Oralsodium Nitrite Enhanced Oxidative Stress and Inflammatory Cytokines Levels in Testicular Tissue

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Abstract
Building bases of scientific evidence about nitrite in humans continues to oppose the general safety in human health. Therefore, we investigate the testicular tissue inflammation caused by oral administration of sodium nitrite. Twenty adult male SD rats were used. Ten rats received oral 80mg/kg sodium nitrite daily for twelve weeks; the other rats were left as negative control. Testis were weighed and the testicular tissue homogenates were used for measurements of Malondialdehyde (MDA), reduced glutathione, Tumor Necrosis Factor (TNF)-α and Interleukin (IL)-1β. Sodium nitrite resulted in significant increase in testis weight and Gonado-Somatic index as well as testicular tissue level of MDA, TNF-α and IL-1β. Moreover, oral sodium nitrite resulted in significant reduction in testicular tissue concentration of reduced glutathione. In conclusion, chronic oral sodium nitrite induced changes in the weight of rat testis accompanied by elevation in the testicular tissue level of oxidative stress markers and inflammatory cytokines.

Keywords: IL-1β; MDA; Reduced Glutathione; TNF-α

Abbreviations: IL-1β: Interleukin-1β; MDA: Malondialdehyde; TNF-α: Tumor Necrosis Factor-α

Introduction
Nitrates are widely used in food preservation systems [15]. Some epidemiological studies reveal association between foods that contain nitrite, namely cured and processed meats, and cancer [10]. Sodium nitrite is generally accepted as a weak carcinogen. Exposure to higher levels of nitrites has been associated with increased incidence of brain tumors, leukemia and nasopharyngeal tumors in children [9]. We have previously evaluated the sodium nitrite-induced detrimental effect in different body organs of rats due to its oxidative properties, fibrosis and inflammation [2,1, 12-14].

The lack of a beneficial effect of sodium nitrite in the testis empathizes that further investigations are required to confirm sodium nitrite-induced abnormalities in male SD rats. Therefore, we conducted this study to evaluate the health risks on testicular tissue associated with dietary sources of sodium nitrates.

Materials and Methods
Animals and their treatment outlines

Ethical committee in Faculty of Pharmacy, University of Mansoura, approved the animal protocol. Twenty adult male Sprague Dawely (SD) rats weighing 120-140 g were used. Rats were classified into two groups with 10 rats each:

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Control group: Rats received standard diet without any treatment and served as negative control group.

Sodium nitrite group: Rats received standard diet and given daily 80 mg/kg sodium nitrite orally for 12 weeks. The doses and time course of experiments used for sodium nitrite were in the range of those used in other studies [2,1]. In addition, the dose was determined after appropriate preliminary experiments.

Animal scarification and collection of samples
Animals were sacrificed by decapitation. Rats’ testis were removed, cleaned, weighed and chilled on crushed ice. A part of the testis was homogenized in a 10-fold volume of ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl and were centrifuged at 600g at 4°C for 10 minutes. The supernatant was referred to as homogenate, stored at –80°C until used. Gonado-somatic index was determined with the help of the formula [7]:

\[
\text{Gonado-Somatic Index (GSI)} = \frac{\text{Gonad weight/total body weight}}{\times 100}
\]

Where Gonad weight = (weight of the right testis + weight of the left testis)/2

Assessment of Oxidative Stress
Testicular Level of Malondialdehyde (MDA) was measured by thiobarbituric acid as described previously by our group [13].

Testicular level of reduced glutathione was determined by the method of Beutler [3].

Elisa Determination
Testicular levels of TNF-α and IL-1β were measured using ELISA kits (eBioscience Inc., San Diego, CA, USA) in accordance with the manufacturer’s instructions.

Statistical Analysis
The mean values ± standard error was used for quantitative variables. Normality of the sample distribution of each continuous variable was tested with the Kolmogorov–Smirnov (K–S) test. For comparison between two groups student t-test was used. Statistical computations were done on a personal computer using SPSS version 20 (Chicago, IL, USA). Statistical significance was predefined as P ≤ 0.05.

Results
Effect of sodium nitrite on testis weight
Chronic oral treatment of rats with sodium nitrite resulted in significant increase in both testis weight (1.5-fold) and Gonado-Somatic Index (1.8-fold) compared with the control group (Figure 1).

Figure 1: Effect of oral 80mg/kg sodium nitrite for twelve weeks on testis weight (a) and Gonado-somatic index (b)*: significant difference as compared with the control groups at p<0.05.
Effect of sodium nitrite on testicular tissue oxidative stress

Sodium nitrite caused 2.29-fold increase in testicular tissue level of MDA that was associated with 61% reduction in testicular concentration of reduced glutathione as compared with the control group (Figure 2).

Effect of sodium nitrite on inflammatory cytokines

As shown in figure 3, oral administration of 80 mg/kg/day sodium nitrite caused significant increase testicular levels of both TNF-α and IL-1β as compared with the control groups.

Discussion

Sodium nitrite is considered an important antimicrobial that added to food over than 5000 years. It delays the development of botulinum toxin in cured meat. In addition, it enhances the flavor and color of meat [16]. Sodium nitrite may react with food amines to produce nitrosamines and other free radicals, which can be harmful for many body organs. In early 1980s, there were numerous studies of the relation between N-nitrosoamines and human cancer [5]. Although many previous studies illustrated the bad effects of sodium nitrite on different...
body organs, no previous study illustrated such an effect of oral sodium nitrite on rats’ testis. Therefore, the current study was undertaken to determine whether sodium nitrite could enhance the oxidative stress markers and pro-inflammatory cytokines in male rats’ testis.

We found that treatment of rats with sodium nitrite resulted in increase in both testis weight and Gonado-Somatic Index. No previous study illustrated such an effect of oral sodium nitrite on rats’ testis. Next, we tried to find out the mechanism of sodium nitrite harmful effects on testicular tissues. However, sodium nitrite resulted in enhanced oxidative stress markers in testicular tissues. Previous studies illustrated increased oxidative stress markers after sodium nitrite in different rat organs [8,12,1], but no previous study measured oxidative stress in testicular tissue. Nitrite interacts with the amines of food inside body producing nitrosamines and the other endogenous free radicals leading to enhanced lipid peroxidation, DNA lesions, enzyme inactivation and damage to organs [4]. In turn, elevated lipid peroxidation might cause inhibition of Na⁺, K⁺-ATPase activity leading to deterioration of membrane fluidity and subsequent neuropathological conditions, including neurodegenerative disorders [6].

We examined the effect of sodium nitrite on the testicular tissues levels of pro-inflammatory cytokines. We observed significant elevation in TNF-α and IL-1β in sodium nitrite group. Similarly, many studies illustrated increased inflammatory cytokines in different organs of rats apart from the testicular tissues [17, 2, 14]. This result may be attributed to the ability of sodium nitrite to initiate peroxidative damage to the cell as well as to activate pro-inflammatory cytokines. Many stimuli have been reported to upregulate of proinflammatory cytokines, including TNF-α, through oxidative stress and activation of NFκB [11].

**Conclusions**

Sodium nitrite induced elevation in the weight of testis that is accompanied by elevation in the testicular tissue level of oxidative stress and inflammatory cytokines (Figure 4).

**Figure 4**: Schematic representation of the mechanism of action of sodium nitrite-induced damage to testicular tissues of rats.

**References**