

Original Research Article

Attenuating effects of nanocurcumin on arsenic-induced ovarian oxidative damage: involvement of the *Nrf2* pathway

Yasan Karimi¹, Alireza Yeganehfar¹, Maryam Baazm^{2,3}, Nastaran Azarbarz^{2,3,*}

¹Students Research Committee, Arak University of Medical Sciences, Arak, Iran

²Molecular and Medicine Research Center, Arak University of Medical Sciences, Arak, Iran

³Department of Anatomy, School of Medicine, Arak University of Medical Sciences, Arak, Iran

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* Corresponding Author:

n.Azarbarz@gmail.com

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Abstract

Objective: This study investigated the protective effects of nanocurcumin (NC) against arsenic (As)-induced ovarian toxicity, focusing on oxidative stress and the *Nrf2/Keap1* pathway.

Materials and Methods: Twenty-eight female NMRI mice were randomly assigned to four groups (n = 7): control, NC (100 mg/kg/day, intraperitoneal, for 14 days), As (50 mg/L in drinking water for 40 days), and As + NC. Ovarian malondialdehyde (MDA) levels, Superoxide Dismutase and Catalase activities, histopathology, and the mRNA expression of *Nrf2*, *Keap1*, *HO-1*, and *NQO1* were assessed. *Nrf2* and *Keap1* protein levels were evaluated by Western blotting.

Results: Arsenic exposure significantly increased ovarian MDA levels and decreased SOD and CAT activities compared with the control group. Arsenic also induced histopathological alterations and significantly increased *Nrf2* mRNA and protein expression, while significantly decreasing *Keap1* mRNA and protein expression. Compared with the As group, NC significantly reduced MDA levels and increased SOD activity, whereas the increase in CAT activity was not statistically significant. NC also partially attenuated histopathological alterations and further increased *Nrf2* mRNA and protein expression. In contrast, *Keap1* expression did not differ significantly between the As and As + NC groups, while *HO-1* and *NQO1* showed non-significant increases.

Conclusion: NC partially attenuates As-induced ovarian toxicity, potentially by enhancing the *Nrf2*-mediated antioxidant response.

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Introduction

Environmental contamination by arsenic (As), a toxic metalloid, poses a major global health concern due to its presence in food and water sources (Ganie et al. 2024; Grund et al. 2000). Chronic exposure to As has been associated with an increased risk of cancers, liver fibrosis, lung diseases, and

metabolic disorders such as diabetes mellitus (Amirmostofian et al. 2023; Kannan et al. 2004; Kumar et al. 2014; Smith et al. 1992).

The World Health Organization has established a maximum permissible concentration of 10 µg/L in drinking water (WHO, 2004). Despite this guideline,

elevated levels of As have been reported in water supplies across several regions of Asia including China and India, as well as in some Central and South American countries (Amirmostofian et al. 2023; Fu et al. 2010; Hei et al. 2004; Srivastava et al. 2015; Sun et al. 2007).

Mechanistically, As compounds impair antioxidant defense systems, induce DNA damage, and promote lipid peroxidation. Among the biological systems affected, the reproductive system is particularly vulnerable. Previous studies have demonstrated that As exposure impairs reproductive function through both direct and indirect mechanisms (Eslami et al. 2024; Kannan et al. 2004). Specifically, As alters gonadotropin secretion by modulating of pituitary activity, and directly interferes estrogen and progesterone synthesis in the gonads (Chen et al. 2022a).

Exposure to As led to increased production of reactive oxygen species (ROS), impairment of antioxidant systems, and induction of oxidative damage (Jelic et al. 2021). This oxidative imbalance disrupts hormonal homeostasis, impairs ovarian function, and decreases oocyte quality, potentially leading to infertility (Chen et al. 2022b). Under physiological conditions, endogenous antioxidant systems neutralize ROS to prevent cellular damage. A key regulator of this defense is the the pathway involving *Nrf2* and its regulator Keap1 (Song et al. 2021). In the absence of stress, *Nrf2* is sequestered by Keap1 in the cytoplasm (Song et al. 2021; Sykietis 2021). Upon oxidative stress, *Nrf2* is released from Keap1, translocates to the nucleus, and activates the transcription of genes encoding antioxidant enzymes. These enzymes include (NAD(P)H Quinone Dehydrogenase 1, Heme oxygenase-1, Superoxide dismutase, and Catalase, which collectively mitigate ROS-induced damage and restore cellular redox balance (Yu et al. 2021).

Arsenic disrupts this protective pathway, intensifying oxidative damage

and impairing organ function and overall health (Balali-Mood et al. 2021). Therefore, antioxidants administration may provide therapeutic potential in mitigating As-induced oxidative stress (Shiek et al. 2023). Curcumin (CU), a polyphenolic compound derived from turmeric (*Curcuma longa*), exhibits potent antioxidant and anti-inflammatory properties (Fekri et al. 2026; Razavi et al. 2021). By modulating signaling pathways such as Nrf2 and NF- κ B, CU mitigates oxidative stress and promotes tissue repair (Mahdavizadeh et al. 2026; Nouri et al. 2022).

However, CU therapeutic application is limited by poor bioavailability, low water solubility, and rapid clearance (Mahlooji et al. 2022). Advances in nanotechnology have addressed these limitations through the development of nanoparticle-based formulations of curcumin, known as nanocurcumin, which enhance its stability and bioavailability (Hafez Ghoran et al. 2022; Hettiarachchi et al. 2022).

This study aims to investigate the protective antioxidant effects of NC against As-induced ovarian toxicity, with a particular focus on the *Nrf2/Keap1* signaling pathway in female mice. The findings could provide novel therapeutic approaches for oxidative stress-related reproductive disorders resulting from environmental toxins such as As.

Materials and Methods

Reagents and chemicals

NC (C21H20O6) was purchased from Sina curcumin (Tehran, Iran). Arsenic was obtained from Acros Organics (Geel, Belgium). Assay kits for Superoxide dismutase, Catalase and Malondialdehyde were sourced from Kiazist, Iran. All other chemicals and reagents were procured from Sigma-Aldrich Chemical Company (St. Louis, USA).

Animals

A total of 28 female NMRI mice, aged 4-6 weeks and weighing 25-30 g, were

procured from the Pasteur Institute of Iran. The animals were acclimated in the animal facility for one week before starting the experiment. They were housed in standard laboratory cages under controlled conditions, including a 12-hr light/dark cycle, ambient temperature of $22 \pm 2^\circ\text{C}$, and relative humidity of $50 \pm 10\%$. The mice had free access to standard rodent chow and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IR.ARAKMU.AEC.1403.015).

Experimental design

Animals were randomly divided to four distinct experimental groups ($n=7$ each) as follows:

Group I: Control; Group II: Nanocurcumin (NC; 100 mg/kg via intraperitoneal daily injection for 14 days)(Alhusaini et al. 2021; Tohamy et al. 2022; Tousif et al. 2017); Group III: As (50 mg/L in drinking water for 40 days)(Carmean et al. 2020; Duan et al. 2022; Guvvala et al. 2016); Group IV: As +NC (NC was administered during the final 14 days of the 40-day As exposure period). The treatments and dosages of both NC and As were based on previously published studies (Sanghamitra et al. 2008; Wang et al. 2017).

Sample collection

At the end of the experimental period, all animals were euthanized under anesthesia induced by ketamine (75 mg/kg) and xylazine (10 mg/kg) (both from Alfasan, Iran) (Fallah Asl et al. 2023). The ovaries were harvested, with one ovary from each animal fixed in 10% formalin for histological evaluation and the contralateral ovary stored at -80°C for further analyses. Ovarian samples from seven mice per group ($n = 7$) were used for histological and antioxidant assessments. For molecular analyses, samples from four mice per group ($n = 4$) were used for gene expression analysis and samples from four mice per group ($n = 4$) were used for Western

blotting. In one mouse per group, the two ovaries were allocated separately to gene expression analysis and Western blotting.

Oxidative stress marker evaluation

To assess the oxidative stress status in the tissues, the following assays were performed: Lipid peroxidation was measured by quantifying MDA levels using a commercial assay kit, with results expressed as nmol per mg of protein (Sun et al. 2013). MDA levels are expressed as nmol/mg protein. SOD activity was determined spectrophotometrically using a commercial assay kit (Assay Kiazist, Iran). CAT activity was assessed spectrophotometrically by monitoring the decomposition of hydrogen peroxide at 240 nm, according to the kit instructions (Mehboodi et al. 2023), and expressed as units per mg protein.

Histological examination

The right ovaries were paraffin-embedded following a 12-hr fixation in 10% formalin. Serial sections (5 μm thick) were prepared, mounted on glass slides, and stained with hematoxylin and eosin. Every fifth ovarian section was examined using a light microscope. To prevent double-counting the same follicle, only those containing a visible oocyte nucleus were recorded. Given that oocyte nuclei range from 20 to 30 μm in diameter, sampling every fifth section created a 30 μm interval between analyzed planes, thereby reducing the risk of recounting identical follicles (Wang et al. 2017).

Real time polymerase chain reaction analysis (qRT-PCR)

The expression levels of *Nrf2*, *Keap1*, *HO-1*, *NQO1*, *GAPDH* (used as a housekeeping gene) were evaluated by qRT-PCR. Total RNA was first extracted from ovarian tissue using the RNA Mini Kit (Sigma-Aldrich Chemical Company (St. Louis, USA), followed by cDNA synthesis using a Reverse Transcription kit. Each reaction was performed in a final volume of

12.5 μ L, containing 6.25 μ L of SYBR Green qPCR Master Mix 2x, 0.25 μ L each of forward and reverse primers, 2.75 μ L of DNase-free water, and 3 μ L of cDNA (equivalent to 200 ng). The primer

sequences are listed in the Table 1. Each reaction was performed in triplicate, and data were analyzed using the $2^{-\Delta\Delta CT}$ formula.

Table 1. Sequences of the primers used for the target genes

Gene name	Sequence	Lenght bp
<i>GAPDH</i> -mice-F	CATCACTGCCACCCAGAAGACTG	153
<i>GAPDH</i> -mice-R	ATGCCAGTGAGCTTCCCGTTTCAG	
<i>NRF2</i> - mice-F	CAGCATAGAGCAGGACATGGAG	107
<i>NRF2</i> - mice-R	GAACAGCGGTAGTATCAGCCAG	
<i>KEAP1</i> - mice-F	ATCCAGAGAGGAATGAGTGGCG	123
<i>KEAP1</i> - mice-R	TCAACTGGTCCTGCCCATCGTA	
<i>HO-1</i> - mice-F	CACTCTGGAGATGACACCTGAG	115
<i>HO-1</i> - mice-R	GTGTTCTCTGTGTCAGCATCACC	
<i>NQO-1</i> - mice-F	GCCGAACACAAGAAGCTGGAAG	120
<i>NQO-1</i> - mice-R	GGCAAATCCTGCTACGAGCACT	

Western blot analysis

The quantification of Nrf2 and Keap-1 proteins was conducted utilizing western blotting technique. Ovarian tissue lysates were carefully prepared and centrifuged at 12,000 rpm for 10 min. Proteins were then separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membranes. The membranes were incubated overnight at 4°C with a primary mouse monoclonal antibody against Nrf2 (sc-365949, Santa Cruz, USA) and Keap1 (sc-365626, Santa Cruz, USA) at a 1:200 dilution in Tris-buffered saline. Following this, membranes were incubated with a goat anti-mouse IgG secondary antibody (ab150113, Abcam, USA) for one hour, then developed using an enhanced chemiluminescence method (Gibco, USA; Cat. No. 32106). For densitometric quantification, intensity of the specific bands was normalized to GAPDH (Bioss, USA; 1:1000 dilution) in the same blot using Image J software (free Java software provided by the National Institute of Health; Bethesda, USA).

Statistical analysis

Results are presented as the mean $\pm$ standard deviation. Statistical analyses

were performed using GraphPad Prism 8.0. Comparisons among groups were made using one-way analysis variance followed by Tukey's post-hoc test. A p-value less than 0.05 was considered statistically significant.

Results

NC partially attenuates arsenic-induced oxidative stress

Our data showed that arsenic exposure caused a significant increase in MDA levels compared with the control group ($p < 0.001$; Figure 1A). Concurrently, antioxidant enzyme activities including CAT ($p < 0.01$), and SOD ($p < 0.001$) were significantly reduced in the AS group (Figure 1B-C). In the As+NC group, MDA levels were significantly reduced ($p < 0.001$) compared with the As group. Moreover, NC treatment significantly increased SOD activity ($p < 0.01$), whereas the increase in CAT activity was not statistically significant compared with the As group (Figure 1).

Nanocurcumin ameliorates arsenic-induced ovotoxicity

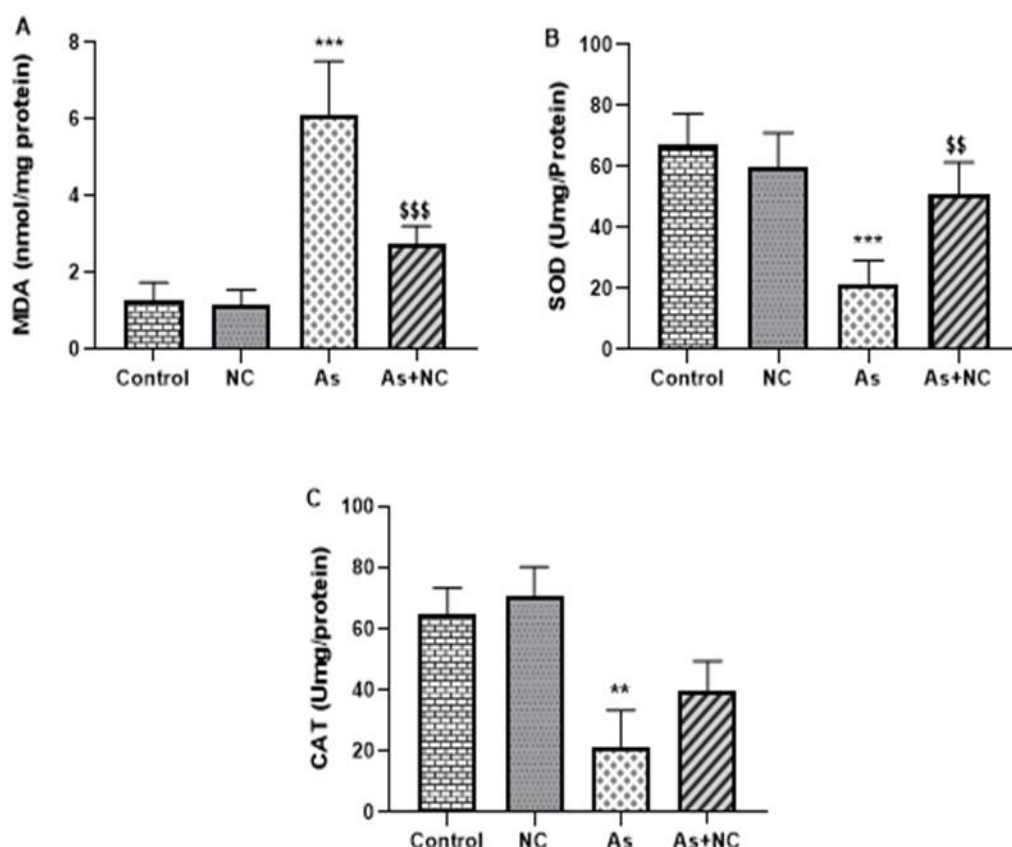


Figure 1. Impact of nanocurcumin on oxidative stress markers (lipid peroxidation and antioxidant enzymes CAT and SOD) in arsenic-induced ovarian toxicity in a mice model. Data represent mean $\pm$ SD (n=7 per group). Statistical significance: * p <0.05, ** p <0.01, *** p <0.001 and **** p <0.0001 vs control group. \$ p <0.05, \$\$ p <0.01, \$\$\$ p <0.001, and \$\$\$\$ p <0.0001 vs arsenic-treated group # p <0.05, ## p <0.01, ### p <0.001 vs Nanocurcumin-treated group. NC: nanocurcumin; As: arsenic; As+NC: arsenic plus nanocurcumin.

Histological examination

Histological examination revealed normal follicular architecture in the control and NC-treated groups, characterized by well-defined granulosa and theca layers, and healthy oocytes devoid of inflammatory infiltrates (lymphocytic, neutrophilic) or fibrosis (Figure 2). In contrast, ovarian tissues from the As group, exhibited significant histopathological damages including follicular degeneration, zona pellucida folding and collapse, increased follicular atresia, vacuolization and pyknosis of granulosa cells, and inflammatory cell infiltration within the ovarian stroma (Figure 3A-F). Treatment with NC partially improved these ovarian

alterations, reducing follicular atresia and granulosa-cell vacuolization (Figure 2).

Quantitative analysis of ovarian follicles showed that, compared with the control group, As exposure significantly decreased the number of primordial follicles (p <0.05) and antral follicles (p <0.05), while significantly increasing the number of atretic follicles (p <0.001), suggesting As exposure impaired follicular development and survival. Compared with the As group, NC treatment significantly reduced the number of atretic follicles (p <0.001), whereas the increase in the number of antral follicles was not statistically significant, NC partially attenuated arsenic-induced follicular damage (Table 2).

Table 2. Comparison of the number of follicles per ovary in different groups (n = 7 per group) NC: nanocurcumin; As: arsenic; As+NC: arsenic plus nanocurcumin.

	Primordial Follicle	Primary Follicle	Preantral Follicle	Antral Follicle	Atretic Follicle
Control	8.4 ± 8.3	2.6 ± 1.2	1.4 ± 0.5	1.8 ± 0.7	1.6 ± 0.8
NC	7.4 ± 3.8	4.0 ± 3.3	2.6 ± 1.5	2.2 ± 1.0	2.0 ± 0.9
As	3.8 ± 4.3*	4.0 ± 1.4	1.6 ± 0.5	1.2 ± 0.4*	8.20 ± 1.1***
As+NC	2.6 ± 2.0	4.8 ± 3.8	1.8 ± 0.7	1.4 ± 1.0	4.0 ± 2.8 \$\$\$

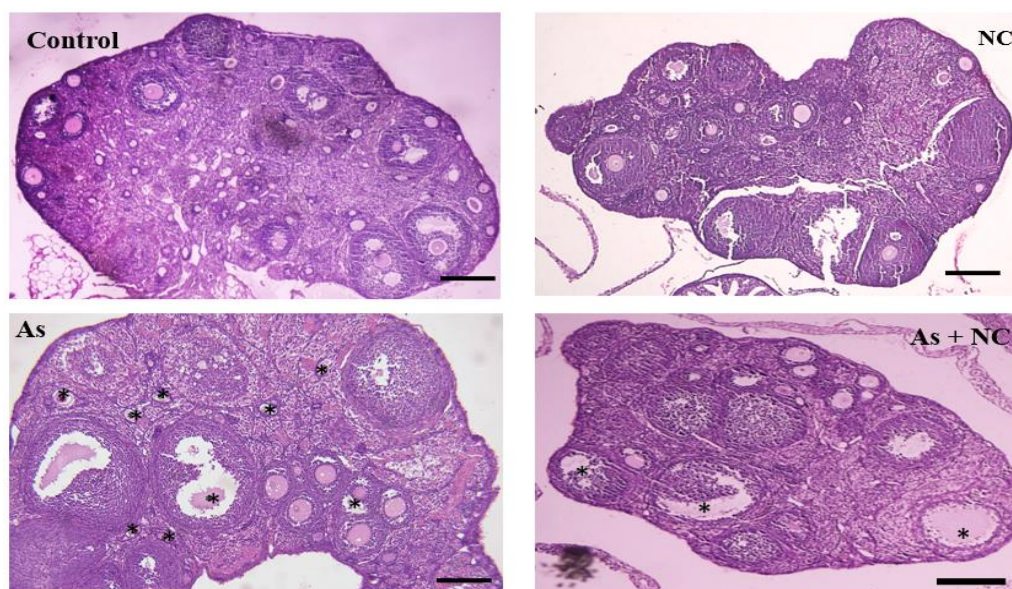


Figure 2. Effect of NC on histopathological changes in arsenic-induced ovototoxicity (H&E Staining, staining original magnification: 40X, Scale bar = 40 μ m) Control group received normal saline; normal histological appearance of ovary, NC group received Nanocurcumin (100 mg/kg/d); normal histological appearance of ovary, Arsenic group received Arsenic (50 mg/L); pathological changes in follicle and ovary tissues, Arsenic +NC: animals received both of them. Stars indicate atretic follicles. NC: nanocurcumin; As: arsenic; As+NC: arsenic plus nanocurcumin.

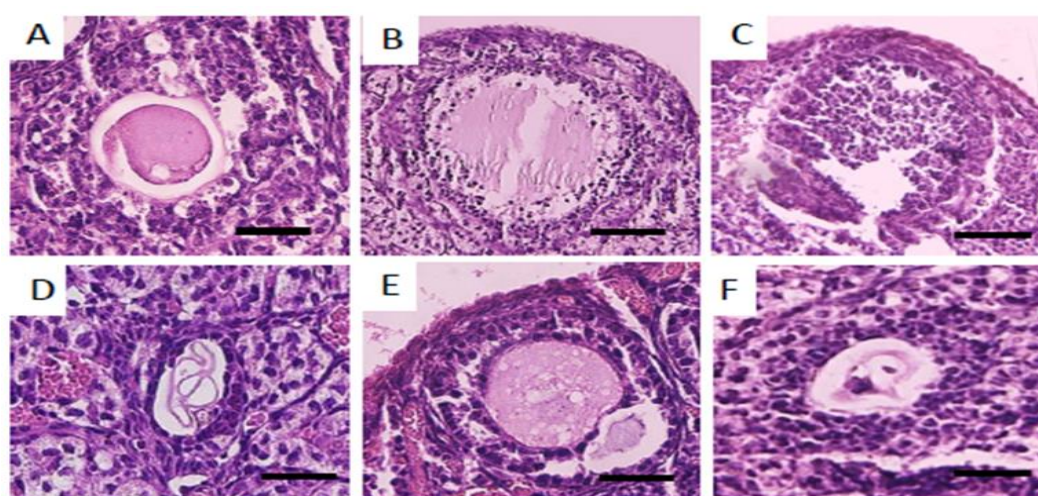


Figure 3. Atretic follicles: Effects of arsenic on follicular histopathological changes (H&E Staining, original magnification: 400X; Scale bar=500 μ m). Atretic follicles : Marked by reduced follicular size, absence of an oocyte, structural distortion and/or granulosa cells with pyknotic nuclei, the zona pellucida becomes folded and collapse as it is slowly broken down within the cavity of the follicle.

NC partially modulates the expression of antioxidant response-related genes

To investigate the effect of NC on arsenic-induced alterations in the antioxidant defense pathway, the mRNA expression levels of *Nrf2*, *Keap1*, *HO-1*, and *NQO1* were evaluated (Figure 4A–D). As shown in Figure 4A, *Nrf2* expression was significantly increased in the arsenic-treated group compared with the control group ($p < 0.01$). Notably, treatment with NC resulted in a further significant increase in *Nrf2* expression compared with the As group ($p < 0.05$).

In contrast, *Keap1* expression was significantly decreased in the arsenic group compared with the control group ($p < 0.01$) (Figure 4B). Although *Keap1* expression remained lower in the As + NC group, no significant difference was observed between the As and As + NC groups.

Regarding the downstream antioxidant response genes, *HO-1* and *NQO1* expression was increased in the As + NC group compared with the arsenic group; however, this difference did not reach statistical significance (Figure 4C–D).

NC partially modulates Nrf2 and Keap-1 protein expression

To evaluate the effect of NC on As-induced alterations in the antioxidant response pathway, the relative protein expression levels of Nrf2 and Keap-1 were quantified by Western blot analysis. As illustrated in Figure 5, a significant increase in Nrf2 protein expression was observed in the As-treated group compared with the control group ($p < 0.001$). In contrast, the NC group showed no significant alteration in Nrf2 protein expression compared with the control group. Notably, co-administration of NC with As resulted in a further significant increase in Nrf2 protein expression compared with the As-treated group ($p < 0.001$).

Keap-1 protein expression was significantly reduced in the As group compared with the control group ($p < 0.001$), whereas no significant differences were observed among the other groups (Figure 5).

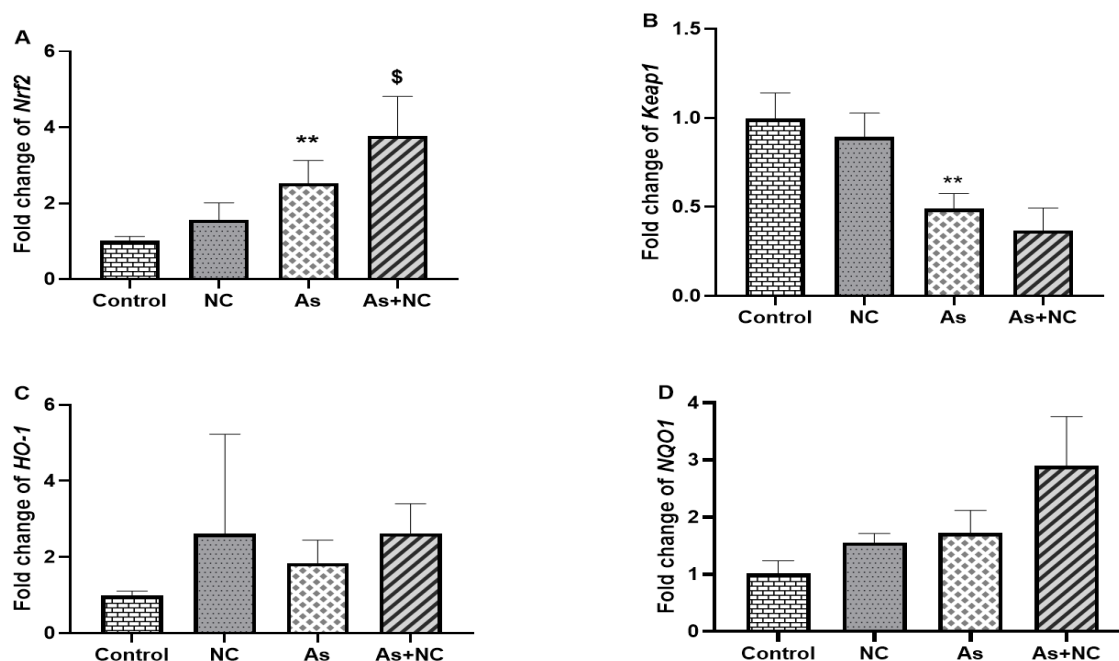


Figure 4. Gene expression profiles (Mean $\pm$ SD, $n=4$). Statistical significance: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs control group. \$ $p < 0.05$ and \$\$ $p < 0.01$ vs arsenic-treated group. # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ vs Nanocurcumin-treated group. NC: nanocurcumin; As: arsenic; As+NC: arsenic plus nanocurcumin.

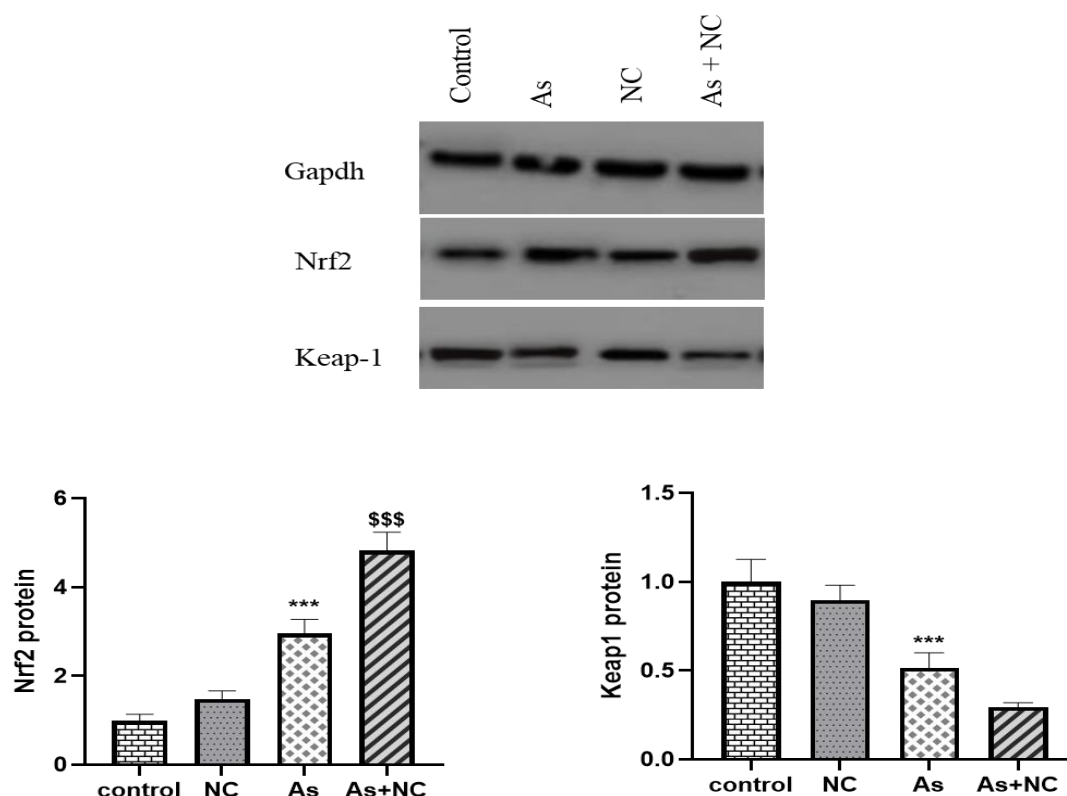


Figure 5. Western blot analysis of Nrf-2 and Keap-1 protein expression levels (Mean $\pm$ SD, $n=4$). Statistical significance: * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ vs control group. \$ $p<0.05$ and \$\$ $p<0.01$ vs arsenic-treated group. # $p<0.05$, ## $p<0.01$, and ### $p<0.001$ vs Nanocurcumin-treated group. NC: nanocurcumin; As: arsenic; As+NC: arsenic plus nanocurcumin.

Discussion

This study investigated the potential of NC as a protective antioxidant molecule against As-induced ovarian injury in a mouse model. Chronic As exposure is known to induce oxidative damage to lipids, proteins, and DNA by promoting free radical generation (Zhang et al. 2025). This damage is exacerbated when endogenous antioxidant defenses are failed to a state of oxidative stress (Najafi et al. 2023). We observed a substantial increase in ovarian MDA levels—a primary marker of lipid peroxidation—concurrent with a significant decrease in the activity of crucial antioxidant enzymes, including SOD and CAT. Histological examination in this study revealed significant As-induced ovarian damage, characterized by a marked increase in atretic follicles. These follicles exhibited classic signs of degeneration,

including reduced size, loss of the oocyte, structural distortion, granulosa cells with pyknotic nuclei, and a collapsed, folded zona pellucida undergoing degradation within the follicular cavity. Our findings align with those of Mehta and Hundal, who reported As-induced follicular atresia and reduced uterine endometrial and myometrial thickness in female rats (Mehta et al. 2016). These structural pathologies were accompanied by significant functional impairment of the ovary, as evidenced by a pronounced state of oxidative stress. Consistent with prior literature, our results confirm the ovarian toxicity of chronic As exposure, which is through oxidative stress. This oxidative damage directly contributes to ovarian dysfunction, manifesting as reduced follicle quality, impaired folliculogenesis, and conditions such as endometriosis and unexplained infertility (Wang et al. 2017).

To evaluate the activation of the endogenous antioxidant defense pathway against As-induced ovarian toxicity, we assessed the expression levels of the *Nrf2* and *Keap1* genes and proteins. Under normal physiological conditions, the concentration of this protein in the cytoplasm is kept at a low and stable level to avoid unnecessary activation of antioxidant mechanisms and to maintain homeostatic balance of Nrf2 levels. This regulation is accomplished through the ubiquitination of Nrf2 in the cytoplasm, followed by its transfer to the proteasome—the cellular machinery responsible for protein breakdown—for degradation (Gu et al. 2024). This suppressive mechanism is driven by ubiquitination, which is facilitated by the ubiquitin ligase complex with the Keap1 protein serving as an adaptor. This inhibitory mechanism is mediated by a process called ubiquitination, facilitated by the ubiquitin ligase complex and the Keap1 protein acting as an adaptor. Keap1 possesses specific structural domains that enable a strong interaction between Nrf2 and Keap1 (Abdellatif et al. 2025). Following the establishment of this interaction, Keap1 functions as an adaptor, linking Nrf2 to the ubiquitin ligase complex, which leads to the ubiquitination and subsequent degradation of Nrf2 in the proteasome. This mechanism helps maintain a low and stable concentration of Nrf2 in the cytoplasm under normal conditions (Gu et al. 2024; Kumar et al. 2020). The cycle of Keap1 binding/dissociation to the ligase complex is critical for regulating Nrf2 levels, and any disruption in the interaction between Keap1 and nuclear enzymes leads to impaired assembly of the ubiquitin ligase complex, resulting in the failure to degrade Nrf2 (Suzuki et al. 2023). This defensive pathway, as a key protective mechanism, shields cells from oxidative damage and toxicity caused by environmental factors (Tripathi et al. 2024). In the As-treated group, *Nrf2* gene expression was elevated, while *Keap1* expression reduced compared

to the control group. The activation of the Nrf2 pathway by As, however, involves a mechanism more complex and multifaceted than that of typical oxidative stressors (Abdellatif et al. 2025).

Arsenic-induced oxidative stress enhances the interaction between Keap1 and the components of its associated E3 ubiquitin ligase complex. This strengthened binding sequesters Keap1, preventing newly synthesized Nrf2 accumulates and its cytoplasmic half-life is prolonged (Tripathi et al. 2024). Moreover,, this altered interaction redirects ubiquitination toward Keap1 itself, targeting it for proteasomal degradation and further reducing Keap1 protein levels—a process that indirectly enhances Nrf2 stability (Louisa et al. 2023). These findings are consistent with a previous report of upregulated *Nrf2* gene expression in rats co-treated with cisplatin and NC (Louisa et al. 2023).

Notably, despite the observed upregulation of Nrf2 in the As-treated group, the activity of key downstream antioxidant enzymes, including SOD and CAT, was significantly reduced. This functional disconnect represents as a recognized paradox in As toxicology, wherein cellular defense signaling is activated yet fails to translate into effective antioxidant capacity (Tao et al. 2016). This indicates a fundamental disruption in the coordination between *Nrf2* pathway induction and the functional expression of its transcriptional targets. Under physiological conditions, *Nrf2* activation robustly enhances the synthesis and activity of antioxidant enzymes. In the context of As exposure, however, this regulatory axis is subverted. Arsenic directly impairs enzyme function through mechanisms such as the covalent modification of critical thiol groups within the SOD and CAT protein structures, thereby inhibiting their catalytic activity (Wang et al. 2008). Furthermore, As reduces the transcription of mRNA for antioxidant enzymes through direct or indirect epigenetic mechanisms, including DNA methylation and histone

modifications, thereby suppressing SOD and CAT expression at the transcriptional level (Sarawi et al. 2022). This collective functional disconnect demonstrates that AS effectively neutralizes the cytoprotective genetic program orchestrated by Nrf2. Consequently, even when *Nrf2* is activated and its target genes are transcribed, the resulting protein, such as SOD and CAT, are rendered non-functional due to As-induced molecular damage or direct inhibition. This dual-level impairment—transcriptional suppression and post-translational inactivation—ultimately highlights the cellular vulnerability to oxidative stress and exacerbates As-induced toxicity. To mitigate the destructive effects of As on the ovary, we employed NC as an antioxidant intervention due to its enhanced efficacy and cellular protective potential (Alhashemi et al. 2022).

A study by Vashistha et al. demonstrated that both CU and NC exhibit hepatoprotective effects in rats exposed to As. Nevertheless, NC provided superior protection compared to conventional CU (Vashistha et al. 2023). Also Embaby and Abdel-Kawi found that CU and NC improved cadmium-induced liver damage, with NC showing greater protective effects (Embaby et al. 2021).

In the present study, a significant upregulation of both gene and protein expression of Nrf2 was observed in As+NC group. Moreover, antioxidant enzyme activity showed a differential pattern: SOD activity increased significantly, whereas CAT activity showed a non-significant elevation. Similarly, downstream target genes of *Nrf2* such as *HO-1* and *NQO1* demonstrated an increasing trend without reaching statistical significance.

SOD constitutes the first line of defense against oxidative stress by catalyzing the dismutation of superoxide radicals into hydrogen peroxide. Therefore, its rapid and significant upregulation may reflect an immediate compensatory response to increased superoxide production. In

contrast, CAT functions downstream by converting hydrogen peroxide into water and oxygen. In contrast, CAT functions downstream by converting hydrogen peroxide into water and oxygen. The absence of a significant increase in CAT activity may be explained by the level of oxidative insult may have been sufficient to trigger SOD activation but not strong enough to induce robust CAT transcription.

The significant elevation of Nrf2 gene and protein expression, SOD activity alongside non-significant upward trend in CAT, *HO-1* and *NQO1* expression further supports the notion of early activation of the *Nrf2* signaling cascade. It is possible that the duration of treatment or sample size limited the ability to detect statistically significant changes in these downstream targets.

In animals exposed to As, NC treatment resulted in the highest level of *Nrf2* gene expression, a significant reduction in MDA levels, marked histological improvement, and a partial restoration of SOD and CAT activities. Consistent with our study, Perveen et al. demonstrated that curcumin extensively attenuated As-mediated oxidative stress and lipid peroxidation in the uterus of Wistar rats (Perveen et al. 2019). Also, Barbhuiya et al. reported that curcumin and quercetin exerted protective effects against As-associated female reproductive toxicity, significantly reducing oxidative stress (Nath Barbhuiya et al. 2025).

These findings highlight the complex pharmacological interaction between AS and NC. While the results confirm NC potential as a protective agent against As-induced ovarian injury, they also indicate that its efficacy may be enhanced by complementary therapeutic strategies or by optimizing its dosing regimen to overcome As sustained toxic pressure.

The results demonstrate that As induces significant ovarian oxidative stress and follicular degeneration. Treatment with NC effectively mitigated this damage by attenuating oxidative stress, preserving

histoarchitecture, and amplifying the *Nrf2*-mediated antioxidant response. These indicate a synergistic interaction : As primes the *Nrf2* pathway, and NC potentiates it, leading to enhanced—though not complete—protection against As-induced ovarian toxicity. These findings underscore NC therapeutic potential and highlight the value of *Nrf2* pathway modulation in combating such injury.

A limitation of the present study is that only a single dose of NC (100 mg/kg/day) was investigated. Therefore, the dose-dependent effects of nanocurcumin on arsenic-induced ovarian toxicity could not be determined. In addition, the present study did not allow identification of the minimum effective dose or the optimal dose that provides the greatest protective effect with minimal exposure. Future studies using multiple doses and extended treatment regimens are warranted to establish a dose–response relationship, identify the effective dose range, and further clarify the efficacy and safety of NC in attenuating As-induced ovarian toxicity.

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Conflicts of interest

No conflicts of interest are declared by the authors.

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Ethical Considerations

This project was conducted according to the guidelines established by the Animal Ethics Committee of our organization.

Code of Ethics

IR.ARAKMU.AEC.1403.015

Authors' Contributions

Yasan karimi: Writing – original draft, Visualization, Validation, Investigation, Formal analysis. Alireza yeganehfard: Writing – original draft, Visualization, Validation, Investigation. Maryam baazm: Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation, Formal analysis. Nastaran Azarbarz: Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis.

Abbreviations

As, arsenic; CAT, catalase; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; NC, nanocurcumin; NQO1, NAD(P)H quinone dehydrogenase 1; Nrf2, nuclear factor erythroid 2-related factor 2; SOD, superoxide dismutase.

References

- Abdellatif HA, Azab M, El-Sayed EH, et al (2025) Cullin-3 and regulatory biomolecules profiling in vitiligo: integrated docking, clinical, and in silico insights. *Biomolecules* 15(7):1053
- Alhashemi SH, Mohammadpour AH, Heidari R, Nikoo MH, Nemati MH, Vazin A (2022) The effect of nanocurcumin on the incidence of atrial fibrillation, and markers of inflammation and oxidative stress level after coronary artery bypass graft surgery: a randomized, double-blind, placebo-controlled clinical study. *Avicenna J Phytomed* 12(5):503–514
- Alhusaini A, Alhumaidan S, Almogren R, Alsaif S, Alsultan E, Hussein I (2021) Nano-curcumin protects against sodium nitrite-induced lung hypoxia through modulation of mitogen-activated protein kinases/c-Jun NH2-terminal kinase signaling pathway. *Dose Response* 19(3):15593258211033148
- Amirmostofian M, Akbari F, Hashemzaei M, et

- al (2023) Hormetic effects of curcumin on oxidative stress injury induced by trivalent arsenic in isolated rat hepatocytes. *Avicenna J Phytomed* 13(6):641–650
- Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair M, Sadeghi M (2021) Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Front Pharmacol* 12:643972
- Carmean CM, Kirkley AG, Landeche M, et al (2020) Arsenic exposure decreases adiposity during high-fat feeding. *Obesity* 28(5):932–941
- Chen P, Luo Q, Lin Y, et al (2022a) Arsenic exposure during juvenile and puberty significantly affected reproductive system development of female SD rats. *Ecotoxicol Environ Saf* 242:113857
- Chen Y, Sun Y, Zhao A, et al (2022b) Arsenic exposure diminishes ovarian follicular reserve and induces abnormal steroidogenesis by DNA methylation. *Ecotoxicol Environ Saf* 241:113816
- Duan X, Xu G, Li J, et al (2022) Arsenic induces continuous inflammation and regulates Th1/Th2/Th17/Treg balance in liver and kidney in vivo. *Mediators Inflamm* 2022:8414047
- Embaby A, Abdel-Kawi S (2021) Histological study on the effect of curcumin and curcumin nanoparticles on cadmium-induced liver damage in adult male albino rats. *J Med Histol* 5(2):119–133
- Eslami H, Askari FR, Mahdavi M, Taghavi M, Ghaseminasab-Parizi M (2024) Environmental arsenic exposure and reproductive system toxicity in male and female and mitigatory strategies: a review. *Environ Geochem Health* 46(10):420
- Fallah Asl H, Jalali Mashayekhi F, Zendedel A, Baazm M (2023) The time course of nod-like receptor family pyrin domain containing 3 inflammasome complex expressions in the testis tissue of an experimental varicocele rat model: an experimental study. *Int J Reprod Biomed* 21(7):577–584
- Fekri K, Haghani Borujeni M, Rahmati S, et al (2026) The protective effect of curcumin capsules on cisplatin-induced nephrotoxicity: a double-blind randomized controlled trial. *Avicenna J Phytomed* e28176.
<https://doi.org/10.22038/ajp.2026.28176>
- Fu J, Woods CG, Yehuda-Shnaidman E, et al (2010) Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: involvement of cellular adaptive response to oxidative stress. *Environ Health Perspect* 118(6):864–870
- Ganie SY, Javaid D, Hajam YA, Reshi MS (2024) Arsenic toxicity: sources, pathophysiology and mechanism. *Toxicol Res* 13(1):tfad111
- Grund SC, Hanusch K, Wolf HU (2000) Arsenic and arsenic compounds. In: *Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.
https://doi.org/10.1002/14356007.a03_113.pub2
- Gu L, Du Y, Chen J, et al (2024) Cullin 3 RING E3 ligase inactivation causes NRF2-dependent NADH reductive stress, hepatic lipodystrophy, and systemic insulin resistance. *Proc Natl Acad Sci U S A* 121(17):e2320934121
- Guvvala PR, Sellappan S, Parameswaraiiah RJ (2016) Impact of arsenic (V) on testicular oxidative stress and sperm functional attributes in Swiss albino mice. *Environ Sci Pollut Res* 23(18):18200–18210
- Hafez Ghoran S, Calcaterra A, Abbasi M, Taktaz F, Nieselt K, Babaei E (2022) Curcumin-based nanoformulations: a promising adjuvant towards cancer treatment. *Molecules* 27(16):5236
- Hei TK, Filipic M (2004) Role of oxidative damage in the genotoxicity of arsenic. *Free Radic Biol Med* 37(5):574–581
- Hettiarachchi SS, Perera Y, Dunuweera SP, Dunuweera AN, Rajapakse S, Rajapakse RMG (2022) Comparison of antibacterial activity of nanocurcumin with bulk curcumin. *ACS Omega* 7(50):46494–46500
- Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU (2021) Oxidative stress and its role in cancer. *J Cancer Res Ther* 17(1):22–28
- Kannan GM, Flora SJ (2004) Chronic arsenic poisoning in the rat: treatment with combined administration of succimers and an antioxidant. *Ecotoxicol Environ Saf* 58(1):37–43
- Kumar A, Khushboo, Pandey R, Sharma B (2020) Modulation of superoxide dismutase activity by mercury, lead, and arsenic. *Biol Trace Elem Res* 196(2):654–661
- Kumar S, Yedjou CG, Tchounwou PB (2014)

- Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. *J Exp Clin Cancer Res* 33:42
- Louisa M, Putera AM, Sidqi AA, et al (2023) Attenuation of cisplatin-induced hepatotoxicity by nanocurcumin through modulation of antioxidative and anti-inflammatory pathways. *J Appl Pharm Sci* 13(3):060–070
- Mahdaviadeh V, Hosseini M, Saberi R, Bahramfar MM, Beheshti F (2026) Curcumin improved liver and renal function in a systemic inflammation model induced by lipopolysaccharide in rats. *Avicenna J Phytomed* e27578. <https://doi.org/10.22038/ajp.2026.27578>
- Mahlooji MA, Heshmati A, Kheiripour N, et al (2022) Evaluation of protective effects of curcumin and nanocurcumin on aluminium phosphide-induced subacute lung injury in rats: modulation of oxidative stress through SIRT1/FOXO3 signalling pathway. *Drug Res (Stuttg)* 72(2):100–108
- Mehboodi M, Asgharpour H, Hosseini SA, RezaeeShirazi R (2023) The effect of swimming and resveratrol on antioxidant markers and lipid peroxidation index in liver tissue. *J Guilan Univ Med Sci* 31(4):286–299
- Mehta M, Hundal S (2016) Effect of sodium arsenite on reproductive organs of female Wistar rats. *Arch Environ Occup Health* 71(1):16–25
- Najafi N, Rezaee R, Hayes AW, Karimi G (2023) A review of mechanisms underlying the protective effects of natural compounds against arsenic-induced neurotoxicity. *Biomaterials* 36(4):799–813
- Nath Barbhuiya S, Barhoi D, Dey P, Giri A, Giri S (2025) Curcumin and quercetin can ameliorate arsenic and smokeless tobacco induced reproductive toxicity in female mice. *Drug Chem Toxicol* 48(6):1342–1356
- Nouri M, Sohaei S, Shalaby MN, Mehrabani S, Ramezani A, Faghih S (2022) Effect of curcumin on body mass index and glycemic indices in females with PCOS: a systematic review and meta-analysis of randomized controlled trial. *Nutr Food Sci* 52(7):1129–1141
- Perveen H, Chattopadhyay S, Maity M, Dash M, Islam SS (2019) Involvement of proinflammatory cytokines and metallothionein in the repairing of arsenic-mediated uterine tissue damage by curcumin. *J Basic Clin Physiol Pharmacol* 30(4):20170179
- Razavi BM, Ghasemzadeh Rahbardar M, Hosseinzadeh H (2021) A review of therapeutic potentials of turmeric (*Curcuma longa*) and its active constituent, curcumin, on inflammatory disorders, pain, and their related patents. *Phytother Res* 35(12):6489–6513
- Sanghamitra S, Hazra J, Upadhyay S, Singh R, Amal RC (2008) Arsenic induced toxicity on testicular tissue of mice. *Indian J Physiol Pharmacol* 52(1):84–90
- Sarawi WS, Alhusaini AM, Fadda LM, et al (2022) Nano-curcumin prevents copper reproductive toxicity by attenuating oxidative stress and inflammation and improving Nrf2/HO-1 signaling and pituitary-gonadal axis in male rats. *Toxics* 10(7):356
- Shiek SS, Sajai ST, Dsouza HS (2023) Arsenic-induced toxicity and the ameliorative role of antioxidants and natural compounds. *J Biochem Mol Toxicol* 37(3):e23281
- Smith AH, Hopenhayn-Rich C, Bates MN, et al (1992) Cancer risks from arsenic in drinking water. *Environ Health Perspect* 97:259–267
- Song MY, Lee DY, Chun KS, Kim EH (2021) The role of NRF2/KEAP1 signaling pathway in cancer metabolism. *Int J Mol Sci* 22(9):4376
- Srivastava R, Bhattacharya S, Chakraborty A, Chattopadhyay A (2015) Differential in vivo genotoxicity of arsenic trioxide in glutathione depleted mouse bone marrow cells: expressions of Nrf2/Keap1/P62. *Toxicol Mech Methods* 25(3):223–228
- Sun G, Xu Y, Li X, Jin Y, Li B, Sun X (2007) Urinary arsenic metabolites in children and adults exposed to arsenic in drinking water in Inner Mongolia, China. *Environ Health Perspect* 115(4):648–652
- Sun H, Wang W, Geng L, Chen Y, Yang Z (2013) In situ studies on growth, oxidative stress responses, and gene expression of juvenile bighead carp (*Hypophthalmichthys nobilis*) to eutrophic lake water dominated by cyanobacterial blooms. *Chemosphere* 93(2):421–427
- Suzuki T, Takahashi J, Yamamoto M (2023) Molecular basis of the KEAP1-NRF2 signaling pathway. *Mol Cells* 46(3):133–

- Sykietis GP (2021) Keap1/Nrf2 signaling pathway. *Antioxidants* 10(6):828
- Tao S, Rojo de La Vega M, Quijada H, et al (2016) Bixin protects mice against ventilation-induced lung injury in an NRF2-dependent manner. *Sci Rep* 6:18760
- Tohamy HG, El Okle OS, Goma AA, Abdel-Daim MM, Shukry M (2022) Hepatorenal protective effect of nano-curcumin against nano-copper oxide-mediated toxicity in rats: behavioral performance, antioxidant, anti-inflammatory, apoptosis, and histopathology. *Life Sci* 292:120296
- Tousif S, Singh DK, Mukherjee S, et al (2017) Nanoparticle-formulated curcumin prevents posttherapeutic disease reactivation and reinfection with *Mycobacterium tuberculosis* following isoniazid therapy. *Front Immunol* 8:739
- Tripathi S, Kharkwal G, Mishra R, Singh G (2024) Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling in heavy metals-induced oxidative stress. *Heliyon* 10(18):e37719
- Vashistha L, Singh M, Verma Y, Rana S (2023) Nano-curcumin ameliorates arsenic induced hepatotoxicity in female rats. *J Environ Biol* 44(6):775–783
- Wang XJ, Sun Z, Chen W, Li Y, Villeneuve NF, Zhang DD (2008) Activation of Nrf2 by arsenite and monomethylarsonous acid is independent of Keap1-C151: enhanced Keap1–Cul3 interaction. *Toxicol Appl Pharmacol* 230(3):383–389
- Wang XN, Zhang CJ, Diao HL, Zhang Y (2017) Protective effects of curcumin against sodium arsenite-induced ovarian oxidative injury in a mouse model. *Chin Med J* 130(9):1026–1032
- Yu C, Xiao JH (2021) The Keap1-Nrf2 system: a mediator between oxidative stress and aging. *Oxid Med Cell Longev* 2021:6635460
- Zhang Y, Zhou M, Wang D, et al (2025) Arsenic exposure and oxidative damage to lipid, DNA, and protein among general Chinese adults: a repeated-measures cross-sectional and longitudinal study. *J Environ Sci* 147:382–391