادة المعنوية من وزن الجسم مقارنة مع قيم ما قبل المعالجة. أثبتت هذه الدراسة وجود اختلاف معنوي في المجموعة الخامسة ٠,٨% باستعمال الاسكوربيك أسيد مع نترات الصوديوم ٠,٤% مقارنة بالمجموعة الرابعة ٠,٤% نترات الصوديوم فقط، وهذا يعني أن الأسكوربيك أسيد ذات تأثير مضاد لعلاج التسمم بالنترات.

Introduction

Nitrate poisoning has been recorded in numerous studies (1,2) and it can happen in man and animals (3). Through entero hepatic metabolism of nitrate due to nitrite being an intermediate may cause poisoning of nitrate (4). The iron atoms in hemoglobin oxidized by nitrites converted from ferrous (Fe^{+2}) to ferric iron (Fe^{+3}), which leads to its inability to carry oxygen, this mechanism can lead to which is called methemoglobinemia it is a generalized deficiency of oxygen in organ tissue and a serious case, usually nitrite converts to ammonia, but if there is an increase in nitrite than can be converted, slowly the human and animal suffers from a deficiency of oxygen (5,6). In human nitrate is reduced to nitrite before ingestion in saliva and in the gastrointestinal tract (3,7). In ruminants; cattle, sheep, and goat, the conversion of nitrate to nitrite is carried out by rumen bacteria (7,8). The poisoning of nitrate produces different and complex neurobehavioral effects in the human and various animal species and represents one of the medical and veterinary problems currently (9,10). The poisoning is being diagnosed with a rising frequency as heavy maturing with nitrogenous compounds becoming more widely used (11). Neurobehavioral tests are available in different types to detect the acute, subacute or chronic poisoning of nitrate compounds in laboratory animals (12, 13). Automated motor activity measurement and functional observational batters of tests have been lately used to assess the neural poisoning of nitrate compounds and other compounds like cholinesterase inhibitors (14). Al-khafaji and Rhaymah (15) had adapted several neurobehavioral tests (open-field activity, negative geotaxis, and ataxia as well as food intake) to evaluate behavioral changes induced by nitrite intoxication in the rat. In order to furthermore develop and upholding such findings, the present study assessed the neurobehavioral changes of persisting exposure to a non-overtly toxic dose of the nitrate compound in mice, and if the ascorbic acid can effect of them or not. Incubation ascorbic acid along with the nitrate was able to significantly decrease METHB formation in a dose-dependent manner in both rats and humans (16). It has potentials to scavenge free radicals and protect cells from oxidative damage. Recycling of α tocopherol by ascorbate has been demonstrated in cellular organelles and erythrocyte membranes (17). It also acts as a co-factor for nicotinamide adenine dinucleotide phosphate (NADP) reductase required for glutathione metabolism (18). Furthermore, ascorbic acid can directly reduce methemoglobin and is proven to treat cyanosis effectively (16).

The objective of this study was to assess the neurobehavioral influences which were resulted from persist dosing of sodium nitrate (as food additive) in mice and the effect using of vitamin C (ascorbic acid) as antidote.

Materials and methods

The experiment was consisting of 50 mice of sexes, their body weights ranged between 30-35 g and their ages between 60-80 days. They were divided in to 5 equal groups ten for each; they were housed at room temperature with 10/14 light - dark cycle. The first group were gave a fed concentrated forage as a control group (Barley, Wheat, Soybeans, Corn and Bran) whereas, the treated groups were fed the same concentration forage with the addition of sodium nitrate (Gerhard Buchman Tuttingeen, Germany) in different concentrations 2nd group add 0.2% of sodium nitrate, 3rd group add 0.2% of sodium nitrate with 0.4% ascorbic acid, 4th group add 0.4% of sodium nitrate only and 5th group add 0.4% of sodium nitrate with 0.8% ascorbic acid as antidote respectively for five weeks. We based in our choices of sodium nitrate dosage on preliminary experiments in mice and they did not cause overt signs of toxicity in mice.

The behavior of animals was recorded on the first day of each week within the test period. The general behavior tests included: 3 minutes for the open-field activity (general locomotors activity) including the counting of the ambulation or squares crossed and rearing in a 60×60×30 cm box divided in to 24 equal squares (15). The investigations also involve measurement of negative geotaxis test was operated by placing the mice in a head down the location on the sloping surface at an angle of 45 °, and the time wanted (maximum 60 seconds) to complete 180° turn was measured (19). The body weights were organized on day 0 (pretreatment day -base line value) and thereafter throughout the study period on the first day weekly for 5 following weeks. All investigates were performed between 8:30 - 12:30 A.M. The data was subjected to two-way analysis of variation followed by the least significant difference test LSD according to Robert *et al.* (20). The measurement of body weight was statistically analyzed by frequent measurement analyzed of variance (21). The level of significance was at $P \leq 0.05$.

Results

In the present experiment the concentrations of sodium nitrate 0.2% did not produce overt signs of intoxication, but sodium nitrate only in a concentration 0.4% (group 4) lead $P \leq 0.05$ significantly reduced of rearing in 2nd, 3rd and 4th weeks in comparison with the control value (group 1), and also significantly decreased contrast with time 0 and with dose 0.2 (group 2), also a significant $P \leq 0.05$ increase of (group 5) in comparison with (group 4) in 2nd, 3rd and 4th weeks and increased significantly $P \leq 0.05$ value of 5th week in compared with the 3rd and 4th weeks in (group 4) (Table 1).

There were significant decrease number of squares crossed (open-field activity test) in (group 2) at 4th week comparing with the control group (group 1). The group treated with sodium nitrate 0.2% and ascorbic acid 0.4% (group 3) in 4th week significant P≤0.05 increase comparison with the sodium nitrate 0.2% alone (group 2), otherwise, in (group 4) lead P≤0.05 significantly decrease the number of squares in 3rd and 4th weeks comparing with group 1, group 2 and with the values at time 0. In 5th week (group 4) significantly P≤0.05 increased comparing with values for same group in 3rd and 4th weeks. (group 5) showed significantly P≤0.05 rise in 3rd and 4th weeks comparing with (group 4) at the same period (Table 2). The result of body weight test appeared the sodium nitrate at different doses led to a significant P≤0.05 increase of values compared with those of pretreatment values. Also, in the dose 0.4% mixed with ascorbic acid 0.8% (group 5) it caused increase significantly in comparing with the dose 0.4% (group 4) (Table 3). The result in the present study manifested a significant increase of negative geotaxis values in 3rd and 4th weeks in (group 4) compared with the control values (group 1), also it led to a significant P≤0.05 increase in comparing with the value of (group 5), and also similarly in (group 4) caused in 4th week increased significantly comparing with pretreatment and 5th week (Table 4).

Table 1: The effect of sodium nitrate on rearing in mice

Groups	Sodium nitrate concentration on rearing in mice : Mean ± SE (10 mice/group)					
	0 pre-treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
1st group	15.86±1.48	14.95±1.05	15.26±0.70	13.93±0.50	15.0±1.40	14.60±0.99
2nd group	15.10±1.27	13.9±0.65	13.50±0.78	12.64±0.65	12.65±0.92	13.90±0.90
3rd group	15.6±0.81	14.7±2.32	14.8±0.60	13.2±1.67	13.1±0.51	14.1±1.45
4th group	15.2±0.52	12.01±0.65	11.09±0.58*+a	5.24±1.65*+a	5.62±1.43*+a	12.62±0.56 cd
5th group	15.7±1.38	14.6±1.36	15.1±1.09b	14.49±1.09b	14.51±1.13b	14.8±0.49

+= vs. control group, *= P≤0.05, a= vs. 0.2% concentration, b= vs. 0.4% concentration, c= at 3 weeks, d= at 4 weeks.

Table 2: The effect of sodium nitrate on the open-field in mice / 3 minutes

Groups	Squire crosses mean (3 minutes) ± SE (10 mice/group)					
	0 pre-treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
1st group	55.7±2.99	57.4±3.01	57.1±2.83	58.1±8.51	59.0±7.83	57.5±6.98
2nd group	54.4±2.01	53.3±2.13	50.8±4.01	51.3±3.20	45.1±3.21+	54.3±5.98
3rd group	59.13±3.83	58.1±2.83	60.1±3.90	59.2±4.89	59.0±5.25a	58.9±5.10
4th group	64.0±2.81	57.1±3.01	48.4±7.02	25.2±7.89*+a	30.1±7.01*+a	58.4±1.65cd
5th group	59.62±3.37	58.5±4.20	62.4±1.01	62.9±6.51 b	61.9±4.80 b	59.2±3.96

+= vs. control group, *= P≤0.05, a= vs. 0.2% concentration, b= vs. 0.4% concentration, c= at 3 weeks, d= at 4 weeks.

Table 3: The effect of sodium nitrate on body weight (g) in mice

Groups	Body weight mean (g) ± SE (10 mice/group)					
	0 pre-treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
1st group	30.5±8.12	32.0±7.7*	33.7±7.2*	34.9±7.8*	35.1±6.81*	36.8±5.50*
2nd group	30.6±2.81	33.9±7.8*+	34.1±3.71*	34.6±2.98*	35.9±2.31*	37.1±4.41*
3rd group	30.5±4.18	33.8±0.41*	34.0±3.12*	34.5±3.61*	36.0±7.99*	37.5±7.21*
4th group	30.7±5.13	33.9±0.91*	34.2±7.09*	34.3±6.60*	35.0±9.12*	36.2±8.36*
5th group	32.3±7.01	35.2±3.49+b	36.1±7.04*+b	36.4±4.11*+b	36.9±3.67*+b	38.9±2.81*+b

+= vs. control group, *= P≤0.05, a= vs. 0.2% concentration, b= vs. 0.4% concentration, c= at 3 weeks, d= at 4 weeks.

Discussion

The main important results in the present experiment were dosed continuously dosing (in a food additive) with sodium nitrate caused behavioral alteration in mice. These effects indicate the general locomotors such as open-field activity, neuromotor performance and coordination as negative geotaxis as previously reported by Matt and Jennifer (19). These outcomes further support speculation that nitrate compound makes various behavioral changes in the laboratory animals (13,14) that could be observed by a battery of neurobehavioral tests evaluating different

functional aspects of the animals (22). Habituation to open-field activity test is expected in rodents (23). However, Habituation in this study was not a determining factor to modify the overall conclusion that explain nitrate effect which leads to decreases the open-field activity and negative geotaxis in mice because of offspring's is not the target here but only the adult.

Table 4: The effect of sodium nitrate on negative geotaxis in mice / 60 second

Groups	Geotaxis rate mean (60 second) ± SE (10 mice/group)					
	0 pre-treatment	1 week	2 weeks	3 weeks	4 weeks	5 weeks
1st group	6.1±0.98	5.7±1.09	6.0±1.54	5.8±1.19	6.1±2.12	6.2±0.99
2nd group	6.0±0.72	6.1±1.39	6.6±1.12	7.6±1.67	8.4±1.71	7.4±1.42
3rd group	6.1±0.45	5.7±0.67	6.1±2.01	5.7±1.43	5.6±0.53	6.0±0.99
4th group	7.0±0.37	7.4±2.30	8.1±1.21	10.2±2.12^{+}	12.0±2.7^{*+}	8.4±1.50
5th group	6.1±1.31	6.1±1.52	6.4±1.32	6.1±1.21^{b}	6.2±1.21^{b}	6.4±1.73

+= vs. control group, *= P≤0.05, a= vs. 0.2% concentration, b= vs. 0.4% concentration, c= at 3 weeks, d= at 4 weeks.

The outcomes with 0.2% concentration sodium nitrate was not significant decrease, may be due to the animal becoming adapted to nitrate (24) Similar to with those obtained by Shehata (25). The neurobehavioral effects of nitrate were appeared clear in the 4th week of treatment in a concentration 0.4% and gradually retrain in the 5th week in time 0 value (pretreatment) and that indicate the animal had habituated to nitrate because of same level feeding to sodium nitrate during a long period (26).

The overall results were manifested that ascorbic acid in 0.8% was the best antagonism effect of sodium nitrate with 0.4%, in addition to rise in body weights through all periods of experiment due to give all groups good concentrated forage these results not agree with those reported by Bassuny *et al*. (27). In 4th group a significant increscent was seen at zero time because adding nitrogenous compound (NH_3) to forage for a long period without ascorbic acid. This possibly due to antioxidant of ascorbic acid that has an inhibitory effect on conversion of nitrate to nitrite and nitric oxide, so it is known that nitrite is eight times toxic more than nitrate (28), and Suspected because ascorbic acid at high concentration has potential to scavenge free radicals and protect cells from oxidative damage (16). Although the mice in the present test was susceptible enough to detect behavioral changes throughout the course of the exposure for the early revealing of the subtle toxic influence of nitrate compounds, the animal manifests apparently healthful and that compatible to previous studies (4, 29,30).

Conclusion

Depending on the objectives of the present research and the results we deduced that the ascorbic acid gave an advantageous result when used for remedy of nitrate poisoning in animals. The results support the concept that in the absence of explicit sign of poisoning neurobehavioral tests could be used to identify adverse behavioral changes generated by sodium nitrate.

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