Research Article



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Sodium Nitrite Effects on Some Blood and Biochemical Parameters in Glutathione Treated Male Rats

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Abstract

In industrial food production, food preservatives like sodium nitrite are used more frequently. The use of some food preservatives has been linked to teratogenic and carcinogenic effects. In order to investigate the effects of food additives and natural antioxidant substances on rats forty adult male albino rats, approximately one month and a half were divided into four groups as follows, Group 1 serves as control group supplement with (Nacl 0.9% normal saline). Group 2 received only sodium nitrite supplement (30 mg/kg BW), group 3 supplemented initially with sodium nitrite and glutathione and group 4 supplemented with glutathione alone (6.42 mg/kg BW). A variety of parameters were measured, including the number of red and white blood cells (RBCs & WBCs), hemoglobin (Hb) level, hematocrit (Hct) value, glucose level, and serum alanine transaminase and aspartate transaminase (ALT & AST) activity analysis. The results clearly showed that (RBCs), (Hb) concentration, and (Hct) value of rats treated with sodium nitrite for two months exhibit significant decrease when compared to the control and treated groups, rats given with either glutathione alone or glutathione plus sodium nitrite showed significant decrease in their serum glucose levels and there was discernible elevation in the serum activity of AST and ALT in rats treated with glutathione alone. Rats treated with sodium nitrite showed a substantial decrease (P < 0.05) in AST activity after 2months when compared with the control group, whereas significant increase (P < 0.05) in ALT activity were detected after 2months. These results indicate the harmful effects of using artificial supplements and colorings and also prove the improving effect of glutathione. Due to the potentially harmful effects of food additives, it is advised that the use of S. N. as an additive be kept to a minimum. Key Words: Sodium nitrite, Food additive, Glutathione, Rats, carcinogenic.

Introduction

Elements that are purposefully added to food as food additives either manufactured or natural, one of these main preservatives are nitrites, which can be found as free acids or salts. Sodium nitrite is frequently used as a preservative in cooked meat products like sausages (1). The proportion of nitrite content daily food consumption may be higher than the allowable amount due to the usage of multiple types of such foods (2). As food additives for humans, nitrites are mostly employed to produce a particular flavor and preserve meat products. Many organic nitrites and nitrates have been employed in clinical settings, but sodium nitrite is the only inorganic nitrite that has been shown to be therapeutic (3).

Environmental contaminants found in food and water such as nitrites and nitrates may play a role in the development of liver and renal disorders as well as immune system issues in domestic poultry)4) to prevent oxidative damage, glutathione (GSH) may function as a free radical acceptor. Leichtweis and Ji showed oral GSH administration raises plasma GSH levels and that dietary GSH can be absorbed intact from the intestinal lumen (5). Because GSH is a substrate for glutathione peroxidase, it helps shield cells from harmful substances and oxidative stress (3). It was mentioned by (6) a sensitive biochemical biomarker of chemical contamination is the glutathione system. Our study's objective was to elucidate the deleterious impacts of sodium nitrate on specific blood images and physiological metrics, as well as the prophylactic role of glutathione.

Material and Methods

In this investigation, forty adult male albino rats, approximately one month and a half old were used. Rats range in weight from 100 to 150 g. The cages housing the animals were hygienic. Water and food were added freely. Experimental animals were divided into four groups (10 for each/group) as follows:

Group 1 serves as control group and supplements with Nacl 0.9% normal saline.

Group 2 received a sodium nitrite supplement 30 mg/kg bw.

Group 3 supplemented initially with sodium nitrite and glutathione.

Group 4 supplemented with glutathione 6.42 mg/kg bw.

For two months, these therapies were administered orally through a stomach tube. Animals were decapitated at the end of the experiment, and blood samples were collected in sterile, dry centrifuge tubes. Before analysis, the serum was separated and stored at -20 °C. Heparinized capillary tubes were used to collect blood samples for hematological examination at the same time. Measured Parameters:

A number of variables were recorded. The count of white and red blood cells, Hemoglobin, Hematocrit, glucose value and serum levels of aspartate transaminase and alanine transaminase (ALT & AST) activity was measured (7).

Results and Discussion

The red blood corpuscles (RBCs), hemoglobin (Hb) concentration, and hematocrit (Hct) value of rats treated with sodium nitrite for two months exhibit significant decrease when compared to the control and treated groups according to data in table (1). Additionally; table (1) showed that there was a noteworthy reduction in the quantity of white blood cells across all three groups when compared to the control group, with the glutathione-treated group exhibiting the greatest significant rise.

Table 1: The effect of sodium nitrite and glutathione alone and their combination on blood parameters in male rats.

Parameters\group	RBC(n×106/mm ³)	Hb(g\dl)	PCV%	WBC(n×103/mm ³)
	7.846a	15.151 b	47.071 c	11.518 a
Group (1)	±0.032	± 0.024	± 0.358	± 0.034
	5.388 d	13.110 d	38.357 d	7.432 d
Group (2)	± 0.031	± 0.041	± 0.048	±0.023
	7.519 c	16.110 a ±	48.421 b	9.632 C
Group (3) Group (4)	± 0.016	0.040	± 0.013	±0.023
	7.629b	14.569 c	50.722 a	10.174 b
	± 0.025	± 0.040	±0.516	± 0.037
LSD	0.11	0.58	1.35	0.54

*Small different letters denote significant differences between groups (p≤0.05).

Table 2's data showed that, after two months, rats given with either glutathione alone or glutathione plus sodium nitrite showed significant decrease in their serum glucose levels. After two months, there was discernible elevation in the serum activity of AST and ALT in rats treated with glutathione alone. On the other hand, rats treated with sodium nitrite showed a substantial decrease (P< 0.05) in AST activity after 2months when compared with the control group, whereas significant increase (P< 0.05) in ALT activity were detected after 2months. The RBC count, Hb concentration, and Hct level in the sodium nitrite-treated groups decreased significantly, according to the study's data due to the potentially harmful effects of sodium nitrite on the liver, spleen, and bone marrow, the decline may be explained by microcytic and/or hypochromic anemia (8). The authors (8 and 9) report that methmoglobin (met Hb) levels increased noticeably along with this decline, indicating that treatment with sodium nitrite may have been the cause (9).

Parameters \group	Glucose (mg\dl)	AST(IU\L)	ALT(IU\L)
Group (1)	99.334 a	27.920 с	24.522 c
	± 0.025	± 0.014	±0.013
Group (2)	82.833 d	55.400 a	38.331 a
	± 1.964	±0.253	± 0.017
Group (3)	95.522 c	31.925 b	26.721 b
	± 0.035	± 0.015	± 0.012
Group (4)	96.433 b	25.628 d	21.132 d
	± 0.042	± 0.016	± 0.017
LSD	0.91	2.29	2.19

 Table 2: The effect of sodium nitrite and glutathione alone and their combination on biochemical parameters glucose, ALT and AST on male rats.

*Small different letters denote to significant differences between groups (p≤0.05).

There was a notable decline in met hemoglobin (met Hb) levels concurrent with this decrease suggesting that sodium nitrite treatment may have caused it (9).

It is well known that the nitrite changes the ferrous to ferric ions in hemoglobin. Sodium nitrite rise met Hb, but had no effect on RBC hemolysis (10 & 11).

When there is toxicity, the liver performs an essential physiological role by getting rid of the toxicants once they have digested and have dropped dramatically in just two months. Our results are in agreement with (12). The current results showed a minimal decline in serum glucose levels in rats treated with sodium nitrite. The liver is an essential physiological organ in cases of poisoning because it gets rid of the toxicants when they are broken down and digested. This leads to a breach in the integrity of the cell membrane, which increases the amount of enzymes in the blood (12). According to the current findings, rats given sodium nitrite had significantly higher serum AST and ALT activity. Increased blood transfer activity has been used as a marker for tissue injury. However, additional elements are taken into account for this process, such as changes in the cell membrane's permeability, an increase in the synthesis of the enzyme, or a decrease in the pace at which the enzyme degrades (13).

Correspondingly to recent researches the breakdown and necrosis of liver cells, along with cell wall destruction and cytolysis, are the causes of the elevation in serum AST and release a significant amount of these enzymes mitochondrial into the bloodstream. On the other hand, oxidation of significant iron-containing enzymes, such as cytochromes, which are involved in cellular respiration and other oxidation-reduction processes, may be the cause of nitrite's harmful effects on the liver. Rats given sodium nitrite treatment showed а

substantial rise in blood AST and ALT activity, according to (14).

Also other researchers hypothesized that the observed enhancement of ALT activity was due to sodium nitrite contact with the enzyme molecule rather than with the tissues (15). As highly sensitive liver indicators, the activities of ALT and AST are generally markedly elevated after the administration of numerous hepatotoxic drugs, resulting in hepatocellular destruction acute or extrahepatic blockages Petra et al., linked increased ALT and AST activities to liver cell injury, while (16) claim that coloring chemicals cause damage to liver tissue and significantly increase ALT, AST, and alkaline phosphatase.

Statistical analysis

All normally distributed data were reported as means \pm SEM and were analyzed using the independent sample t-test and Pearson correlations, respectively, (SPSS, version 20.0, Chicago, IL, USA), to examine the differences in physiological and biochemical values for correlation analysis.When P < 0.05, the differences were deemed significant in (17).

Conclusion

Food additives such as sodium nitrite can have dangerous effects; thus, it is best to utilize toxic compounds sparingly and offset any harmful effects with antioxidants like glutathione.

References

1. Fletcher M. T. and Netzel G. (2020): Editorial Food Safety and Natural Toxins. Toxins; 12(4):236.

- Soltan S. S. A. and Shehata M. M. E. (2021): The Effects of Using Color Foods of Children on Immunity Properties and Liver, Kidney on Rats," *Food and Nutrition Sciences*, 3 (7), 2012, pp. 897-904.
- Helal E. G. E. (2001): The Protective Role Of Royal Jelly Against Sodium Nitrate And Sun-Set Yellow Toxicity In Albino Rats *The Egyptian Journal of Hospital Medicine* 2: 121 – 137.
- 4. Hall J.O. (2018): Nitrate- and Nitrite-Accumulating Plants. Gupta RC, ed. *Veterinary Toxicology; (3)*; 941–946.
- Leichtweis S. and Ji L. (2001): Glutathione deficiency intensifies ischaemia-reperfusion induced cardiac dysfunction and oxidative stress. *Acta Physiol Scand.; 172* (1):1-10.
- Paskerová H., Hilscherová K., Bláha L
 (2012): Oxidative stress and detoxification biomarker responses in aquatic freshwater vertebrates exposed to microcystins and cyanobacterial biomass. *Environ Sci Pollut Res. Int.* 19(6):2024-37.
- 7. Zhou Q., Zhu J., Liu B., Qiu J., Lu X., Curtin B., Ji F. and Yu D.(2021): Effects of High-Dose of Copper Amino Acid Complex on Laying Performance, Hematological and Biochemical Parameters, Index. Organ and Histopathology in Laying Hens. Biological Trace Element Research 199:3045-3052.
- Kraemer M. V., Fernandes A. C., Chaddad M. C. C., Uggioni P. L., Rodrigues V. M., Bernardo G. L., and Proença R. P. (2022): Food additives in childhood: a review on consumption and health consequences Rev. *Saude. Publica.*; 56: 32.
- 9. Hou Y, Michiels J, Kerschaver CV, Vandaele M, Majdeddin M, Vossen E and Degroote J.(2023). The kinetics of glutathione in the gastrointestinal tract

of weaned piglets supplemented with different doses of dietary reduced glutathione. *Front Vet Sci.Aug* 10:10:1220213.

- Al-Attar, A. and Zari, T. (2007): Modulatory effects of ginger and clove oils on physiological responses in streptozotocin-induced diabetic rats. *International Journal of Pharmacology*, 3, 34-40.
- Mondal S., Jalal M. R., M., Khan S. A., Kumar U., Rahman R., Hamidul H., (2013): "Hydro-Meteorological Trends in Southwest Coastal Bangladesh: Perspectives of Climate Change and Human Interventions. *American Journal* of Climate Change. 2 (1); 2167-9509.
- 12. Samanta P, Mukherjee AK, Ghosh AR. (2014): Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. *Biomed Res Int., Jun 12*: 425159.

- 13. Al-Logmani A. and Zari T. (2011): Long-term effects of Nigella sativa L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. *Journal of Diabetes Mellitus, 1*, 46-53.
- Eman, G.E., Samir, A.M., Hamdy, A., (2000): Effect of some food colorants (syntheticand natural products) of young albino rats liver and kidney functions. *Egypt.J. Hosp. Med. 1*, 103–113.
- 15. Petra A., Hana K.,and Jana R. (2015). Health safety issues of synthetic food colorants Regulatory *Toxicology and Pharmacology*. 73(3); 914-922.
- 16. Marlissa C. (2000): Evidence on developmental and reproductive toxicity of sodium nitrite.
- 17. Walters SJ, Campbell MJ, Machin D (2021). Medical statistics: A textbook for the health sciences.

تأثير نتريت الصوديوم على بعض المعايير الدموية والكيميائية الحيوية في ذكور الجرذان المعالجة بالجلوتاثيون

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الخلاصة

في إنتاج الأغذية الصناعية يتم استخدام المواد الحافظة الغذائية مثل نتريت الصوديوم بشكل متكرر. تم ربط استخدام بعض هذه المواد الحافظة الغذائية بالتأثير إت المسخية والمسرطنة. من أجل التحقق من تأثير المضافات الغذائية والمواد الطبيعية المضادة للأكسدة على الجرذان الأربعين من ذكور الجرذان البيضاء البالغة، تم تقسيمهم لمدة شهر ونصف تقريباً إلى أربع مجموعات على النحو التالي، المجموعة (1) هي مجموعة السيطرة اعطيت المحلول الملحي الاعتيادي بنسبة 9و0% تلقت المجموعة (2) نتريت الصوديوم لوحده (30 مجم / كجم من وزن الجسم) والمجموعة (3) اعطيت نتريت الصوديوم (30 مجم / كجم من وزن الجسم) والجلوتاثيون(642 مجم / كجم من وزن الجسم). والمجموعة (4) اعطيت الجلوتاثيون لوحده (642 مجم / كجم من وزن الجسم). تم قياس مجموعة متنوعة من المعاملات، بما في ذلك عدد خلايا الدم الحمراء والبيضاء ومستوى خضاب الدم والهيماتوكريت. ومستوى الجلوكوز وتحليل نشاط ناقلات أمين الألانين والاسبارتات. أظهرت النتائج بوضوح أن عدد كرات الدم الحمراء وتركيز الهيموجلوبين ومستوى الهيماتوكريت في الجرذان المعاملة بنتريت الصوديوم لمدة شهرين اظهرت انخفاضا معنويا عند مقارنتها بمجموعتي السيطرة والمعاملة, الجرذان التي أعطيت إما الجلوتاثيون وحده أو الجلوتاثيون زائد نتريت الصوديوم اظهرت انخفاضًا معنويا في مستويات الجلوكوز في الدم وكان هناك ارتفاع ملحوظ في نشاط الجرذان المعاملة بنتريت الصوديوم اظهرت انخفاضا واضحا في نشاط ALT &AST. ناقلات الاسبارتات بعد شهرين من المعاملة عند مقارنتها مع مجموعة السيطرة. بينما كان هنالك زيادة معنوية(< 0.05) في نشاط ناقلات الالانين التي تمت ملاحظتها بعد شهرين من المعالجة. تشير هذه النتائج إلى الآثار الضارة لاستخدام المكملات الغذائية والملونات الصناعية وتثبت أيضًا التأثير المحسن للجلوتاثيون, ونظرًا للآثار الضارة المحتملة للمضافات الغذائية يُنصح بتقليل استخدام نتريت الصوديوم كمضافات غذائبة الي الحد الادني.

الكلمات المفتاحية: ، نتريت الصوديوم, المضافات الغذائية, الجلوتاثيون، الجرذان ، مسرطن.