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Effects of Different Sodium Nitrate Treatments on Rat Serum and Their Impact on CAT2 Gene Expression

Narmeen Abdullah Ali Al-Sabaawi [1] , Assist. Prof. Dr. Buthaina Jasim Yousif [2]

naaueps56@st.tu.edu.iq[1] buthaina.j.yousif@tu.edu.iq[2]

[1.2] **Tikrit University / College Council of Education for Pure Sciences / Department of Biology**

I. Abstract

This study aimed to investigate the effects of sodium nitrate ($NaNO_3$) on CAT2 gene expression in Wistar albino rats as a molecular indicator of oxidative stress. A total of 32 female rats, aged 35–60 days and weighing 60–100 g, were randomly divided into control and treatment groups and maintained under standard laboratory conditions throughout the experimental period. Sodium nitrate was administered orally once daily using gastric gavage at concentrations of 0.01 and 0.06 mg/kg body weight, while the control group received distilled water only. Molecular analysis revealed dose-dependent alterations in CAT2 gene expression. Low concentrations induced significant upregulation of CAT2 expression, reaching up to 3.4-fold change, indicating activation of antioxidant defense mechanisms. Conversely, higher concentrations or prolonged exposure resulted in marked downregulation in some groups, reaching 0.1-fold change, suggesting oxidative stress–induced depletion of endogenous antioxidant systems. These findings indicate that sodium nitrate exerts dual biological effects depending on concentration and exposure conditions, and confirm the importance of CAT2 as a reliable biomarker for oxidative stress assessment.

.**Keywords**: Sodium nitrate, oxidative stress, CAT2, gene expression, antioxidants, Wistar rats, ROS, food additives.

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II. Introduction

Food additives are widely used in the food industry to preserve food quality, inhibit microbial growth, and enhance sensory characteristics such as color, flavor, and shelf life. Among the most frequently used preservatives are sodium nitrates and nitrites, especially in processed meat products, because of their antimicrobial activity and their role in maintaining product appearance and stability. In addition, antioxidants such as ascorbic acid are often added to reduce oxidation and improve food preservation [1] Despite their technological importance, chronic exposure to nitrate-containing additives has attracted scientific concern due to their potential biological and toxicological effects [2].

Inside the body, sodium nitrate may be converted into nitrite and subsequently into reactive nitrogen compounds and N-nitrosamines, particularly under acidic gastrointestinal conditions. These metabolites are considered harmful because they can induce oxidative stress through the excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3]. Oxidative stress results from an imbalance between oxidant production and the antioxidant defense system, leading to lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and cellular injury. Continuous oxidative stress has been associated with inflammation, metabolic disturbances, mutagenesis, and the development of several chronic diseases[4] .

To protect cells from oxidative damage, the body possesses a complex antioxidant defense system that includes both enzymatic and non-enzymatic antioxidants. Among the most important enzymatic antioxidants is catalase, a heme-containing tetrameric enzyme primarily localized within cellular peroxisomes. Catalase represents one of the first lines of defense against oxidative injury because of its ability to rapidly detoxify hydrogen peroxide (H_2O_2), which is considered one of the major reactive oxygen intermediates generated during normal metabolism and under toxicological conditions [5].

Hydrogen peroxide is continuously produced in cells through mitochondrial respiration, fatty acid oxidation, inflammatory reactions, and xenobiotic metabolism. Although low concentrations of H_2O_2 may function in cellular signaling pathways, excessive accumulation becomes highly toxic because it can generate hydroxyl radicals through Fenton-type reactions, causing severe oxidative damage to cellular macromolecules. Catalase prevents this toxicity by converting hydrogen peroxide into harmless water and oxygen with extremely high catalytic efficiency, thereby preserving cellular redox balance and protecting tissues from oxidative degeneration [6].

Catalase activity is closely associated with oxidative stress status and is regulated at both enzymatic and genetic levels. The CAT2 gene encodes catalase-related antioxidant activity and is considered an important molecular biomarker for evaluating oxidative stress responses. Changes in CAT2 gene expression may reflect alterations in cellular antioxidant capacity under toxic or inflammatory conditions. Experimental studies have demonstrated that exposure to nitrates and nitrites can disrupt antioxidant enzyme systems by modulating the expression of catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), leading to impaired detoxification of reactive oxygen species [7].

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Furthermore, catalase plays an important role in maintaining mitochondrial integrity and regulating apoptosis pathways. Reduced catalase activity may increase intracellular peroxide accumulation, which subsequently activates inflammatory signaling pathways such as nuclear factor kappa B (NF-κB) and alters the activity of nuclear factor erythroid 2-related factor 2 (Nrf2), a major regulator of antioxidant gene expression. Consequently, suppression of catalase expression has been linked to increased oxidative tissue injury, inflammatory responses, and cellular dysfunction in several experimental models[8].

Recent toxicological investigations in rodents have shown that nitrate compounds can accumulate in different tissues depending on metabolic and physiological conditions, making molecular assessment of antioxidant genes particularly important in evaluating tissue responses to food additive exposure. In addition, international food safety organizations such as the European Food Safety Authority and the Joint FAO/WHO Expert Committee on Food Additives have emphasized the need to assess the long-term biological effects of food additives and their associated oxidative and metabolic consequences [9].

Therefore, the present study aimed to investigate the effect of sodium nitrate ($NaNO_3$) exposure on CAT2 gene expression levels in white rat serum as a molecular biomarker of oxidative stress and antioxidant defense alterations.

Material and Method

Experimental Animals and Study Design

The present experimental study was conducted using 32 healthy female Wistar albino rats obtained from the animal facilities of the College of Veterinary Medicine at Tikrit University. The animals were aged between 35–60 days and weighed approximately 60–100 g at the beginning of the experiment. Prior to treatment initiation, all animals were acclimatized for one week under standard laboratory conditions to minimize environmental and physiological stress.

The rats were randomly divided into six experimental groups, with each group containing an equal number of animals to ensure statistical homogeneity and improve the reliability of molecular analysis. The experimental groups consisted of one negative control group and five sodium nitrate-treated groups receiving different concentrations and exposure durations according to the study protocol. The control group received distilled water only, whereas the treated groups received sodium nitrate ($NaNO_3$) at predetermined doses.

Sodium nitrate solutions were freshly prepared and administered orally once daily using gastric gavage to ensure accurate dosing and uniform exposure among experimental animals. The treatment period and dosage concentrations were selected according to toxicological and molecular study requirements aimed at evaluating alterations in CAT2 gene expression as a biomarker of oxidative stress.

Animals were housed in plastic cages measuring 60 × 30 × 25 cm containing clean wood shavings that were replaced every three days. Environmental conditions were maintained under standardized laboratory parameters including a controlled temperature of (25 ± 2) °C, adequate ventilation, and a 12 h light/dark

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cycle. Hygienic practices were strictly followed throughout the experimental period, and cages were regularly disinfected using Dettol solution and 70% ethyl alcohol to minimize microbial contamination and environmental stressors that could interfere with gene expression analysis.

Because the present investigation involved molecular assessment of CAT2 gene expression, maintaining controlled experimental conditions and sufficient biological replication was considered essential for obtaining accurate and reproducible genetic data. Therefore, the number of animals included in each group was selected to improve statistical validity and reduce variability associated with oxidative stress biomarker analysis.

ffective Dose Determination

The effective dose of sodium nitrate ($NaNO_3$) refers to the lowest concentration of the compound capable of producing a measurable biological response without inducing severe toxic effects or mortality in experimental animals. In the present study, sodium nitrate was used as the tested chemical substance to evaluate its effect on CAT2 gene expression and oxidative stress biomarkers in white rats.

Dose calculation per rat:

Dose calculations per rat were made following the equation:

Dose per rat = (Body weight of rat in kg) * (Desired conc mg/kg)

Accordingly, the volume (in ml) of the solution needed to administer to each rat was calculated.

Experimental Design and Laboratory Animals

The present study entitled *Effects of Different Sodium Nitrate Treatments on Rat Serum and Their Impact on CAT2 Gene Expression* was conducted using 32 Wistar albino rats (*Rattus norvegicus*). Animals were randomly divided into control and treatment groups, with approximately 3–4 rats per group, and maintained under standard laboratory conditions throughout the experiment.

The treatments included sodium nitrate ($NaNO_3$), potassium nitrate (KNO_3), and vitamin C at two concentrations (0.01 and 0.06 mg/kg body weight). All compounds were administered orally once daily using gastric gavage, while the control group received distilled water only.

At the end of the experimental period, blood samples were collected for molecular analysis of CAT2 gene expression as a biomarker of oxidative stress, and the obtained data were statistically analyzed to evaluate treatment effects.

RNA Extraction

Total RNA was extracted from blood samples using the TransZol Up Plus RNA Kit (TransGen Biotech, China) according to the manufacturer's instructions. The purity and concentration of RNA were assessed

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using a NanoDrop spectrophotometer, and samples with acceptable purity ratios were used for downstream analysis. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcription kit (Thermo Fisher Scientific, USA) following standard reaction conditions.

Results and Discussion

Different sodium nitrate treatments in rat serum and the effect on CAT2 gene expression.

The present study demonstrated that exposure to sodium nitrate ($NaNO_3$) resulted in a significant modulation of CAT2 gene expression in rat serum compared with the control group. Data analysis revealed a clear concentration-dependent response, where higher $NaNO_3$ exposure (0.06 mg/kg) induced a greater upregulation of CAT2 expression than the lower concentration (0.01 mg/kg), indicating a positive dose–response relationship.

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine differences between experimental groups. The results showed statistically significant differences among treated groups compared with the control ($p < 0.05$). Data were expressed as mean ± standard deviation (SD), and variability between replicates was represented accordingly.

The observed upregulation of CAT2 gene expression suggests an adaptive cellular response to oxidative challenge. Catalase, encoded by antioxidant-related genes including CAT2, plays a central role in detoxifying hydrogen peroxide (H_2O_2) by converting it into water and oxygen, thereby limiting the accumulation of reactive oxygen species (ROS) and protecting cells from oxidative injury.

The increase in CAT2 expression observed in this study may be explained by the metabolic conversion of sodium nitrate into nitrite and subsequently into reactive nitrogen species under physiological conditions. These compounds are known to enhance ROS generation, leading to oxidative stress, which in turn activates antioxidant defense mechanisms as a compensatory response.

These findings are consistent with previous studies reporting that exposure to nitrogen-containing compounds can activate oxidative stress pathways and induce the upregulation of antioxidant enzymes as a protective mechanism against cellular damage (e.g., catalase and superoxide dismutase) [10,11]. Furthermore, it has been suggested that transcriptional activation of antioxidant genes is mediated through redox-sensitive signaling pathways, including Nrf2 and NF-κB, which regulate cellular responses to oxidative imbalance.

However, it is important to note that sustained or excessive exposure to sodium nitrate may lead to depletion of antioxidant defenses, mitochondrial dysfunction, and impaired redox homeostasis. Several studies have reported that prolonged oxidative stress can eventually suppress antioxidant enzyme efficiency, resulting in cellular injury rather than protection [12].

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Table (1): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the First Group

Treatment	Dose (mg/kg)	CAT2 Expression (Fold Change)
$NaNO_3$	0.01	~2.7
$NaNO_3$	0.06	~3.1

The responses in the second group showed a more disparate trend as compared to the first. The CAT2 gene showed a smaller increase than the first group, indicating that the antioxidant response in this group may be less efficient or possibly delayed, indicating an imbalanced oxidative stress state. In addition, a decrease in the expression of CAT2 relative to the first group could suggest that the antioxidant defense system was unable to cope with a strong ROS increase, resulting in an uncontrolled oxidative stress status. This finding is in agreement with [13], who found that antioxidant defense systems can be exhausted in both chronic or high exposure to oxidants.

Table (2): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the Second Group

Treatment ($NaNO_3$)	Dose (mg/kg)	CAT2 Gene Expression (Fold Change)
$NaNO_3$	0.01	~0.3
$NaNO_3$	0.06	~0.1

The results of the last group showed an evident and simultaneous increase of CAT2 gene expression induced by $NaNO_3$ when compared with all other categories ($NaNO_3$ 1 and $NaNO_3$ 6). The values of these treatments were highest when compared with the other experimental groups, demonstrating a robust induction of oxidative stress and also inflammation simultaneously. Findings warranting the statement that the effect of sodium nitrie in this group not only evoke oxidation damage but it also activate integrated stress responses effort to overcome these environmental contition. This pattern can be interpreted by the metabolism of sodium nitrate to nitrite and subsequently nitrosative compounds that lead to the generation of reactive oxygens species (ROS) and consequently, oxidative stress-induced activation of antioxidant defense systems – ↑ CAT2. As reported, CAT2 expression increase is aimed to improve hydrogen peroxide detoxification and thus mitigate the risk of oxidative damage. This interpretation is in accodance with [14], who reported that the nitrate-nitrite-nitric oxide pathway is associated with higher oxidative stress in certain conditions. Moreover, these results confirm the dual metabolic effect of nitrates as they become harmful and protective with increasing doses [15].

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Table (3): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the Third Group

Treatment ($NaNO_3$)	Dose (mg/kg)	CAT2 Gene Expression (Fold Change)
$NaNO_3$	0.01	~1.6
$NaNO_3$	0.06	~2.8

The gene expression levels of the fourth experimental group were significantly impacted by sodium nitrate ($NaNO_3$) treatments, with distinct variations for the applied concentrations. The expression of the CAT2 gene was significantly up- regulated in plants treated with low concentration ($NaNO_3$ 1) and attained the both highest relative expression level, which suggested that the mechanisms related to the stimulation by oxidative stress had been strongly stimulated. At the higher concentration ($NaNO_3$ 6), a trend of stimulation was still observed, but much less pronounced since the expression level of CAT2 was lower than at the low concentration. According to these results, at low concentrations sodium nitrate stimulates genes related to oxidative stress and inflammation, while increasing the concentration may reduce it, leading to a dose-dependent response. This may relate to induction of cellular adaptive mechanisms or a certain inhibition of known pathways targeted by the gene expression at greater intensifying conditions [16]

The findings lay among regularity with analysis [17], that showed that vegetable nitrates hold the stability of oxidative stress and Modulation of expression of genes related with Antioxidants and cellular operation by reducing the reactive oxygen species (ROS).

Table (4): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the Fourth Group

Treatment ($NaNO_3$)	Dose (mg/kg)	CAT2 Gene Expression (Fold Change)
$NaNO_3$	0.01	~3.4
$NaNO_3$	0.06	~1.8

In the fifth group, treatments with sodium nitrate ($NaNO_3$) showed a rather mild stimulation of RNA level in most of the examined genes in comparison with the other investigated groups. Low concentration ($NaNO_3$ 1) the CAT2 gene expression was only slightly increased, which indicated the osmotic stress response is weak. At the higher concentration ($NaNO_3$ 6), a similar pattern continued with little change because CAT2 expression remained at moderate levels without a significant increase. These observations

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suggest that sodium nitrate in this group did not produce a potent activation of oxidative stress pathways because of a weak cellular response or an adaptive capacity that may have blunted the compound's effect on gene expression at low and high concentrations [16].

Table (5): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the Fifth Group

Treatment ($NaNO_3$)	Dose (mg/kg)	CAT2 Gene Expression (Fold Change)
$NaNO_3$	0.01	~1.1
$NaNO_3$	0.06	~1.0

$NaNO_3$ treatments from the sixth group had a more defined (but also more variable) effect on genes expression than the fifth group (concentration-dependent). The expression of CAT2 gene showed a moderate increase at the low concentration ($NaNO_3$ 1). The more pronounced response, observed in the higher concentration ($NaNO_3$ 6), corroborated the stress effect since CAT2 expression was significantly increased as compared to the low concentration, reflecting a strong activation of antioxidant defense [18].

A new study [19] showed that Biological effects of nitrates exhibit dose dependency, low doses beneficial with antioxidant activity, gene regulation [19]and high doses exert adverse effects or functional inhibition.

Table (6): Effect of Different Sodium Nitrate ($NaNO_3$) Treatments on CAT2 Gene Expression in the Sixth Group

Treatment ($NaNO_3$)	Dose (mg/kg)	CAT2 Gene Expression (Fold Change)
$NaNO_3$	0.01	~1.2
$NaNO_3$	0.06	~1.4

Conclusions:

The present study demonstrated that sodium nitrate ($NaNO_3$) induced dose-dependent alterations in CAT2 gene expression in rat serum, reflecting its direct impact on oxidative stress and antioxidant defense mechanisms. The findings revealed that low concentrations stimulated CAT2 gene upregulation, with some experimental groups exhibiting marked increases reaching 3.4-fold change, indicating activation of antioxidant defense systems. In contrast, high concentrations or prolonged exposure resulted in significant downregulation of gene expression in certain groups, reaching as low as 0.1-fold change, suggesting depletion and impairment of endogenous antioxidant defenses. These findings highlight the

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significance of CAT2 as a valuable molecular biomarker for evaluating oxidative stress and the biological effects associated with nitrate-containing food additives.

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