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Research Article:

Protective Effects of Sulforaphane and Kojic Acid Against Arsenic-Induced Inflammatory and Apoptotic Toxicity in HaCaT Cells

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Abstract

Background: Arsenic is an environmental toxicant associated with skin injury, inflammation, and apoptosis-related cellular responses. Human keratinocyte (HaCaT) cells are widely used to investigate the mechanisms of arsenic-induced skin toxicity and potential protective interventions. Sulforaphane and kojic acid have been reported to possess cytoprotective and anti-inflammatory properties in various experimental models. **Aim:** To evaluate the protective effects of sulforaphane and kojic acid against arsenic-induced cytotoxicity and alterations in inflammation- and apoptosis-related gene expression in HaCaT cells. **Methods:** HaCaT cells were exposed to arsenic (5 μ M) alone or in combination with sulforaphane (5, 10, and 20 μ M) or kojic acid (50, 100, and 200 μ M). Cell viability was assessed using the MTT assay. The expression of tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), BCL-2-associated X protein (BAX), and B-cell lymphoma 2 (BCL-2) were evaluated by quantitative real-time polymerase chain reaction (qPCR). **Results:** Arsenic exposure significantly reduced cell viability and increased TNF- α , IL-6, and BAX expression while decreasing BCL-2 expression. Treatment with sulforaphane or kojic acid improved cell viability and attenuated arsenic-induced changes in gene expression. Both compounds reduced TNF- α , IL-6, and BAX expression and partially restored BCL-2 expression compared with the arsenic-treated group. Sulforaphane generally produced a more consistent protective effect than kojic acid across the evaluated inflammatory and apoptosis-related markers. **Conclusion:** Arsenic induced cytotoxicity and significant transcriptional alterations associated with inflammatory and apoptosis-related responses in HaCaT cells. Sulforaphane and kojic acid mitigated these effects, with sulforaphane showing a more consistent protective profile. These findings suggest that both compounds may help attenuate arsenic-induced keratinocyte injury and provide a basis for further mechanistic investigation.

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1. Introduction

Arsenic exposure remains a major public health concern, particularly in populations chronically exposed to contaminated drinking water (1). The skin is one of the primary target organs, and prolonged exposure has been associated with pigmentary abnormalities, hyperkeratosis, and cutaneous carcinogenesis (2). Experimental studies have shown that arsenic acts as a cocarcinogen with ultraviolet radiation, induces chromosomal alterations, and promotes keratinocyte-derived growth factor dysregulation, all of which contribute to skin pathology (3–5). Arsenic toxicity is associated with multiple mechanisms, including oxidative damage, inflammatory activation, mitochondrial

dysfunction, and dysregulation of cell survival pathways (6–11). In human keratinocytes, arsenic has been shown to induce oxidative damage through reactive oxygen and nitrogen species generation and to alter signaling pathways involved in cellular proliferation and survival (10,12). Recent studies have further linked arsenic exposure to cellular senescence and tissue injury (13,14). Because the skin is a major target of arsenic toxicity, HaCaT cells (human immortalized keratinocytes) provide a biologically relevant *in vitro* model for studying arsenic-induced cellular injury.

Sulforaphane is a naturally occurring isothiocyanate that has demonstrated cytoprotective, anti-inflammatory, and anti-apoptotic activities in various experimental models. Previous studies have reported activation of nuclear factor erythroid 2-related factor 2 (Nrf2)-associated pathways, sirtuin 1 (SIRT1), and AMP-activated protein kinase/peroxisome proliferator-activated receptor gamma coactivator-1 alpha (AMPK/PGC-1 α), resulting in reduced cellular stress and improved survival (15–24). In contrast, kojic acid is primarily known as a tyrosinase inhibitor used in dermatological depigmenting products (25–28). However, recent evidence indicates that kojic acid and its derivatives

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also possess anti-inflammatory and cytomodulatory properties (29–34). Despite these observations, its potential protective role against arsenic-induced keratinocyte injury remains poorly understood. The chemical structures of sulforaphane and kojic acid are shown in Figure 1 and 2, respectively.

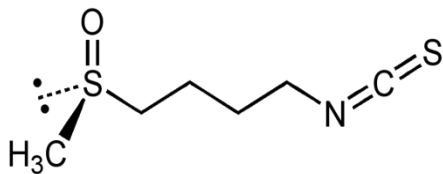


Figure 1. Chemical structures of sulforaphane

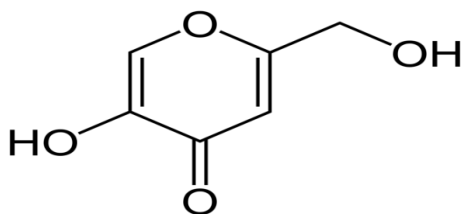


Figure 2. Chemical structure of kojic acid

Although sulforaphane has been extensively investigated as a cytoprotective compound, direct comparisons between sulforaphane and kojic acid in arsenic-exposed keratinocytes are lacking. Furthermore, the effectiveness of kojic acid in attenuating arsenic-induced inflammatory and apoptosis-associated responses has not been adequately characterized. Therefore, the present study investigated the effects of sulforaphane and kojic acid on arsenic-induced toxicity in HaCaT cells by evaluating cell viability and the expression of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), BCL-2-associated X protein (BAX), and B-cell lymphoma 2 (BCL-2). The primary objective was to directly compare the protective effects of sulforaphane and kojic acid under identical experimental conditions.

2. Materials and methods

2.1. Materials and reagents

Human immortalized keratinocyte (HaCaT) cells were used as the experimental model. Dulbecco's Modified Eagle Medium (DMEM), penicillin-streptomycin solution, and TRIzol EX reagent were obtained from DNA Biotech (Iran). Fetal bovine serum (FBS) was used as a culture supplement. Sulforaphane (Sigma, USA) and kojic acid (Merck, Germany) were used as the protective compounds, while sodium arsenite 1 (Danapharmed, Iran) was used to induce cellular toxicity. Cell viability was evaluated using MTT reagent (Deehan-Riedel, Germany) and dimethyl sulfoxide (DMSO; Merck, Germany). Complementary DNA (cDNA) synthesis was performed using a commercial cDNA synthesis kit (Pars Tous, Iran), and quantitative real-time PCR was conducted using SYBR Green Master Mix (Zist Virayesh, Iran). Gene-specific primers for TNF- α , IL-6, BAX, BCL-2, and GAPDH were used for gene expression analysis. Major equipment included a CO₂ incubator, inverted phase-contrast microscope, refrigerated centrifuge, microplate reader, spectrophotometer, vortex mixer, water bath, and a real-time PCR system (Bio-Rad, USA).

2.2. Cell culture

HaCaT cells (passage 3) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂. The culture medium was replaced every 2–3 days. Cell morphology and growth were monitored regularly using an inverted phase-contrast microscope. When cells reached approximately 80–90% confluence, they were washed with phosphate-buffered saline (PBS), detached using trypsin-EDTA, and subcultured for subsequent experiments.

2.3. Preparation of treatment solutions

Stock solutions of sodium arsenite, sulforaphane, and kojic acid were prepared according to standard laboratory procedures. Working concentrations were freshly prepared before each experiment by diluting stock solutions in complete culture medium.

2.4. Determination of arsenic cytotoxicity and IC₅₀

HaCaT cells were seeded into 96-well plates at a density of 1×10^3 cells/well and allowed to attach overnight. Cells were subsequently exposed to sodium arsenite at concentrations of 0, 0.5, 1, 2, 5, 10, and 20 μ M for 72 h. Cell viability was evaluated using the MTT assay. Dose-response data were analyzed using GraphPad Prism software, and the half-maximal inhibitory concentration (IC₅₀) was estimated by nonlinear regression analysis. Based on the calculated IC₅₀ value (approximately 5 μ M), this concentration was selected for subsequent experiments.

2.5. Protective treatment protocol

To evaluate the protective effects of kojic acid and sulforaphane against arsenic-induced toxicity, HaCaT cells were assigned to different treatment groups. For kojic acid experiments, the groups included: control, arsenic (5 μ M), arsenic + kojic acid (50 μ M), arsenic + kojic acid (100 μ M), and arsenic + kojic acid (200 μ M). For sulforaphane experiments, the groups included: control, arsenic (5 μ M), arsenic + sulforaphane (5 μ M), arsenic + sulforaphane (10 μ M), and arsenic + sulforaphane (20 μ M). Based on the cell viability results, 100 μ M kojic acid and 20 μ M sulforaphane were selected for subsequent gene expression analyses.

2.6. MTT cell viability assay

Cell viability was determined using the MTT assay. HaCaT cells were seeded into 96-well plates at a density of 1×10^3 cells/well and treated for 72 h. Following treatment, the culture medium was replaced with fresh medium containing MTT solution (0.5 mg/mL). Cells were incubated for 4 h at 37°C to allow the formation of formazan crystals. The medium was then removed, and the crystals were dissolved in 100 μ L DMSO. Absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control group. Each treatment condition was analyzed in triplicate wells, and the entire experiment was independently repeated three times.

2.7. RNA extraction

Following 72 h of treatment, total RNA was extracted from HaCaT cells using TRIzol EX reagent according to the manufacturer's instructions. Cells were washed with cold PBS before RNA isolation. RNA concentration and purity were determined spectrophotometrically by measuring absorbance at 260 and 280 nm. RNA samples with acceptable purity were stored at -80°C until further analysis.

2.8. Complementary DNA synthesis

Complementary DNA (cDNA) was synthesized from 1000 ng of total RNA using a commercial cDNA synthesis kit according to the manufacturer's instructions. The resulting

cDNA samples were stored at -20°C until quantitative real-time PCR analysis.

2.9. Quantitative real-time PCR

The expression levels of inflammation-related genes (TNF- α and IL-6) and apoptosis-related genes (BAX and BCL-2) were evaluated using quantitative real-time PCR. GAPDH was used as the reference housekeeping gene. Each reaction mixture contained SYBR Green Master Mix, gene-specific primers, cDNA template, and nuclease-free water. Amplification was performed using a Bio-Rad real-time PCR system. All reactions were performed in triplicate. Relative gene expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method and expressed as fold change relative to the control group. The primer sequences used in this study are presented in **Table 1**.

Table 1. Primer sequences used for quantitative real-time PCR

Gene	Forward primer	Reverse primer
TNF- α	5'AGCCCTGGTATGAGCCCATGTA 3'	5'CCGGACTCCGTGATGTCTAAGT 3'
IL-6	5'TCCTACCCCAACTTCCAATGCTC 3'	5'TTGGATGGTCTTGGTCCTTAGCC 3'
BCL-2	5'ACTTCTCTCGTCGCTACCGTCGC 3'	5'AGAGCGATGTTGTCCACCAGGG 3'
BAX	5'CCAGGACGCATCCACCAAGAAG 3'	5'CCCAGTTGAAGTTGCCGTCTGC 3'
GAPDH	5'CCCCAATGTATCCGTTGTG 3'	5'TAGCCCAGGATGCCCTTTAGT 3'

2.10. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). All experiments were performed independently three times. Statistical analyses were conducted using GraphPad Prism software. Differences among groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Arsenic-induced cytotoxicity in HaCaT cells

Exposure of HaCaT cells to increasing concentrations of sodium arsenite for 72 h resulted in a concentration-dependent reduction in cell viability. Cell viability decreased from $99.7 \pm 2.3\%$ in the control group to $89.7 \pm 5.9\%$, $72.7 \pm 6.9\%$, $60.3 \pm 8.6\%$, $38.5 \pm 6.1\%$, and $27.0 \pm 5.4\%$ following treatment with 0, 0.5, 1, 2, 5, 10, and 20 μM arsenic, respectively. Nonlinear regression calculated $\text{IC}_{50} = 5.6 \mu\text{M}$. Therefore, 5 μM arsenic was selected for subsequent protective treatment experiments. Detailed quantitative data are provided in **Table 2**, while the arsenic dose-response curve and IC_{50} estimation are presented in **Figure 3**. The present table presents the complete quantitative data underlying all cell viability and gene expression analyses, including mean values, standard deviations, sample sizes ($n = 3$), and fold-change values for all experimental groups.

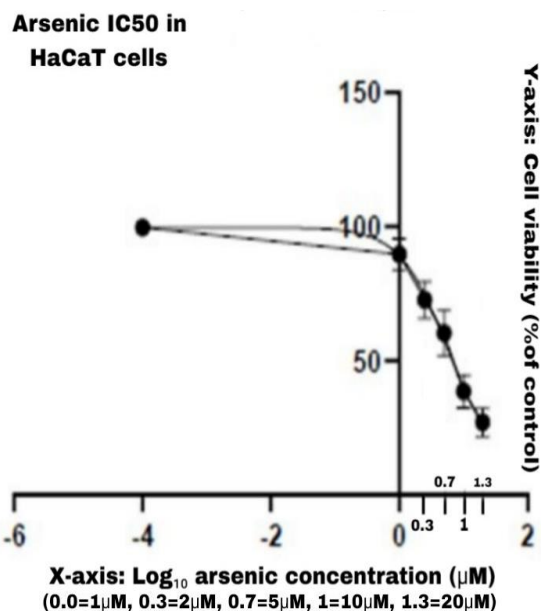


Figure 3. Determination of arsenic IC_{50} in HaCaT cells following 72 h exposure using the MTT assay. Cells were treated with sodium arsenite at concentrations of 0.5, 1, 2, 5, 10, and 20 μM . Cell viability was expressed as a percentage of the untreated control group. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$). The X-axis represents $\log_{10}$ -transformed arsenic concentrations. Nonlinear regression analysis calculated $\text{IC}_{50} = 5.6 \mu\text{M}$; 5 μM was selected as the working concentration.

Table 2. presents the complete quantitative data underlying all cell viability and gene expression analyses, including mean values, standard deviations, sample sizes (n = 3), and fold-change values for all experimental groups.

Experiment	Group	Mean	SD	n
Arsenic Cytotoxicity (MTT)	Control	99.7	2.3	3
	Arsenic 1 μ M	89.7	5.9	3
	Arsenic 2.5 μ M	72.7	6.9	3
	Arsenic 5 μ M	60.3	8.6	3
	Arsenic 10 μ M	38.5	6.1	3
	Arsenic 20 μ M	27.0	5.4	3
	Calculated IC ₅₀	5.6 μ M	—	—
Cell Viability (Kojic Acid)	Control	99.7	2.3	3
	Arsenic (5 μ M)	60.3	8.6	3
	Arsenic + KA (50 μ M)	67.0	4.8	3
	Arsenic + KA (100 μ M)	83.7	4.5	3
	Arsenic + KA (200 μ M)	73.0	6.2	3
Cell Viability (Sulforaphane)	Control	99.7	2.3	3
	Arsenic (5 μ M)	57.3	8.1	3
	Arsenic + SFN (5 μ M)	63.8	10.5	3
	Arsenic + SFN (10 μ M)	76.2	5.3	3
	Arsenic + SFN (20 μ M)	80.0	9.8	3
TNF- α Expression (Fold Change)	Control	1.0	0.7	3
	Arsenic	5.9	4.2	3
	Arsenic + KA	2.1	0.4	3
	Arsenic + SFN	2.6	0.7	3
	KA alone	1.7	0.9	3
	SFN alone	2.4	1.3	3
IL-6 Expression (Fold Change)	Control	1.1	0.2	3
	Arsenic	4.5	0.5	3
	Arsenic + KA	2.9	1.3	3
	Arsenic + SFN	2.1	0.9	3
	KA Alone	1.1	0.3	3
	SFN Alone	1.5	0.5	3
BAX Expression (Fold Change)	Control	1.0	0.3	3
	Arsenic	4.1	0.3	3
	Arsenic + KA	1.9	0.1	3
	Arsenic + SFN	2.0	0.4	3
	KA Alone	1.0	0.4	3
	SFN Alone	1.2	0.1	3
BCL-2 Expression (Fold Change)	Control	1.0	0.1	3
	Arsenic	0.2	0.1	3
	Arsenic + KA	0.6	0.2	3
	Arsenic + SFN	0.7	0.1	3
	KA Alone	0.9	0.1	3
	SFN Alone	1.0	0.1	3

3.2. Protective effects of kojic acid and sulforaphane on cell viability

Treatment with kojic acid improved the viability of arsenic-exposed HaCaT cells in a concentration-dependent manner,

as shown in **Figure 4**. Cell viability increased from $60.3 \pm 8.6\%$ in the arsenic-treated group to $67.0 \pm 4.8\%$, $83.7 \pm 4.5\%$, and $73.0 \pm 6.2\%$ following co-treatment with 50, 100, and 200 μ M kojic acid, respectively. The greatest protective effect was observed at 100 μ M kojic acid.

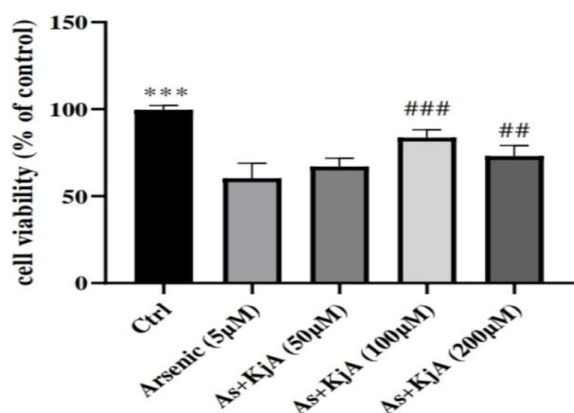


Figure 4. Effect of kojic acid on arsenic-induced reduction in HaCaT cell viability. Cells were treated with arsenic (5 µM) alone or in combination with kojic acid (50, 100, or 200 µM) for 72 h. Cell viability was assessed using the MTT assay. Data are presented as mean ± SD from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 versus control; #P < 0.05, ##P < 0.01, ###P < 0.001 versus arsenic-treated group.

Sulforaphane also improved cell viability following arsenic exposure, as shown in **Figure 5**. Viability increased from $57.3 \pm 8.1\%$ in the arsenic-treated group to $63.8 \pm 10.5\%$, $76.2 \pm 5.3\%$, and $80.0 \pm 9.8\%$ following treatment with 5, 10, and 20 µM sulforaphane, respectively. The highest viability was observed in cells treated with 20 µM sulforaphane.

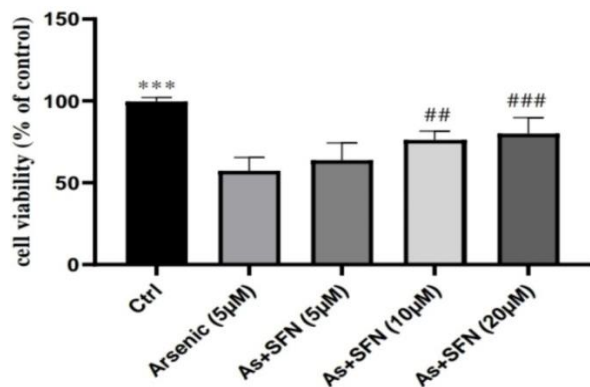


Figure 5. Effect of sulforaphane on arsenic-induced reduction in HaCaT cell viability. Cells were treated with arsenic (5 µM) alone or in combination with sulforaphane (5, 10, or 20 µM) for 72 h. Cell viability was assessed using the MTT assay. Data are presented as mean ± SD from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 versus control; #P < 0.05, ##P < 0.01, ###P < 0.001 versus arsenic-treated group.

3.3. Effects of kojic acid and sulforaphane on inflammatory gene expression

Arsenic exposure significantly increased TNF-α expression compared with the control group (5.9 ± 4.2 -fold versus 1.0 ± 0.7 -fold). Co-treatment with kojic acid or sulforaphane reduced TNF-α expression to 2.1 ± 0.4 -fold and 2.6 ± 0.7 -fold, respectively. Expression levels in cells treated with

kojic acid alone (1.7 ± 0.9 -fold) or sulforaphane alone (2.4 ± 1.3 -fold) remained lower than those observed in the arsenic-treated group. Arsenic exposure increased IL-6 expression from 1.1 ± 0.2 -fold in control cells to 4.5 ± 0.5 -fold. Co-treatment with kojic acid and sulforaphane reduced IL-6 expression to 2.9 ± 1.3 -fold and 2.1 ± 0.9 -fold, respectively. Expression levels in cells treated with kojic acid alone (1.09 ± 0.28 -fold) or sulforaphane alone (1.47 ± 0.50 -fold) remained lower than those observed in the arsenic-treated group. Both kojic acid and sulforaphane reduced IL-6 expression compared with the arsenic-treated group. Numerically, sulforaphane produced a greater reduction than kojic acid. The effects on TNF-α and IL-6 expression are shown in **Figure 6** and **7**, respectively.

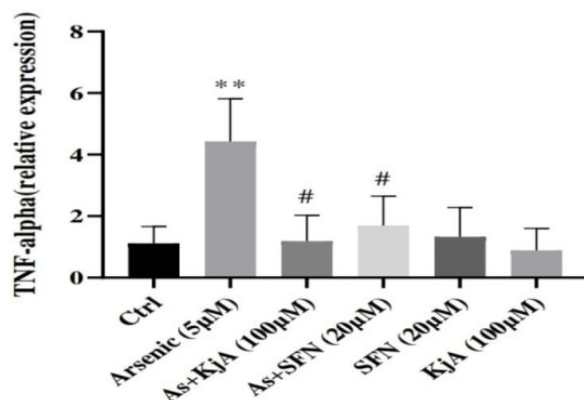


Figure 6. Effect of kojic acid and sulforaphane on TNF-α gene expression in arsenic-exposed HaCaT cells. Relative gene expression was determined by quantitative real-time PCR and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Treatment groups included control, arsenic (5 µM), arsenic + kojic acid (100 µM), arsenic + sulforaphane (20 µM), kojic acid alone (100 µM), and sulforaphane alone (20 µM). Data are presented as mean ± SD from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. P < 0.05, **P < 0.01, ***P < 0.001 versus control; #P < 0.05, ##P < 0.01, ###P < 0.001 versus arsenic-treated group.

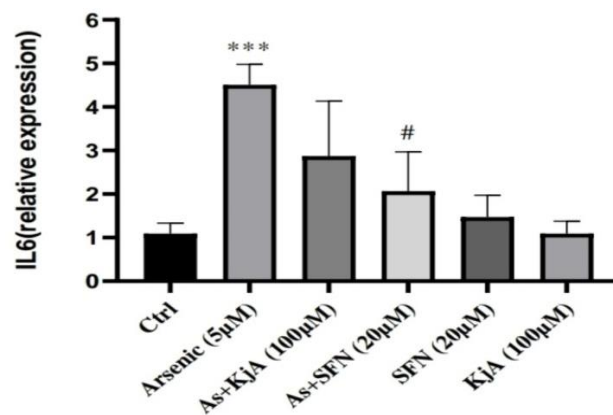


Figure 7. Effect of kojic acid and sulforaphane on IL-6 gene expression in arsenic-exposed HaCaT cells. Relative gene expression was determined by quantitative real-time PCR and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Treatment groups included control, arsenic (5 µM), arsenic + kojic acid (100 µM), arsenic + sulforaphane (20 µM), kojic acid alone (100 µM), and sulforaphane alone (20 µM). Data are presented as mean ± SD from three independent experiments (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. P < 0.05, **P < 0.01, ***P < 0.001 versus control; #P < 0.05, ##P < 0.01, ###P < 0.001 versus arsenic-treated group.

3.4. Effects of kojic acid and sulforaphane on apoptosis-related gene expression

BAX expression increased from 1.0 ± 0.3 -fold in control cells to 4.1 ± 0.3 -fold following arsenic treatment. Co-treatment with kojic acid or sulforaphane reduced BAX expression to 1.9 ± 0.1 -fold and 2.0 ± 0.4 -fold, respectively. Expression levels remained near baseline in cells treated with kojic acid alone (1.0 ± 0.4 -fold) and sulforaphane alone (1.2 ± 0.1 -fold). Arsenic exposure reduced BCL-2 expression from 1.0 ± 0.1 -fold in the control group to 0.2 ± 0.1 -fold. Co-treatment with kojic acid and sulforaphane increased BCL-2 expression to 0.6 ± 0.2 -fold and 0.7 ± 0.1 -fold, respectively. Cells treated with kojic acid alone or sulforaphane alone exhibited expression levels similar to those observed in control cells. The effects on BAX and BCL-2 expression are shown in Figure 8 and 9, respectively.

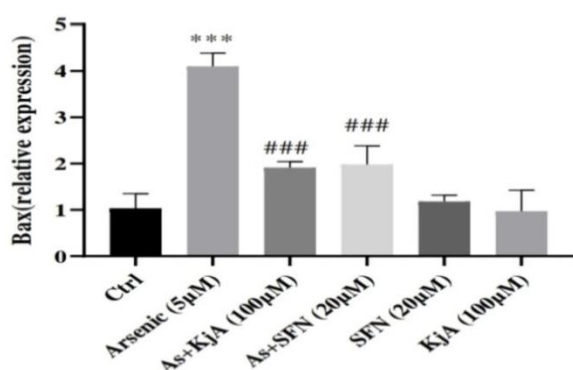


Figure 8. Effect of kojic acid and sulforaphane on BAX gene expression in arsenic-exposed HaCaT cells. Relative gene expression was determined by quantitative real-time PCR and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Treatment groups included control, arsenic (5 µM), arsenic + kojic acid (100 µM), arsenic + sulforaphane (20 µM), kojic acid alone (100 µM), and sulforaphane alone (20 µM). Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus arsenic-treated group.

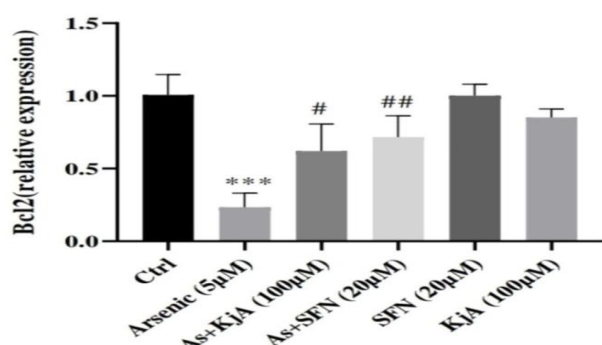


Figure 9. Effect of kojic acid and sulforaphane on BCL-2 gene expression in arsenic-exposed HaCaT cells. Relative gene expression was determined by quantitative real-time PCR and normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Treatment groups included control, arsenic (5 µM), arsenic + kojic acid (100 µM), arsenic + sulforaphane (20 µM), kojic acid alone (100 µM), and sulforaphane alone (20 µM). Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control; # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ versus arsenic-treated group.

4. Discussion

The present study demonstrated that arsenic exposure reduced HaCaT cell viability and induced significant increases in TNF- α , IL-6, and BAX expression, accompanied by reduced BCL-2 expression. These findings are consistent with previous reports describing arsenic as a potent cellular toxicant. Shi et al. (2004) and Flora (2011) highlighted the central role of oxidative and cellular stress pathways in arsenic toxicity, while Ding et al. (2005) demonstrated that inorganic arsenic compounds induce oxidative damage in human keratinocytes through the generation of reactive oxygen and nitrogen species. Similarly, Germolec et al. (1996) reported that arsenic alters growth factor expression in human keratinocytes, and Yamaguchi et al. (2016) showed that arsenic promotes cellular senescence and inhibits proliferation. Collectively, these studies support the observed reduction in viability and the inflammatory and apoptosis-associated transcriptional changes detected in the present investigation.

Sulforaphane treatment significantly improved cell viability and attenuated arsenic-induced alterations in TNF- α , IL-6, BAX, and BCL-2 expression. These findings agree with previous studies demonstrating the cytoprotective effects of sulforaphane. Li et al. (2021) reported that sulforaphane protects endothelial cells against oxidative stress through the miR-34a/SIRT1/Nrf2 axis, whereas Zhang and Wu (2021) demonstrated activation of the AMPK/SIRT1/PGC-1 α pathway in injured epithelial cells. Chen et al. (2021) further showed that sulforaphane attenuates oxidative stress-induced apoptosis, and Thangapandian et al. (2024) reported protective effects against arsenic-induced cardiotoxicity. The improved viability and normalization of inflammatory and apoptosis-related gene expression observed in the present study are therefore consistent with the established cytoprotective profile of sulforaphane. Kojic acid also improved cell viability and partially reversed arsenic-induced transcriptional alterations. Although traditionally recognized as a depigmenting agent (Kim and Uyama, 2005; Parvez et al., 2007), increasing evidence suggests broader biological activities. Khan et al. (2021) reported antioxidative and anti-inflammatory effects of kojic acid, whereas Ali et al. (2024) demonstrated suppression of inflammatory signaling through modulation of the TLR4/NF- κ B pathway. Likewise, Li et al. (2021) reported anti-inflammatory activity of kojic acid derivatives, and Da Costa et al. (2018) observed immunomodulatory effects in human monocytes. The present findings extend these observations by suggesting that kojic acid may also provide protection against arsenic-induced injury in keratinocytes.

This study compared sulforaphane and kojic acid as separate protective interventions under identical arsenic-exposure conditions. A combined sulforaphane-kojic acid treatment group was not included; therefore, possible additive or synergistic effects remain unknown. Sulforaphane was selected as a well-characterized cytoprotective comparator, whereas kojic acid was selected because of its dermatological relevance and emerging anti-inflammatory activity. However, the effects of both compounds may be concentration- and cell-type dependent. For example, Suthiram et al. reported that kojic acid induced oxidative stress while also showing anti-inflammatory effects in HepG2 cells (19). Therefore, future studies should evaluate compound safety, combined treatment effects, and formulation suitability before considering topical applications.

A notable aspect of the present study is the direct comparison between sulforaphane and kojic acid under identical experimental conditions. Although both compounds improved cell viability and attenuated arsenic-induced transcriptional changes, sulforaphane generally showed numerically greater effects in several measured endpoints. Importantly, the protective potential of kojic acid in arsenic-exposed HaCaT cells has received limited attention in previous studies. Therefore, the present work contributes new evidence supporting the potential use of both compounds, particularly sulforaphane, as protective agents against arsenic-induced keratinocyte toxicity. Although numerical differences were observed between sulforaphane and kojic acid treatment groups, the present study was not specifically designed to perform head-to-head statistical comparisons between the two compounds. Therefore, conclusions regarding superiority of one treatment over the other should be interpreted cautiously.

5. Study limitations and future directions

The present study has several limitations that should be considered when interpreting the findings. First, inflammatory and apoptosis-related responses were evaluated only at the mRNA level. Although arsenic exposure altered TNF- α , IL-6, BAX, and BCL-2 gene expression, these transcriptional changes do not necessarily reflect protein abundance or biological activity. Therefore, the present findings should be interpreted as evidence of transcriptional modulation rather than definitive confirmation of inflammatory or apoptotic processes. Second, oxidative stress biomarkers were not measured. Consequently, potential involvement of oxidative stress pathways remains based on previously reported evidence rather than direct experimental verification. Third, the study was performed using an *in vitro* HaCaT cell model, which cannot fully replicate the complexity of human skin tissue and chronic arsenic exposure *in vivo*. Future studies should include protein-level validation of inflammatory and apoptosis-related markers using approaches such as ELISA, Western blotting, or caspase activity assays. Assessment of oxidative stress biomarkers, including ROS generation, lipid peroxidation, antioxidant enzyme activity, and glutathione status, would further clarify the mechanisms underlying the observed protective effects. Additional investigations using primary keratinocytes, three-dimensional skin models, or *in vivo* systems may provide further insight into the therapeutic potential of sulforaphane and kojic acid against arsenic-induced skin toxicity.

6. Conclusion

The present study demonstrated that arsenic exposure reduced cell viability and induced transcriptional alterations associated with inflammatory and apoptosis-related responses in HaCaT cells. Both sulforaphane and kojic acid attenuated these changes, indicating protective effects against arsenic-induced cellular injury. Sulforaphane generally produced a more consistent improvement in inflammatory and apoptosis-related gene expression, whereas kojic acid also showed significant protective activity despite being less extensively studied in this context. These findings contribute to the growing understanding of potential protective strategies against arsenic-induced skin toxicity and provide a foundation for future mechanistic and translational investigations.

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التأثيرات الوقائية للسلفورافان وحمض الكوجيك ضد السمية الالتهابية والاستماتية الناجمة عن الزرنيخ في خلايا HaCaT

الخلاصة

المقدمة: يُعد الزرنيخ مادة سامة بيئية ترتبط بتلف الجلد والالتهاب والاستجابات الخلوية المرتبطة بالموت الخلوي المبرمج. تُستخدم خلايا الكيراتين البشرية (HaCaT) على نطاق واسع لدراسة آليات سمية الزرنيخ على الجلد والتدخلات الوقائية المحتملة. وقد أشارت الدراسات إلى أن السلفورافان وحمض الكوجيك يمتلكان خصائص وقائية للخلايا ومضادة للالتهابات في نماذج تجريبية مختلفة. الهدف: تقييم التأثيرات الوقائية للسلفورافان وحمض الكوجيك ضد سمية الزرنيخ على الخلايا والتغيرات في التعبير الجيني المرتبط بالالتهاب والموت الخلوي المبرمج في خلايا HaCaT. **المنهجية:** عُرضت خلايا HaCaT للزرنيخ (5 ميكرومولار) بمفرده أو بالاشتراك مع السلفورافان (5، 10، 20 ميكرومولار) أو حمض الكوجيك (50، 100، 200 ميكرومولار). تم تقييم حيوية الخلايا باستخدام اختبار MTT. تم تقييم التعبير عن عامل نخر الورم ألفا (TNF- α)، والإنترلوكين-6 (IL-6)، والبروتين المرتبط بـ BAX (BCL-2)، والليفيما للخلايا البائية 2 (BCL-2) باستخدام تفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي (qPCR). **النتائج:** أدى التعرض للزرنيخ إلى انخفاض ملحوظ في حيوية الخلايا وزيادة في التعبير عن TNF- α وIL-6 وBAX، بينما انخفض التعبير عن BCL-2. حسن العلاج بالسلفورافان أو حمض الكوجيك من حيوية الخلايا وخفف من التغيرات التي يُحدثها الزرنيخ في التعبير الجيني. قلل كلا المركبين من التعبير عن TNF- α وIL-6 وBAX، واستعاد جزئيًا التعبير عن BCL-2 مقارنةً بالمجموعة المعالجة بالزرنيخ. أظهر السلفورافان عمومًا تأثيرًا وقائيًا أكثر اتساقًا من حمض الكوجيك عبر المؤشرات الالتهابية ومؤشرات موت الخلايا المبرمج التي تم تقييمها. **الخلاصة:** أدى الزرنيخ إلى سمية خلوية وتغيرات جينية كبيرة مرتبطة باستجابات التهابية وموت خلوي مبرمج في خلايا HaCaT. وقد خفف كل من السلفورافان وحمض الكوجيك من هذه التأثيرات، مع إظهار السلفورافان فعالية وقائية أكثر اتساقًا. تشير هذه النتائج إلى أن كلا المركبين قد يساهمان في تخفيف تلف الخلايا الكيراتينية الناجم عن الزرنيخ، ويوفران أساسًا لمزيد من الدراسات الآلية.

الكلمات المفتاحية: الاستماتة؛ الزرنيخ؛ خلايا HaCaT؛ حمض الكوجيك؛ السلفورافان