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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Introduction

Nitrate poisoning has been recorded in numerous studies (1,2) and it can happen in man and animals (3). Through enterohepatic 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 (Fe²⁺) to ferric iron (Fe³⁺), 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 Tuttinegen, 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 60x60x30 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 investigate 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≤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≤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≤0.05 increase of (group 5) in comparison with (group 4) in 2nd, 3rd and 4th weeks and increased significantly P≤0.05 value of 5th week in compared with the 3rd and 4th weeks in (group 4) (Table 1).
There were significant decreases in the 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** | 5.24±1.65** | 5.62±1.43** | 12.62±0.56** |
| 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** | 30.1±7.01** | 58.4±1.65ed |
| 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±4.18 | 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±3.49b | 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 locomotor 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
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±1.21b | 6.2±1.21b | 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).

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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Reference

1. Anjana, Umar S, Iqbal M. Nitrate accumulation in plants, factors affecting the process, and human health implications. A review. Agron Sustain develop. 2007; 27:45-57. https://doi.org/10.1051/agrsc006021
2. Jaafar, R.S. Bioremediation of lead and cadmium and the strive role of Pediococcus pentosaceus probiotic. Iraqi Journal of Veterinary Sciences,2020;34(1):51-57. https://doi.org/10.33899/ijvs.2019.12581.1092
3. Diane B, Ian A, Andrew C, Jean C, Eugenia D, Alessandro DD, Maria LF, Peter F, Johanna FG, Corrado LG, Philippe G, Jadwiga G, Gerhard H, Niklas I, Antonio M, Josef S, Rolaf VL, Carlos Van P, Philippe V. Nitrate as undesirable substances in animal feed. EFSA J. 2009;10:17:1-47. https://doi.org/10.2903/j.efsa.2009.1017
4. Alicia M, Fernando A, Riccardo C. Re-evaluation of sodium nitrate (E 251) and potassium nitrate (E 252) as food additives. EFSA J. 2017;15(6): https://doi.org/10.2903/j.efsa.2017.4787
5. Gladwin MT, Kim DB, Patel RP, Hogg N. Between nitrite and hemoglobin: the role of nitrite in hemoglobin-mediated hypoxic vasodilation. J Inorganic Biochem. 2005;99:237-246. https://doi.org/10.1016/j.jinorgbio.2004.10.034
6. Stoltenow, C. and Greg L. Nitrate Poisoning of Livestock. Fargo, ND. North Dakota State University. 1998. Accessed August 19, 2008. http://www.ag.ndsu.edu/pubs/anSci/livestoc/v839w.htm.
7. Jimmy T. Keeton. History of Nitrite and Nitrate in Food. Nitrite and Nitrate in Human Health and Disease. Book Chapter published 2017; p 85 – 97. https://doi.org/10.1007/978-3-319-46189-2_7
8. Andrew C, Gianfranco B, Maria LF, Davide A, Luisa RB, Bruce C, Carlos Van P, Jean LD. Nitrite in feed: From animal health to human health. Toxicology and Applied Pharmacology, 21 Nov 2010; 270 (3): 209-217. https://doi.org/10.1016/j.taap.2010.11.008
9. EFSA European Food Safety Authority. Nitrate in vegetables: Scientific opinion of the panel on contaminants in the food chain. EFSA J. 2008; 689:1-79. https://doi.org/10.2903/j.efsa.2008.689
10. EFSA European Food Safety Authority. Nitrite as undesirable substance in animal feed: Scientific opinion of the panel on contaminants in the food chain. EFSA J. 2009; 1017: 1-47. https://doi.org/10.2903/j.efsa.2009.1017
11. Mensinga TT, Speijers GJ, Meulenbelt J. Health implications of exposure to environmental nitrogenous compounds. Toxicol. 2003; 22 (1): 41-51. https://doi.org/10.2165/00139709-200322010-00005
12. Tanaka T. The relationships between litter size, offspring weight, and behavioral development in laboratory mice Mus musculus. Mammal. Study. 2004; 29: 147-153. https://doi.org/10.3106/mammalstudy.29.147
13. Toyohito T. Effects of maternal clothianidin exposure on behavioral development in F1 generation mice. 2012;28 (8): 1-5. https://doi.org/10.1177/0748233711422726
14. Douglas W. Mouse behavioral testing. 1st ed. New York: Academic Press; 2010; 12 (2): 288-288. https://doi.org/10.1111/j.1601-183x.2012.00864.x
15. Al-Khafaji NJ, Rhaymah MS. Changes in rat behavior induced by nitrite intoxication. Iraqi J Vet Sci. 1999; 12: 21-28. https://doi.org/10.33899/ijvs.2010.5570
16. Nihad A, Seyedeh PY, Seydeh MJ, Hamid S. Antioxidant effect of different vitamins on methemoglobin production: An in vitro study. Vet Res Forum. 2012; 3 (2): 97-101. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4312803/
17. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional food: Impact on human health. Pharmacogn Rev. 2010; 4 (8): 118-126. https://doi.org/10.4103/0973-7847.70902
18. Sebastian JP, Mark L. Vitamin C physiology: The known and the unknown and goldilocks. Oral Dis. 2016; 22 (6): 463-493. https://doi.org/10.1111/odi.12446
19. Matt C, Jennifer S. Animal behavior: Guide to research techniques in neuroscience. 2nd ed. New York: Blackwell; 2015; 39-71. https://doi.org/10.1016/b978-0-12-800511-8.00002-2
20. Robert GDS, James HT, David AD. Principles and procedures of statistics: A biometrical approach subsequent edition. Publisher: McGraw-Hill College; Subsequent edition 1996; p. 672. https://www.amazon.com/Principles-Procedures-Statistics-Biometrical-Approach/dp/0070610282
21. Thomas W. Mac F, Ian MY. Nonparametric Statistics for the Biological Sciences. Book Chapter published 2016 in Introduction to Nonparametric Statistics for the Biological Sciences Using R on 2016; 1-50. https://doi.org/10.1007/978-3-319-30634-6_1
22. Wier M, Anderson LM. Demand for organic foods attitudes, values and purchasing behaviors newsletter. Danish Res Center Farm. 2003; (2):1-3. http://orgprints.org/00001829
23. Anil K. Behavioral and neurochemical evidence of deltamethrin anxiogenic-like effects in rats. Braz J Vet Res Anim Sci. Evaluation of toxicological and behavioral symptoms on deltamethrin treated albino rats. J. Artic. published 23 Feb 2018 in MOJ Anatomy & Physiology. 2018; 5(1). https://doi.org/10.15406/mojap.2018.05.0016
24. Kvasnicka B, Krysl LJ. Nitrate Poisoning in Livestock. 1996; 1-3. http://www.iowabeefceter.com/bch/NitratoPoisoning.pdf
25. Shehata SA. Nitrate detoxification of drinking water by ascorbic acid in growing rabbits. World Rabbit Sci. 2005;13(2): 93-106. https://doi.org/10.4995/wrs.2005.526
26. Ozlem O, Firdevs M, Sima S, Aytan U. Pathological and toxicological investigations of chronic nitrate poisoning in cattle. 2004; 87 (1): 99-106. https://doi.org/10.1080/02772240400007104
27. Bassuny SM, Shehata SA, Bahgat LB, Mohamed SIA. Nitrate toxicity in rabbits: Effect of nitrate in drinking water on digestion, some blood constituents and growth performance of growing rabbits. Egyptian J Rabbit Sci. 2004; 14: 147-158. https://iopscience.iop.org/article/10.1088/1742-6596/1234/1/012072/pdf
28. Cheeke PR, Shull LR. Natural toxicants in feeds and poisonous plants. Avi publishing company, Westport, conn., USA. No. of pages: XII+492.ISBN-0–87055–482-4,1985; https://doi.org/10.1002/fj.2730010207
29. FASSETT, D.W. Nitrates and nitrates, Toxins occurring naturally in foods. Comm. in Food Protection. Washington D.C.: Natl. Acad. Sci., 1973; 7-25. https://www.nap.edu/read/21278/chapter/3
30. Al-Jammas S and Al-Saraj A. The histological changes induced by Cytarabine on rabbits livers (with and without vitamin E administration). Iraqi J of Veterinary Sciences, 2020; 33 (2): 9-13. DOI:10.33899/ijvs.2020.163564.

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