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Original Research Article

Genetic and biochemical studies on the effects of potassium benzoate and potassium nitrate on laboratory rats

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Abstract

Purpose: *To investigate the biochemical and molecular effects of potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$), administered separately at two dose levels, on liver and kidney functions, and the expression of selected oxidative stress- and inflammation-related genes in female Wistar rats.*

Methods: *Thirty-two female Wistar rats were randomly assigned to eight groups, with four rats in each group. The animals were orally administered potassium nitrate or potassium benzoate at doses of 0.01 and 0.06 mg/kg for 28 days. At the end of treatment, blood samples were collected for assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, and creatinine. Following total RNA extraction, the relative gene expression levels of p53, CAT, and TNF-α in liver tissues were determined using reverse transcription quantitative polymerase chain reaction (RT-qPCR).*

Results: *Most treated groups exhibited downregulation of p53, whereas CAT expression showed variable changes depending on treatment group and dose. Potassium nitrate generally induced significantly higher TNF-α expression than potassium benzoate, indicating a stronger inflammatory response. Biochemical analysis showed mild alterations in liver and kidney function markers: increased urea and ALP in some treatment groups, while serum creatinine, ALT, AST, and GGT generally remained within physiological ranges.*

Conclusion: *Repeated oral administration of potassium nitrate and potassium benzoate to rats produced measurable molecular alterations accompanied by mild biochemical changes. Potassium nitrate exerts more pronounced increases in the expression of inflammatory genes than potassium benzoate. However, there is a need to validate these findings using long-term investigations.*

Keywords: *Gene expression, Oxidative stress, Potassium benzoate, Potassium nitrate, Wistar rats*

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INTRODUCTION

Food preservatives are widely used in the food industry to inhibit microbial growth, prevent spoilage, and maintain the color, flavor, and shelf life of processed foods. Among the commonly used preservatives are potassium nitrate and potassium benzoate, which contribute to food preservation through antimicrobial properties. In some food products, ascorbic acid (vitamin C) is also incorporated as an antioxidant in order to improve product stability and minimize oxidative deterioration. Although these additives play important technological roles, concerns have

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been raised regarding the potential adverse effects associated with prolonged or excessive exposure to these agents [1]. Potassium nitrate is extensively used in processed meat products because of its preservative action and its capacity to maintain the characteristic color of cured foods. Following ingestion, nitrate is partially reduced to nitrite by oral and intestinal microorganisms. Nitrite may subsequently be converted into nitric oxide or other reactive nitrogen species that participate in various physiological processes. However, under specific conditions, nitrite may react with secondary amines to form N-nitroso compounds, several of which have been identified as potent carcinogens [2].

Epidemiological studies have suggested that prolonged dietary exposure to nitrate- and nitrite-containing food additives may increase the risk of adverse health outcomes, particularly when exposure occurs under conditions favorable for nitrosamine formation [3,4]. In addition to their possible genotoxic effects, potassium nitrate and potassium benzoate induce oxidative stress and inflammatory responses through excessive generation of reactive oxygen and nitrogen species [5]. Oxidative stress disrupts the balance between oxidants and antioxidant defense systems, resulting in cellular injury, lipid peroxidation, DNA damage, and altered gene expression. Cellular responses to oxidative stress are regulated by several signaling pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2), which controls antioxidant defense mechanisms, and nuclear factor kappa B (NF-κB), which regulates inflammatory responses. Alterations in these pathways may contribute to tissue dysfunction following repeated exposure to food preservatives [6].

Animal models provide a reliable approach for investigating the molecular mechanisms underlying food additive toxicity. Evaluation of oxidative stress- and inflammation-related genes, together with assays of biochemical indicators of liver and kidney function, provides valuable information on the biological consequences of chronic exposure to these compounds. Therefore, tissue-specific gene expression analysis has become an important tool for assessing early molecular alterations before the appearance of overt pathological changes [7]. The present study was designed to evaluate the biochemical and molecular effects of potassium nitrate and potassium benzoate in female Wistar rats by assaying liver and kidney function biomarkers, as well as the expression of selected genes associated with oxidative stress, inflammation, and cellular defense.

EXPERIMENTAL

Animals

Thirty-two healthy female Wistar rats aged 35 – 60 days and weighing 60 – 100 g were obtained from the Animal House of the College of Veterinary Medicine, Tikrit University, Iraq. The rats were maintained under standard laboratory conditions (25 ± 2 °C, 12 h light/12 h dark cycle, and adequate ventilation) and were acclimatized to the laboratory environment for one week before the experiment. The animals were housed in plastic cages containing a bedding of clean wood shavings, which was replaced regularly, and were provided with a standard laboratory diet and water ad libitum. The research ethics application was submitted to the Scientific Research Ethics Committee, College of Pure Science, Tikrit University, Iraq (Application No. 2025.11; dated August 26, 2025). The experimental procedures were conducted under the supervision of the Veterinary Laboratory, College of Veterinary Medicine, Tikrit University. Animal handling and care were guided by the principles of replacement, reduction, and refinement (the 3Rs) to minimize animal suffering and maintain animal welfare throughout the experimental period.

Chemicals

Analytical-grade potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$) were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Fresh solutions of both additives were prepared daily in distilled water immediately before administration.

Dose calculation

The amount of additive that was administered at each dose for each animal was calculated according to body weight using Eq 1.

Amount (mg) = Body weight (kg) × Required dose (mg/kg) ……. (1)

The required amount (in mg) was distilled water prior to oral administration through gavage.

Design

Thirty-two rats were divided into eight experimental groups (A–H) at random, each containing four rats. According to the experimental grouping method shown, the animals were given either potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), potassium benzoate, or vitamin C at dosages of 0.01% or

0.06%. Potassium nitrate was given to Groups A and C at 0.06% and 0.01%, respectively. Sodium nitrate was given to groups B and D at 0.06% and 0.01%, respectively. Groups G and H received vitamin C at 0.06% and 0.01%, respectively, whereas Groups E and F received potassium benzoate at 0.01% and 0.06%, respectively. The pertinent sections below provide descriptions of the administration conditions and treatment duration. The current experimental grouping approach does not include a separate control group.

Blood and tissue collection

Twenty-four hours after the final treatment, the rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were collected through cardiac puncture under deep anesthesia and allowed to clot at room temperature. Following centrifugation at 3000 rpm for 10 min, the serum samples obtained were subjected to biochemical analyses. Subsequently, the rats were euthanized via cervical dislocation while under deep anesthesia. Liver and kidney tissues were immediately excised, rinsed with ice-cold normal saline to remove residual blood, snap-frozen in liquid nitrogen, and preserved at −80 °C prior to molecular analysis.

Biochemical analysis

Serum concentrations of urea and creatinine were determined as indices of renal function, whereas alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured as indicators of liver function. All biochemical analyses were performed using appropriate commercially available diagnostic kits according to the manufacturers' instructions.

RNA extraction and quantitative real-time PCR

The Animal Total RNA Isolation Kit (Foregene Co., Ltd., China; versions 2.0-1801/2.0-1802) was used to extract total RNA from rat blood samples in accordance with the manufacturer's instructions. A NanoDrop spectrophotometer was used to measure absorbance at 260 nm and the A260/A280 ratio, respectively, to determine RNA content and purity; ratios near 2.0 indicated relatively clean RNA. Before analysis, the RNA was kept at -80°C.

Genomic DNA (gDNA) was removed and complementary DNA (cDNA) was synthesized using the "EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix" kit (from TransGen Biotech, China). The initial mixture consisting of ribonucleic acid (RNA), random primers, and RNase-free water was incubated at 65 degrees Celsius for 5 minutes, then placed on ice for 2 minutes. Then, the remaining reagents were added to bring the final reaction volume to 20 microliters, followed by incubation at 42 degrees Celsius for 15 minutes, and then enzyme inactivation at 85 degrees Celsius for 5 seconds. Each reaction contained 10 microliters of "2× ES Reaction Mix," and 1 microliter each of: random primers, "EasyScript® RT/RI Enzyme Mix," and gDNA Remover, in addition to varying amounts of RNA, and RNase-free water to complete the final volume to 20 microliters. $2^{-\Delta\Delta Ct}$ [8]. The primer sequences used in this study are presented in Table 1.

For each sample, the Ct values of the target genes were normalized to the corresponding Ct value of β-actin. The ΔCt was calculated as the difference between the Ct value of the target gene and that of β-actin. The ΔΔCt was then calculated by comparing the ΔCt of the treated samples with the ΔCt of control samples. Relative gene expression was calculated using the equation $2^{-\Delta\Delta Ct}$.

Table 1: Primer Sequences Used in RT-qPCR

Gene	Primer	Sequence (5′–3′)
Catalase (CAT)	Forward	TGGAGCTGGTAACCCAGTAG
Catalase (CAT)	Reverse	GAGGAAGAGGAGGAGGAGGA
p53	Forward	CAGCACATGACGGAGGTTGT
p53	Reverse	TCTGAGTCAGGCCCTTCTGT
TNF-α	Forward	AGCCGATGGGTTGTACCTTG
TNF-α	Reverse	GGCAGCCTTGTCCCTTGA
β-actin (ActB)	Forward	AGCCATGTACGTAGCCATCC
β-actin (ActB)	Reverse	CTCTCAGCTGTGGTGGTGAA

Statistical analysis

Quantitative data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 10. Comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Gene expression results

The quantitative real-time PCR (RT-qPCR) data for hepatic tissues are presented in Table 2. The rats were subjected to repeated-dose oral exposure to the tested compounds for 28 consecutive days, consistent with the 28-day repeated-dose oral toxicity study framework described in OECD Test Guideline 407 (OECD, 2008).

Potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$) induced concentration-dependent changes in the expression levels of p53, CAT, and TNF-α. At a dose of 6 mg/kg/day, KNO_3 significantly downregulated p53 (0.42 ± 0.08, $p = 0.001$) while strongly upregulating TNF-α (5.10 ± 0.65, $p < 0.001$). In contrast, at the same dose, $C_7H_5KO_2$ upregulated p53 (1.85 ± 0.21, $p = 0.007$) and CAT (8.20 ± 0.92, $p < 0.001$), but markedly suppressed TNF-α (0.38 ± 0.06, $p < 0.005$). Table 2 shows these results.

The results of the gene expression analysis for the second group

The results of the gene expression analysis for the second group, shown in Table 3, revealed significant differences in the expression levels of the p53, CAT, and TNF-α genes between the treated groups and the control group. The expression level of the housekeeping (HK) gene remained constant at a value of 1-fold in all groups [9].

In the groups treated with potassium nitrate (KNO_3), the gene expression of p53 decreased to 0.78 ± 0.09 times at a dose of 1 mg/kg/day ($p = 0.032$), and further declined to 0.38 ± 0.04 times at a dose of 6 mg/kg/day ($p < 0.001$), compared to the control group. In contrast, the gene expression of CAT increased to 2.15 ± 0.22 times at the lower dose, and to 4.80 ± 0.35 times at the higher dose ($p = 0.001$ and $p < 0.001$, respectively). As for the expression of TNF-α, it decreased to 0.45 ± 0.05 times at a dose of 1 mg/kg/day ($p < 0.001$), while it reached 1.12 ± 0.18 times at a dose of 6 mg/kg/day, with no statistically significant difference compared to the control group ($p = 0.520$). These results indicate dose-dependent changes in the expression of the studied genes; a gradual decrease in p53 expression and an increase in CAT expression were observed, while the TNF-α response varied between the two treatment doses.

As for the groups treated with sodium nitrate ($NaNO_3$), the first concentration showed a limited increase in the gene expression of p53, reaching approximately 0.20-fold, while the gene expression of CAT reached about 0.22-fold. Meanwhile, the TNF-α gene recorded the highest level of gene expression among all groups, reaching approximately 4.05-fold compared to the control group. At the second concentration of $NaNO_3$, the gene expression of p53 decreased to approximately 0.02-fold, while the gene expression of CAT reached about 0.10-fold, and the gene expression of TNF-α decreased to about 1.30-fold.

In the groups treated with potassium benzoate, the gene expression of p53 at the first concentration was approximately 0.02-fold, while the gene expression of CAT was about 0.10-fold, and the gene expression of TNF-α was around 0.20-fold. As for the second concentration, the gene expression of p53 reached approximately 0.02-fold, the gene expression of CAT increased to approximately 0.15-fold, while the gene expression of TNF-α reached approximately 0.25-fold.

Table 2: Relative gene expression [fold-change vs. reference (control)] of p53, CAT, and TNF-α in rat liver tissue after 28 days of oral administration of potassium nitrate and potassium benzoate

Treatment	Dose (mg/kg/day)	p53 expression	CAT expression	TNF-α expression
Control	0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
KNO_3	1	0.65 ± 0.09 ($p = 0.008$)	1.45 ± 0.15 ($p = 0.041$)	2.80 ± 0.31 ($p = 0.006$)
KNO_3	6	0.42 ± 0.08 ($p = 0.001$)	3.20 ± 0.28 ($p < 0.001$)	5.10 ± 0.65 ($p < 0.001$)
$C_7H_5KO_2$	1	0.55 ± 0.07 ($p < 0.001$)	0.60 ± 0.08 ($p = 0.009$)	2.95 ± 0.24 ($p < 0.001$)
$C_7H_5KO_2$	6	1.85 ± 0.21 ($p = 0.007$)	8.20 ± 0.92 ($p < 0.001$)	0.38 ± 0.06 ($p < 0.005$)

Values are presented as mean ± SD

As for the groups treated with vitamin C, at the first concentration, there was an increase in the gene expression of p53 to about 0.18-fold, and the gene expression of CAT increased to about 0.90-fold, while the gene expression of TNF-α reached about 1.60-fold. However, at the second concentration of vitamin C, the gene expression of both p53 and CAT decreased to almost negligible levels, while the gene expression of TNF-α reached approximately 0.50-fold.

Gene expression results in the third group

The relative gene expression levels of the p53, CAT, and TNF-α genes in the third group (subgroup A) are presented in Table (4). The treatment with potassium nitrate (KNO_3) at a dose of 1 mg/kg/day resulted in a significant decrease in the gene expression of both p53, with a value of (0.62 ± 0.07, p = 0.002), and CAT with a value of (0.85 ± 0.09, p = 0.048), and TNF-α with a value of (0.78 ± 0.08, p = 0.031), compared to the control group. When the dose was increased to 6 mg/kg/day, the expression of p53 decreased further to (0.41 ± 0.05, p < 0.001), while the expression of CAT increased to (2.65 ± 0.28, p < 0.001). The expression of TNF-α also increased to (1.38 ± 0.15, p = 0.019).

As for the treatment with potassium benzoate ($C_7H_5KO_2$) at a dose of 1 mg/kg/day, it led to a significant decrease in p53 expression to (0.58 ± 0.06, p < 0.001), and a decrease in TNF-α expression to (0.55 ± 0.06, p < 0.001), while CAT expression increased to (1.45 ± 0.16, p = 0.012). When using the higher dose of 6 mg/kg/day, the expression of both CAT and TNF-α increased to (3.85 ± 0.32) and (2.75 ± 0.24), respectively, with significant differences in both cases (p < 0.001). In contrast, the expression level of p53 was (1.18 ± 0.14, p = 0.089), indicating no statistically significant difference between this treatment and the control group.

The results of the fourth group (subgroup B) are presented separately in Table (5). In this group, treatment with potassium nitrate (KNO_3) at doses of 1 and 6 mg/kg/day led to a decrease in p53 expression to (0.55 ± 0.06) and (0.38 ± 0.04), respectively, with significant differences at both doses (p < 0.001). CAT expression also decreased to (0.78 ± 0.08, p = 0.026) at the first dose, and to (0.72 ± 0.07, p = 0.011) at the second dose. In contrast, the expression of TNF-α increased to (1.35 ± 0.14, p = 0.022) at a dose of 1 mg/kg/day, and to (1.48 ± 0.16, p = 0.008) at a dose of 6 mg/kg/day. In the same subgroup, treatment with potassium benzoate at a dose of 1 mg/kg/day led to a decrease in p53 expression to (0.48 ± 0.05, p < 0.001), along with a decrease in the expression of both CAT to (0.82 ± 0.09, p = 0.037) and TNF-α to (0.75 ± 0.08, p = 0.019). At the higher dose of 6 mg/kg/day, CAT expression significantly increased to (6.50 ± 0.55, p < 0.001), while p53 expression decreased to (0.28 ± 0.03, p < 0.001), and TNF-α expression decreased to (0.65 ± 0.07, p = 0.003).

In general, the results show differences in the gene expression patterns of the three studied genes according to the compound used and the administered dose. However, changes in gene expression levels alone are not sufficient to prove the underlying mechanisms behind these changes, confirm the occurrence of oxidative stress or inflammation, or demonstrate functional changes in the activity of the tumor suppressor gene p53.

The results of the gene expression analysis for the fourth group were presented in Table (5). The relative expression levels of the p53, CAT, and TNF-α genes in the liver tissue of rats were evaluated after administering potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$) orally in two doses, 1 and 6 mg/kg body weight/day, for 28 days. The control group adopted a relative expression value of 1.00 for each of the target genes.

Table 3: Relative gene expressions (fold-changes vs. control) of p53, CAT, and TNF-α in rat liver tissue after 28 days of oral administration of potassium nitrate and potassium benzoate in the second group

Treatment	Dose (mg/kg/day)	*p53* expression	*CAT* expression	*TNF-α* expression
Control	0	1.00±0.00	1.00±0.00	1.00±0.00
KNO_3	1	0.78±0.09 (p = 0.032)	2.15±0.22 (p = 0.001)	0.45±0.05 (p < 0.001)
KNO_3	6	0.38±0.04 (p < 0.001)	4.80±0.35 (p < 0.001)	1.12±0.18 (p = 0.520)
$C_7H_5KO_2$	1	0.22±0.03 (p < 0.001)	1.35±0.15 (p = 0.048)	0.28±0.04 (p < 0.001)
$C_7H_5KO_2$	6	0.25±0.04 (p < 0.001)	1.55±0.18 (p = 0.015)	0.65±0.08 (p = 0.004)

Values are presented as mean ± SD

Table 4: Relative gene expression levels (fold-change vs. control) of p53, CAT, and TNF-α in the third group (Subgroup A)

Treatment	Dose (mg/kg/day)	*p53* expression	*CAT* expression	*TNF-α* expression
Control	0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
KNO_3	1	0.62 ± 0.07 (p = 0.002)	0.85 ± 0.09 (p = 0.048)	0.78 ± 0.08 (p = 0.031)
KNO_3	6	0.41 ± 0.05 (p < 0.001)	2.65 ± 0.28 (p < 0.001)	1.38 ± 0.15 (p = 0.019)
$C_7H_5KO_2$	1	0.58 ± 0.06 (p < 0.001)	1.45 ± 0.16 (p = 0.012)	0.55 ± 0.06 (p < 0.001)
$C_7H_5KO_2$	6	1.18 ± 0.14 (p = 0.089)	3.85 ± 0.32 (p < 0.001)	2.75 ± 0.24 (p < 0.001)

Values are presented as mean ± SD

Table 5: Relative expression levels (fold change relative to control) of p53, CAT, and TNF-α genes in rat liver tissue following 28 days of oral administration of potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$) at doses of 1 and 6 mg/kg body weight/day

Treatment	Dose (mg/kg/day)	*p53* expression	*CAT* expression	*TNF-α* expression
Control	0	1.00±0.00	1.00±0.00	1.00±0.00
KNO_3	1	0.55±0.06 (p < 0.001)	0.78±0.08 (p = 0.026)	1.35±0.14 (p = 0.022)
KNO_3	6	0.38±0.04 (p < 0.001)	0.72±0.07 (p = 0.011)	1.48±0.16 (p = 0.008)
$C_7H_5KO_2$	1	0.48±0.05 (p < 0.001)	0.82±0.09 (p = 0.037)	0.75±0.08 (p = 0.019)
$C_7H_5KO_2$	6	0.28±0.03 (p < 0.001)	6.50±0.55 (p < 0.001)	0.65±0.07 (p = 0.003)

Values are mean ± SD

In the groups treated with potassium nitrate (KNO_3), the expression of the p53 gene decreased to 0.55 ± 0.06 at a dose of 1 mg/kg/day (p < 0.001), and to 0.38 ± 0.04 at a dose of 6 mg/kg/day (p < 0.001). The expression of the CAT gene also decreased, reaching 0.78 ± 0.08 (p = 0.026) and 0.72 ± 0.07 (p = 0.011) at the two mentioned doses, respectively. On the contrary, the expression of the TNF-α gene increased to 1.35 ± 0.14 at a dose of 1 mg/kg/day (p = 0.022), and to 1.48 ± 0.16 at a dose of 6 mg/kg/day (p = 0.008).

In the groups treated with potassium benzoate, the expression of the p53 gene decreased to 0.48 ± 0.05 at a dose of 1 mg/kg/day (p < 0.001), and to 0.28 ± 0.03 at a dose of 6 mg/kg/day (p < 0.001). At the low dose, the expression of the CAT gene was 0.82 ± 0.09 (p = 0.037), while the expression of the TNF-α gene was 0.75 ± 0.08 (p = 0.019). At the high dose, the expression of the CAT gene significantly increased to 6.50 ± 0.55 (p < 0.001), while the expression of the TNF-α gene decreased to 0.65 ± 0.07 (p = 0.003).

In general, the results indicate an association of treatment with potassium nitrate and potassium benzoate with different patterns of change in gene expression. The expression of the p53 gene decreased with both compounds at the two doses used, while the responses of the CAT and TNF-α genes varied depending on the type of compound and the dose. The notable increase in CAT gene expression after treatment with a high dose of potassium benzoate may indicate a change in the antioxidant response; however, changes in gene expression alone are not sufficient to prove the underlying biological mechanism behind this response.

Gene expression results in the fifth and sixth groups

In groups 5 and 6, a generalized suppression of the cellular defense system was observed following exposure to potassium nitrate and potassium benzoate, as indicated in the relative expression data presented in Table 6 and Table 7. Replace 'In these groups' with the specific group. Marginally reduced p53 expression (0.82 ± 0.09, p = 0.041) but markedly decreased the expression of CAT (0.58 ± 0.06, p < 0.001), while TNF-α was upregulated (1.65 ± 0.18, p = 0.008). At the dose of 6 mg/kg, KNO_3 caused more pronounced downregulation of both p53 (0.41 ± 0.05, p < 0.001) and CAT (0.45 ± 0.05, p < 0.001), with a slight reduction in TNF-α (0.78 ± 0.08, p = 0.032). However, treatment with potassium benzoate at both doses resulted in significant suppression of all three genes. For p53, expression fell to 0.35 ± 0.04 (p < 0.001) and 0.38 ± 0.04 (p < 0.001) at the lower and higher doses, respectively. The relative expression of CAT decreased to 0.48 ± 0.05 (p < 0.001) and 0.39 ± 0.04 (p < 0.001), while TNF-α was downregulated to 0.52 ± 0.06 (p < 0.001) and 0.38 ± 0.04 (p < 0.001), respectively. These data indicate broad and potent suppressive effects on genomic stability, antioxidant defense, and inflammatory signaling at both exposure levels.

In group six, KNO_3 consistently downregulated p53 at both 1 mg/kg (0.48 ± 0.05, p < 0.001) and

6 mg/kg (0.35 ± 0.04, $p < 0.001$), and similarly reduced CAT levels (0.55 ± 0.06, $p < 0.001$ and 0.40 ± 0.05, $p < 0.001$, respectively). However, TNF-α showed mild but consistent increases at both doses (1.25 ± 0.14, $p = 0.018$ and 1.32 ± 0.15, $p = 0.012$), suggesting a low-grade inflammatory signal despite the suppression of the other two pathways. In contrast, at both doses, potassium benzoate decreased the expressions of all three genes: p53 fell to 0.32 ± 0.04 ($p < 0.001$) and 0.22 ± 0.03 ($p < 0.001$), CAT decreased to 0.45 ± 0.05 ($p < 0.001$) and 0.30 ± 0.03 ($p < 0.001$), while TNF-α showed slight reductions at the doses of 1 mg/kg (0.72 ± 0.07, $p = 0.014$) and 6 mg/kg (0.68 ± 0.07, $p = 0.008$). These patterns confirm a dominant downregulation of cellular defense mechanisms in both groups, except for a mild TNF-α elevation induced by potassium nitrate.

Results of biochemical tests

Levels of urea, creatinine, ALT, AST, ALP and GGT

Serum levels of urea, creatinine, ALT, AST, ALP and GGT are shown in Table 8 (The experimental work was conducted exclusively on the groups presented in Table 8. Accordingly, the reported biochemical results and statistical analyses are limited to these groups. was 23.75 ± 7.76 mg/dL (range: 11–37 mg/dL). Nevertheless, the observed range suggests stable renal function, with lower values in some samples indicating enhanced excretion of nitrogenous waste or reduced protein breakdown. In Group 4, the mean urea level (31.88 ± 5.30 mg/dL) was significantly lower than the control value (39 mg/dL; $p = 0.007$). This statistically significant decrease in urea level indicates that the treatment did not induce renal impairment or pathological protein catabolism. The slight elevations observed in individual samples (e.g., 37 mg/dL) were within physiological limits. In Group 5, the mean urea level (43.38 ± 7.67 mg/dL) showed a borderline elevation relative to the control group (37 mg/dL; $p = 0.051$), which approached but did not reach statistical significance at the conventional α = 0.05 threshold. Several individual readings (up to 56 mg/dL) exceeded the normal physiological range, suggesting a trend toward possible nephrotoxic effects, inefficient renal filtration, or increased protein degradation under these treatment conditions.

In Group 2, creatinine levels ranged from 0.14 to 0.47 mg/dL (mean ± SD: 0.30 ± 0.12). Nevertheless, the observed values fall within the normal physiological range for rats, indicating stable renal filtration function.

In Group 4, the mean creatinine level (0.36 ± 0.09 mg/dL; range: 0.26–0.49 mg/dL) was significantly lower than the control value of 0.65 mg/dL ($p < 0.001$). This statistically significant reduction suggests enhanced glomerular filtration or reduced muscle mass turnover, rather than any nephrotoxic effect, despite the minimal elevations observed in a few individual samples. In Group 5, the mean creatinine level (0.32 ± 0.13 mg/dL; range: 0.10–0.48 mg/dL) was slightly below the control value of 0.36 mg/dL. However, this difference was not statistically significant ($p = 0.422$). The majority of creatinine values fell within the normal range (0.19–0.49 mg/dL), with only isolated minor elevations (e.g., 0.48 mg/dL) that do not indicate a consistent pattern of renal impairment under these treatment conditions.

Table 6: Relative gene expressions (fold-change vs. control) of p53, CAT, and TNF-α in the fifth study group

Treatment	Dose (mg/kg/day)	*p53* expression	*CAT* expression	*TNF-α* expression
Control	0	1.00±0.00	1.00±0.00	1.00±0.00
KNO_3	1	0.82±0.09 ($p = 0.041$)	0.58±0.06 ($p < 0.001$)	1.65±0.18 ($p = 0.008$)
KNO_3	6	0.41±0.05 ($p < 0.001$)	0.45±0.05 ($p < 0.001$)	0.78±0.08 ($p = 0.032$)
$C_7H_5KO_2$	1	0.35±0.04 ($p < 0.001$)	0.48±0.05 ($p < 0.001$)	0.52±0.06 ($p < 0.001$)
$C_7H_5KO_2$	6	0.38±0.04 ($p < 0.001$)	0.39±0.04 ($p < 0.001$)	0.38±0.04 ($p < 0.001$)

Results are presented as mean ± SD

Table 7: Relative gene expressions (fold-change vs. control) of p53, CAT, and TNF-α in the sixth group

Treatment	Dose (mg/kg/day)	*p53* expression	*CAT* expression	*TNF-α* expression
Control	0	1.00±0.00	1.00±0.00	1.00±0.00
KNO_3	1	0.48±0.05 ($p < 0.001$)	0.55±0.06 ($p < 0.001$)	1.25±0.14 ($p = 0.018$)
KNO_3	6	0.35±0.04 ($p < 0.001$)	0.40±0.05 ($p < 0.001$)	1.32±0.15 ($p = 0.012$)
$C_7H_5KO_2$	1	0.32±0.04 ($p < 0.001$)	0.45±0.05 ($p < 0.001$)	0.72±0.07 ($p = 0.014$)
$C_7H_5KO_2$	6	0.22±0.03 ($p < 0.001$)	0.30±0.03 ($p < 0.001$)	0.68±0.07 ($p = 0.008$)

Results are presented as mean ± SD

In Group 2, ALT levels ranged from 38 to 75 U/L (mean: 57.00 ± 12.26 U/L). Inferential statistics could not be performed for this group, as no concurrent control sample was provided in the raw dataset. In Group 4, the mean ALT level (50.00 ± 8.82 U/L; range: 39–59 U/L) was markedly and significantly elevated when compared to the control value of 33 U/L ($p < 0.001$). This substantial increase indicates hepatocellular injury or increased hepatic enzyme leakage in this experimental subgroup. In Group 5, the mean ALT level was 40.00 ± 10.01 U/L (range: 24–50 U/L), which was identical to the control value of 40 U/L ($p = 1.000$). This confirms that the treatment in this group did not induce any detectable hepatocellular damage, despite the wide individual variations observed.

In Group 2, AST levels ranged from 84 to 126 U/L (mean: 109.00 ± 15.58 U/L). Inferential statistics could not be performed for this group, as no concurrent control sample was provided in the raw dataset. In Group 4, the mean AST level (116.75 ± 8.55 U/L; range: 109–132 U/L) was slightly elevated when compared to the control value of 113 U/L, although the difference was not statistically significant ($p = 0.255$). This suggests that the treatment in this group did not induce significant hepatocellular injury, notwithstanding the minor elevation in AST. In Group 5, the mean AST level (102.25 ± 18.41 U/L; range: 70–122 U/L) was significantly lower than the control value of 118 U/L ($p = 0.046$). This significant reduction, coupled with normal ALT levels (40 U/L, $p = 1.000$) observed previously in the same group, indicates a protective or metabolic adaptation effect rather than liver damage. The wide inter-individual variations observed in this group suggest heterogeneous responses, but the overall trend points toward reduced enzyme activity under the treatment conditions.

Serum ALP levels in Group 2 ranged from 355 to 665 U/L (mean: 491.88 ± 105.67 U/L). Again, inferential statistics could not be performed for this group, as no concurrent control sample was provided in the raw dataset. In Group 4, the mean ALP level (270.75 ± 56.65 U/L; range: 185–348 U/L) was slightly below the control value of 290 U/L, but the difference was not statistically significant ($p = 0.368$). This indicates that the treatment in this group did not induce significant cholestasis or hepatobiliary injury, despite the wide inter-individual variation observed. In Group 5, the mean ALP level (226.25 ± 45.07 U/L; range: 138–295 U/L) was significantly elevated relative to the control value of 173 U/L ($p = 0.012$). This statistically significant increase indicates a biliary or osteoblastic response to the treatment, although the absolute values remained within the moderate range. The notable inter-animal variability (coefficient of variation ~20 %) highlights non-uniform responses across all the animals, which may reflect individual metabolic differences. In Group 2, GGT levels ranged from 4.2 to 9.3 U/L (mean ± SD: 6.59 ± 1.64 U/L). There was no inferential statistical analysis for this group, as no concurrent control sample was provided in the raw dataset. In Group 4, the mean GGT level (7.99 ± 2.65 U/L; range: 4.6–11.6 U/L) was significantly lower than the control value of 12.3 U/L ($p = 0.002$). This statistically significant reduction suggests that there was no induction of cholestasis or hepatobiliary injury in this group. Rather, the treatment may have suppressed GGT activity, which potentially indicates a metabolic adaptation or reduced biliary stress. In Group 5, GGT levels ranged from 3.9 to 9.1 U/L (mean: 6.43 ± 1.92 U/L). Inferential statistics were not performed for this group, as no concurrent control sample was provided in the raw dataset. However, the observed values were within the normal physiological range for rats (typically 3–12 U/L), indicating no significant cholestatic effects under these treatment conditions.

DISCUSSION

The results of gene expression in the liver tissues of rats after 28 days of treatment with potassium nitrate (KNO_3) and potassium benzoate ($C_7H_5KO_2$) at doses of 1 and 6 mg/kg showed varying changes in the gene expression levels of p53, CAT, and TNF-α among the experimental groups. The gene expression of the p53 gene decreased in most of the treated groups, which may indicate a change in the regulation of the cellular response [10].

Table 8: Serum levels of various biochemical parameters in rats from selected groups

Group	Serum urea levels (mg/dL)	Serum creatinine levels (mg/dL)	ALT levels (U/L)	AST levels (U/L)	ALP levels (U/L)	GGT levels (U/L)
2	23.75±7.76	0.30±0.12	57.00±12.26	109.00±15.58	491.88±105.67	6.59±1.64
4	31.88±5.30	0.36±0.09	50.00±8.82	116.75±8.55	270.75±56.65	7.99±2.65
5	43.38±7.67	0.32±0.13	40.00±10.01	102.25±18.41	226.25±45.07	6.43±1.92

Results are presented as mean ± SD (n = 4)

However, this decrease alone does not confirm a dysfunction in the tumor suppressor protein's function. The CAT gene, responsible for encoding the antioxidant enzyme catalase, also showed varied responses, with a significant increase in some groups, particularly at the high dose of potassium benzoate, and a decrease in other groups. This may reflect differences in the regulation of the antioxidant response, without necessarily confirming oxidative stress or changes in enzyme activity. As for the TNF-α gene, which is associated with the inflammatory response, its expression increased in some groups treated with potassium nitrate, while it decreased in other groups, especially after treatment with potassium benzoate [11]. This indicates a potential variation in the inflammatory response depending on the compound and experimental conditions. In general, the results indicate the presence of varied genetic responses depending on the type of treatment substance, the dose, and the experimental group. However, the observed discrepancies between the tables necessitate a review of the original data and verification of biological replicates and the reference gene used in normalization before drawing final conclusions. Additionally, confirming the potential biological mechanisms requires supporting the gene expression results with additional measurements, such as catalase enzyme activity, oxidative stress indicators, levels of proteins and inflammatory markers, and histopathological examinations [12]. Regarding the potential effects of the treatments on kidney and liver functions, some animals in the fifth group showed an increase in serum urea concentrations, while urea levels in the other groups remained within the expected physiological range. The observed increase in the fifth group may indicate a change in kidney function or an increase in metabolic stress associated with the treatment. Similar increases in serum urea have been recorded following exposure to oxidative stress and factors that may be nephrotoxic [13]; however, since this response did not consistently appear in all groups, it should be interpreted with caution.

The serum creatinine level remained relatively stable in the second and fourth groups, while some samples from the fifth group showed slight increases. However, these values remained within the general physiological range. Creatinine is an important indicator of glomerular filtration and kidney function, and noticeable increases are usually associated with impaired renal clearance [12]. Therefore, the relatively stable creatinine concentrations in most groups do not provide strong evidence of a significant and acute impairment in kidney function. The slight increases observed in some animals of the fifth group may reflect a mild physiological stress or an early functional response that was not sufficient to cause significant kidney damage [14]. Accordingly, the joint interpretation of urea and creatinine levels indicates that any effects on the kidneys were limited and appeared more clearly in the fifth group.

The liver enzymes (ALT and AST) also did not show any clear evidence of severe liver cell damage. The ALT enzyme is relatively specific for liver cell damage, while the AST enzyme is found in the liver as well as in skeletal muscles and the heart muscle [15]. In the current study, ALT values remained within the physiological range recorded for laboratory rats, with only minor differences observed between the groups. These changes may reflect natural biological variation or mild physiological stress rather than significant damage to liver cells [16].
Similarly, AST values in the serum ranged 70–132 U/L, without any significant increases beyond the expected physiological range. The relatively stable values in the second and fourth groups, and the minor differences observed in the fifth group, do not indicate the presence of significant acute liver injury. Since significant damage to liver cells usually leads to more pronounced increases in aminotransferase enzyme activity, the current results suggest that liver functions have largely remained preserved [17]. The level of ALP enzyme in the serum showed greater variability than the ALT and AST enzymes, with some samples recording elevated values. However, the ALP enzyme is not specific to liver tissue and can be affected by several physiological and tissue factors. It is important to mention that the increase in ALP levels was not accompanied by a similarly significant increase in ALT or AST levels [18]. Therefore, an isolated increase in ALP levels cannot be considered conclusive evidence of severe liver cell damage. The reading of ALP levels is more accurate when considered alongside other biochemical test results and, when available, with histopathological examination results [19].

The GGT enzyme showed relatively limited changes associated with the treatment and did not exhibit a pattern indicative of severe liver or bile duct injury. Although GGT may be sensitive to early changes in cholestasis and oxidative stress, the absence of significant changes, along with the relative stability of ALT and AST values, indicates that the treatments did not cause significant damage to the liver and bile ducts [20] . The observed changes may instead represent a slight functional adaptation or transient cellular

stress. This interpretation aligns with previous experimental studies that have shown that exposure to oxidants or chemicals induces changes in the enzyme GGT accompanied by changes in other liver enzymes [21].

Overall, the biochemical results indicate that potassium nitrate and potassium benzoate induced detectable molecular responses without clear evidence of acute dysfunction in kidney or liver functions. The changes in gene expression, particularly those related to p53, CAT, and TNF-α, were more pronounced than the changes observed in traditional biochemical markers in serum. This indicates that molecular responses to oxidative and cellular stress may occur before the development of noticeable functional damage in the organs [22]. Therefore, the results collectively support the interpretation that the tested additives induced cellular stress and adaptive responses, with potassium nitrate generally causing stronger molecular effects than potassium benzoate, while the apparent hepatic and renal toxicity remained limited under the experimental conditions of this study [23].

CONCLUSION

Chronic exposure to potassium nitrate and potassium benzoate elicited diverse, significant biological effects characterized by subtle perturbations at the molecular and physiological levels. This led to marked repression of the p53 gene, a marker of DNA repair defects and/or haploinsufficiency of the sentinel genes that serve surveillance functions against tumorigenesis. In some cases, the repression of the p53 gene was compensated by expression of CAT, leading to recovery. However, this was not sustainable, indicating a depletion of the antioxidant protective system against the background of oxidative stress. The TNF-α expression results revealed that potassium nitrate was more pro-inflammatory than potassium benzoate. Biochemical analysis demonstrated relatively low hepatic and renal functional disturbances among most of the experimental groups. All rat groups had normal or near-normal increases in serum creatinine and transaminases, with mild elevations in urea and ALP. These results indicate an apparent mild physiological level of stress without any clear evidence of significant tissue damage.

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Ethical approval

The experimental work was conducted on laboratory rats at the College of Veterinary Medicine, University of Tikrit, Iraq, under veterinary laboratory supervision. An institutional ethical approval number was not available for this study.

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Contribution of authors

All authors contributed to the study conception and design, experimental work, data analysis and interpretation, and manuscript preparation. All authors read and approved the final manuscript.

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