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Hepatoprotective effects of zingerone on sodium arsenite-induced hepatotoxicity in rats: Modulating the levels of caspase-3/Bax/Bcl-2, NLRP3/NF-κB/TNF-α and ATF6/IRE1/PERK/GRP78 signaling pathways

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ABSTRACT

Objective: Long-term exposure to arsenic has been linked to several illnesses, including hypertension, diabetes, hepatic and renal diseases and cardiovascular malfunction. The aim of the current investigation was to determine whether zingerone (ZN) could shield rats against the hepatotoxicity that sodium arsenite (SA) causes.

Methods: The following five groups of thirty-five male Sprague Dawley rats were created: I) Control; received normal saline, II) ZN; received ZN, III) SA; received SA, IV) SA + ZN 25; received 10 mg/kg body weight SA + 25 mg/kg body weight ZN, and V) SA + ZN 50; received 10 mg/kg body weight SA + 50 mg/kg body weight ZN. The experiment lasted 14 days, and the rats were sacrificed on the 15th day. While oxidative stress parameters were studied by spectrophotometric method, apoptosis, inflammation and endoplasmic reticulum stress parameters were measured by RT-PCR method.

Results: The SA disrupted the histological architecture and integrity of the liver and enhanced oxidative damage by lowering antioxidant enzyme activity, such as those of glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) level and increasing malondialdehyde (MDA) level in the liver tissue. Additionally, SA increased the mRNA transcript levels of Bcl2 associated x (Bax), caspases (−3, −6, −9), apoptotic protease-activating factor 1 (Apaf-1), p53, tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), interleukin-6 (IL-6), c-Jun NH2-terminal kinase (JNK), mitogen-activated protein kinase 14 (MAPK14), MAPK15, receptor for advanced glycation endproducts (RAGE) and nod-like receptor family pyrin domain-containing 3 (NLRP3) in the liver tissue. Also produced endoplasmic reticulum stress by raising the mRNA transcript levels of activating transcription factor 6 (ATF-6), protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and glucose-regulated protein 78 (GRP-78). These factors together led to inflammation, apoptosis, and endoplasmic reticulum stress. On the other hand, liver tissue treated with ZN at doses of 25 and 50 mg/kg showed significant improvement in oxidative stress, inflammation, apoptosis and endoplasmic reticulum stress.

Conclusions: Overall, the study's data suggest that administering ZN may be able to lessen the liver damage caused by SA toxicity.

1. Introduction

Arsenic is a naturally occurring, semimetallic element widely distributed in the Earth's crust. Because of its toxicity, sodium arsenite (SA), like other arsenic compounds, presents serious environmental hazards [1,2]. Agricultural runoff, mining, industrial processes, improper disposal of arsenic-containing materials, and industrial processes are the main ways through which it can enter the soil, water, and

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atmosphere. The use of SA in some industrial processes or its disposal in waste industrial waste contaminate water. Arsenic contamination of groundwater makes it unsafe for use by humans and animals. The contamination poses serious health risks to individuals who rely on the affected water sources [3]. Soil contamination may occur from herbicides containing SA or from industrial effluent. When plants absorb arsenic from polluted soil, the pollution may have an impact on plant growth and make its way into the food chain [4]. Certain industrial processes have the potential to release airborne arsenic compounds. This can happen during production, when materials containing arsenic burn, or from natural sources like volcanoes. Particles of arsenic have the potential to accumulate in soil and water, leading to pollution [5]. To reduce its negative effects on the environment and stop widespread contamination, regulations and appropriate disposal techniques are crucial. Reducing the damage that SA causes to the environment requires efforts to develop less harmful alternatives and to limit its use. The liver is the main organ of arsenic's conversion into methylated compounds, which cause free radicals to be produced and damage tissues and organs [6]. By producing excess amount of reactive oxygen species (ROS), reactive nitrogen species (RNS), hydroxyl species, and malondialdehyde (MDA) through lipid peroxidation, arsenic toxicity causes oxidative stress [7,8]. Additionally, it decreases the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) [9]. Oxidative stress also contributes to the inflammatory process by releasing pro-inflammatory cytokines by triggering the c-Jun N-terminal Kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) signal transduction pathways [10]. Chronic exposure to arsenic compounds has been connected to a number of health concerns, such as cancer, neurological disorders, skin blemishes, and cardiovascular ailments [11,12].

Zingerone (ZN) is a naturally occurring chemical that gives ginger its unique smell and scent. It's an antioxidant-rich phenolic substance that may offer some health advantages. According to several studies, ZN may have gastroprotective, anti-inflammatory, and anti-nausea properties [13,14]. It may also be useful in the treatment of diseases such as obesity and diabetes, though additional studies are required to comprehensively understand how it affects these health issues [15]. Recently anti-inflammatory, and anti-apoptotic role of zingerone was established in vancomycin-induced hepatotoxicity by decreasing nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), caspase-3, caspase-8, p53, cytochrome *c*, and Bcl-2 associated X protein (Bax) [14]. ZN also showed an anti-inflammatory effect by reducing the increase in the expression of inflammatory markers such as NF-κB, COX-2, TNF-α and IL-6 in ethanol-induced liver injury in rats [16]. In this regard, the aim of the study was to ascertain whether ZN, a chemical produced from plants, could treat liver damage caused by SA.

2. Materials and methods

2.1. Chemicals

Sodium arsenite ($NaAsO_2$) and ZN (Vanillacetone ≥96 %) were obtained from Sigma-Aldrich, (St. Louis, MO, USA). Other chemicals used in the study were of analytical purity and were used according to the respective protocols.

2.2. Experimental animals

In this study, a total of 35 male Sprague Dawley rats (weighing an average of 230 g at 12–14 weeks of age) with 7 animals per group were used. The animals were housed in clean polypropylene cages bedded with husk under standard conditions of humidity (40%–50 %), temperature (25 °C), and 12-hr light/12-hr dark cycle in the Experimental Research and Application Center, Ataturk University. Additionally, access to standard food and water was *ad libitum*. The experimental protocol was approved by the Atatürk University Animal Experiments Local Ethics Committee (Approval No: 2023–09/145).

2.3. Experimental design

Thirty-five animals were divided into five groups of seven rats each. Animals were allowed to acclimatize for 1 week before the experiment started.

Group I (Control) received normal saline (orally for 14 days).
Group II (ZN) received ZN (50 mg/kg b.w., orally for 14 days) [14].
Group III (SA) received SA (10 mg/kg b.w., orally for 14 days) [8].
Group IV (SA + ZN 25) received SA (10 mg/kg b.w.) and ZN (25 mg/kg b.w.) administered orally for 14 days. ZN was given to the rats 30 min before SA.
Group V (SA + ZN 50) received SA (10 mg/kg b.w.) and ZN (50 mg/kg b.w.) administered orally for 14 days. ZN was given to the rats 30 min before SA.

At the end of the study period, the rats were sacrificed under mild sevoflurane anesthesia. Animals were then killed by decapitation and liver tissues were isolated for biochemical, molecular and histopathological examinations.

2.4. Antioxidant enzyme activities and lipid peroxidation

The liver tissues were ground in liquid nitrogen using the Tissue Lyser II (Qiagen, Netherlands) device. It was then homogenised using suitable buffers for SOD, CAT, GPx, glutathione (GSH), malondialdehyde (MDA) and protein analysis. For SOD, CAT, and MDA analysis, homogenates were centrifuged at 1180 *g* for 15 min at +4 °C. For GSH and GPx analyses, they were centrifuged at 9350 *g* for 20 min at 4 °C. In the liver tissue, CAT, GPx and SOD activity were measured according to methods developed by Aebi [17], Lawrence and Burk [18], Sun, Oberley and Li [19] respectively. GSH and MDA levels were measured according to the methods of Sedlak and Lindsay [20], Placer, Cushman and Johnson [21] respectively. The protein concentration in liver homogenates were determined according to the method of Lowry, Rosebrough, Farr and Randall [22].

2.5. Analysis of mRNA transcript levels by RT-PCR

The relative mRNA transcript levels of Bcl-2, Apaf-1, Caspase-3, Bax, P53, Caspase-6, Caspase-9, NF-κB, TNF-α, IL-1β, IL-6, CHOP, IRE1, PERK, ATF-6, GRP78, RAGE, NLRP3, MAPK14, MAPK15 and JNK genes in liver tissues were analyzed by RT-PCR method. All primer sequences are shown in Table 1. For this, initially, total RNAs were isolated from the tissues with QIAzol Lysis Reagent (79306; Qiagen), and then the RNAs were converted into cDNAs using the iScript cDNA Synthesis Kit (Bio-Rad). Then, cDNAs were reacted with the primers of the relevant genes and iTaq Universal SYBR Green Supermix (BIORAD) in the Rotor-Gene Q (Qiagen) device. While setting up the reaction, the Rotor-Gene Q (Qiagen) device was set for 30 s at 95 °C (1 cycle), 5 s at 95 °C (40 cycles), and 30 s at 60 °C (40 cycles). After the cycles were completed, genes were normalized to β-Actin using the $2^{-\Delta\Delta CT}$ method [23].

2.6. Histopathological analysis

Dissected liver samples from all groups were fixed in neutral buffered 10 % formalin solution for histological examination. They were then dehydrated in increasing concentrations of alcohol and formed into blocks by embedding in paraffin. 5 μm thick sections were prepared from the prepared blocks using a microtome and stained with hematoxylin and eosin (H&E). Stained sections were examined using a binocular Olympus Cx43 light microscope (Olympus Inc., Tokyo, Japan) and photographed with an EP50 camera (Olympus Inc., Tokyo, Japan).

Table 1
Primer sequences.

Gene	Sequences (5′-3′)	Length (bp)	Accession No
Bcl-2	F: GACTTTGCAGAGATGTCCAG R: TCAGGTACTCAGTCATCCAC	214	NM_016993.2
Apaf-1	F: ACCTGAGGTGTCAGGACC R: CCGTCGAGCATGAGCCAA	192	NM_023979.2
Caspase-3	F: ACTGGAATGTCAGCTCGCAA R: GCAGTAGTCGCCTCTGAAGA	270	NM_012922.2
Bax	F: TTTCATCCAGGATCGAGCAG R: AATCATCCTCTGCAGCTCCA	154	NM_017059.2
P53	F: GCGCTTCGAGATGTTCCGA R: AGACTGGCCCTTCTTGGTCT	121	NM_030989.3
Caspase-6	F: GAACGAACGGACCTGTGGA R: CAGTCCAGCTCTGTACCTCG	124	NM_012922.2
Caspase-9	F: ACGTGAACTTCTGCCCTTCC R: GGTCGTTCTTCACCTCCACC	117	NM_031632.2
NF-κB	F: AGTCCCGCCCCTTCTAAAAC R: CAATGGCCTCTGTGTAGCCC	106	NM_001276711.1
TNF-α	F: CTCGAGTGACAAGCCCGTAG R: ATCTGCTGGTACCACCAGTT	139	NM_012675.3
IL-1β	F: ATGGCAACTGTCCCTGAACT R: AGTGACACTGCCTTCCTGAA	197	NM_031512.2
IL-6	F: AGCGATGATGCACTGTCAGA R: GGAACTCCAGAAGACCAGAGC	127	NM_012589.2
CHOP	F: GAAGCCTGGTATGAGGATCT R: GAACTCTGACTGGAATCTGG	209	NM_001109986.1
IRE1	F: GCAGTTCCAGTACATTGCCATTG R: CAGGTCTCTGTGAACAATGTTGA	163	NM_001191926.1
PERK	F: GATGCCGAGAATCATGGGAA R: AGATTCGAGAAGGGACTCCA	198	NM_031599.2
ATF-6	F: TCAACTCAGCACGTTCCTGA R: GACCAGTGACAGGCTTCTCT	130	NM_001107196.1
GRP78	F: CATGCAGTTGTGACTGTACCAG R: CTCTTATCCAGGCCATATGCAA	143	NM_013083.2
RAGE	F: CTGAGGTAGGGCATGAGGATG R: TTCATCACCGGTTTCTGTGACC	113	NM_053336.2
NLRP3	F: TCCTGCAGAGCCTACAGTTG R: GGCTTGCAGCACTGAAGAAC	185	NM_001191642.1
MAPK14	F: GTGGCAGTGAAGAAGCTGTC R: GTCACCAGGTACACATCGTT	170	NM_031020.2
MAPK15	F: TGTTTGAGTCCATGGACACC R: GCATCCAATAGAACGTTGGC	169	NM_173331.2
JNK	F: GAATCAGACCCATGCTAAGC R: CCATGAGCTCCATGACTATG	149	NM_053829.2
β-Actin	F: CAGCCTTCCTTCTTGGGTATG R: AGCTCAGTAACAGTCCGCCT	360	NM_031144.3

2.7. Statistical analysis

All data analyses were performed using SPSS 20.0 software. One-way analysis of variance (ANOVA) and Tukey test were used to determine the difference and significance levels between the groups. Results were expressed as mean ± standard deviations (SDs). $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Antioxidant enzymes and lipid peroxidation in the liver

Biochemical results showed that SA administration significantly increased MDA levels while significantly decreased levels of GSH and SOD, CAT, and GPx activities compared to the control group ($p < 0.05$). In the treatment groups, 25 and 50 mg/kg doses of ZN demonstrated significant differences on SOD, CAT, GPx, GSH, and MDA levels when compared to the AS treated group ($P < 0.05$). It was determined that the 50 mg/kg ZN dose was the most effective dose in all parameters (Table 2).

Table 2
Protective effect of zingerone (ZN) on oxidative stress biomarkers in sodium arsenite (SA)-induced hepatotoxicity in rats.

Parameters	Control	ZN	SA	SA + ZN 25	SA + ZN 50
SOD (U/g tissue)	20.65 ± 1.73^{d}	20.09 ± 1.66^{d}	10.76 ± 0.85^{a}	13.37 ± 0.78^{b}	15.79 ± 0.83^{c}
CAT (katal/g protein)	38.29 ± 1.79^{c}	38.72 ± 1.70^{c}	20.06 ± 1.42^{a}	26.60 ± 3.23^{b}	28.92 ± 1.93^{b}
GPx (U/g tissue)	26.14 ± 1.32^{d}	27.23 ± 1.29^{d}	13.70 ± 0.98^{a}	17.11 ± 1.11^{b}	20.02 ± 0.94^{c}
GSH (nmol/g tissue)	6.78 ± 0.35^{d}	6.82 ± 0.33^{d}	3.76 ± 0.28^{a}	4.64 ± 0.19^{b}	5.81 ± 0.37^{c}
MDA (nmol/g tissue)	13.54 ± 1.17^{a}	14.07 ± 1.35^{a}	25.45 ± 1.25^{d}	21.04 ±0 .96^{c}	17.39 ± 0.67^{b}

Different superscripts (a–d) in the same row indicate significant difference ($p < 0.05$) among groups.

3.2. Evaluation of apoptotic markers in the liver

To evaluate the effects of ZN and AS treatments on the mRNA levels of genes located in apoptotic pathway, Bax, Bcl-2, caspase-3, Apaf1, P53, caspase-6 and caspase-9 markers were analyzed by RT-PCR methods. AS exposure upregulated the expression of Bax, caspase-3, Apaf1, P53, caspase-6, and caspase-9 and downregulated the expression of Bcl-2 in liver tissue. It was determined that the expressions of Bax, caspase-3, Apaf1, P53, caspase-6, and caspase-9 were suppressed and the expression of Bcl-2 was triggered in the liver tissues of rats given ZN. Also, it was determined that a high dose of ZN in the liver was more effective on all apoptotic genes (Fig. 1A–C and Fig. 2A–D).

3.3. Evaluation of inflammatory markers in the liver

The relative mRNA transcript levels of inflammatory markers are presented in Fig. 3A–E and Fig. 4A–D. The results demonstrated that AS could trigger inflammation by increasing TNF-α, NF-κB, IL-1β, IL-6, RAGE, NLRP3, JNK, MAPK14 and MAPK15 genes in liver tissue. On the other hand, transcription levels of these inflammatory parameters decreased in ZN groups administered together with SA. When the doses of ZN were compared, it was found that the higher dose was more effective on inflammation in liver tissue.

3.4. Evaluation of endoplasmic reticulum stress markers in the liver

The effects of ZN and AS treatment on mRNA transcript levels of PERK, IRE1, ATF-6, CHOP and GRP-78 in rat liver tissue are presented in Fig. 5A–E. The results showed that SA caused endoplasmic reticulum stress in the liver, triggering the expression of ATF-6, PERK, IRE1, GRP-78, and CHOP genes, while ZN treatment attenuated endoplasmic reticulum stress and reduced the expression of related genes.

3.5. Histopathological results

To understand the potential effects of ZN and SA on liver tissue, it was stained with H&E and the histological evaluation results are given in Fig. 6. When the control and ZN applied groups were examined, it was observed that the photomicrographs had a normal histological structure (Fig. 6A and B). In contrast, with the application of SA, there were histological changes such as congestion of vessels, dilatation of sinusoids, infiltration of inflammatory cells, and necrosis (Fig. 6C). Treatment with ZN reduced the severity of pathological damage caused by SA. It was observed that most of the liver sections examined in the SA + ZN groups were preserved. There was a significant improvement in sinusoidal dilations, necrosis and inflammation with low-dose and high-dose ZN treatment (Fig. 6D and E). Histopathological findings are summarized in Table 3.

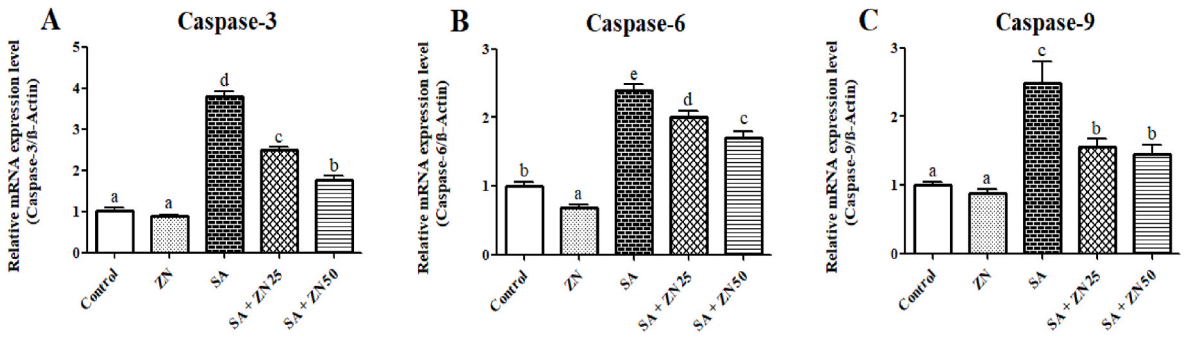

Fig. 1. Effects of ZN and AS treatments on Caspase-3, Caspase-6 and Caspase-9 mRNA transcription levels in liver tissue. A) Caspase-3 mRNA transcript levels, B) Caspase-6 mRNA transcript level, C) Caspase-9 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–e) on the columns show a statistical difference ($p < 0.05$).

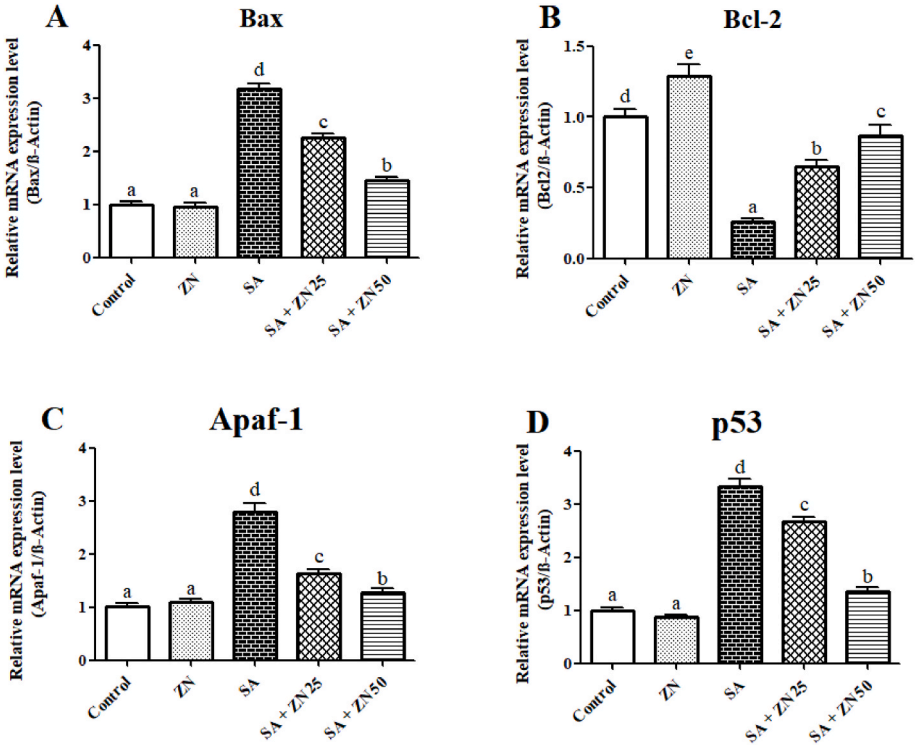

Fig. 2. Effects of ZN and AS treatments on Bax, Bcl-2, Apaf-1 and p53 mRNA transcription levels in liver tissue. A) Bax mRNA transcript levels, B) Bcl-2 mRNA transcript level, C) Apaf-1 mRNA transcript levels, D) p53 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–e) on the columns show a statistical difference ($p < 0.05$).

4. Discussion

It is well known that arsenic is toxic to the liver and kidneys among other human organs. Over time, arsenic buildup in the liver can cause oxidative stress, inflammation, and liver cell destruction [24]. This may make it more difficult for the liver to carry out vital processes including detoxification, metabolism, and nutrient storage. Long-term arsenic exposure may be a factor in liver conditions such as fibrosis, fatty liver, and even liver cancer. This study aimed to assess the ameliorative effect of ZN on SA-induced hepatotoxicity in rats.

It has been proposed that arsenic causes oxidative stress in liver. According to Jomova, Jenisova, Feszterova, Baros, Liska, Hudecova, Rhodes and Valko [11], arsenic-induced oxidative stress is caused by increased formation of ROS, a decrease in the antioxidant capacity of cells, changes in metals to oxidation forms, and the development of inflammatory responses. All of these factors contribute to the intracellular build-up of free radicals. Inorganic arsenic undergoes methylation in the liver to produce methylarsonic acid and dimethylarsinic acid. After being transformed into dimethylarsine, dimethylarsinic acid reacts with oxygen molecules to produce superoxide and dimethylarsinic radicals. Dimethylarsinic peroxyl radicals are produced when a dimethylarsinic radical reacts with another oxygen molecule. Additionally, it destroys cellular DNA and proteins and disables the antioxidant defence mechanism by targeting the mitochondrial respiratory chain [25]. The primary defence mechanism a cell has against arsenic toxicity is the antioxidant response. Several independent studies have revealed an antioxidant imbalance resulting from arsenic exposure [8,26,27]. These studies have demonstrated that a variety of enzymatic and non-enzymatic components aid in the elimination and clearance of ROS, protecting the cells. As a most prevalent non-protein thiol in cells, GSH reacts with electrofilms directly or acts as a cofactor (for the enzymes GPx and GST) to play a vital role in arsenic detoxification and arsenic-induced oxidative stress [28]. Mice and rats that are exposed to either a very high dose of acute or chronic arsenic exposure experience

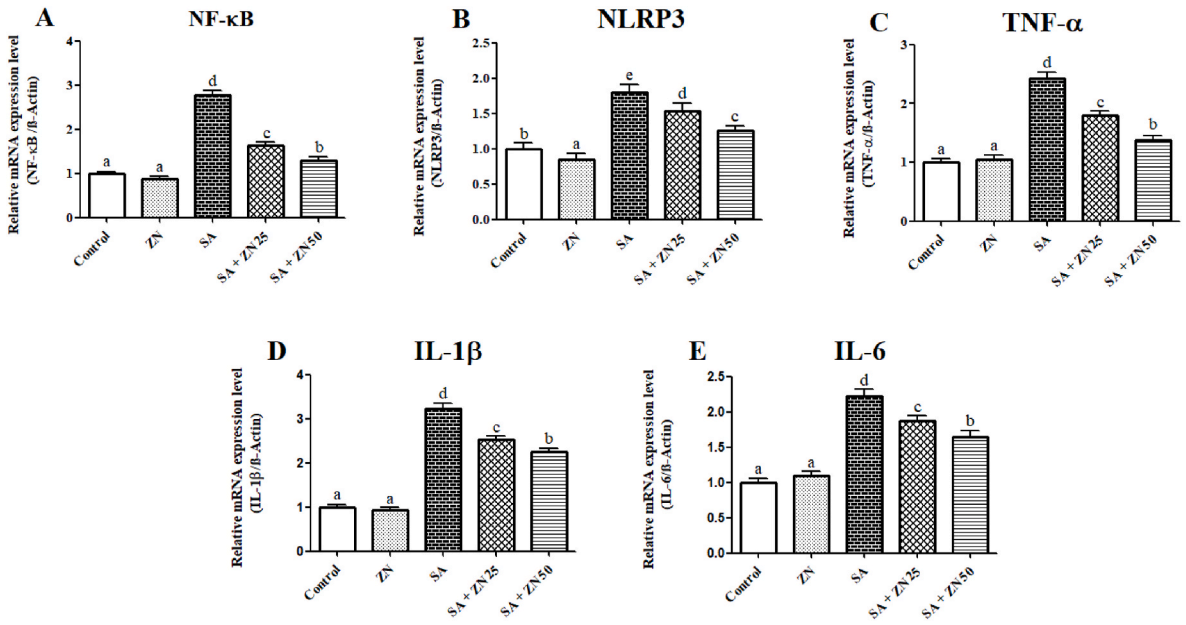

Fig. 3. Effects of ZN and AS treatments on NF-κB, NLRP3, TNF-α, IL-1β and IL-6 mRNA transcription levels in liver tissue. A) NF-κB mRNA transcript levels, B) NLRP3 mRNA transcript level, C) TNF-α mRNA transcript levels, D) IL-1β mRNA transcript levels, E) IL-6 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–e) on the columns show a statistical difference ($p < 0.05$).

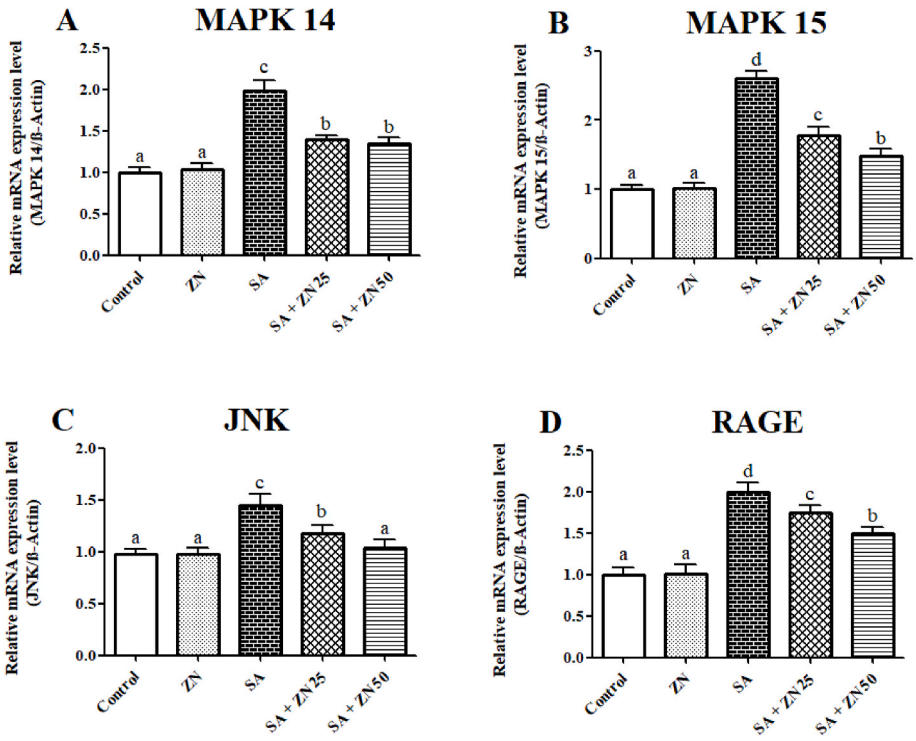

Fig. 4. Effects of ZN and AS treatments on MAPK 14, MAPK 15, JNK and RAGE mRNA transcription levels in liver tissue. A) MAPK 14 mRNA transcript levels, B) MAPK 15 mRNA transcript level, C) JNK mRNA transcript levels, D) RAGE mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$).

GSH depletion [29,30]. The current findings showed that the arsenic-treated group's hepatic GSH content had significantly reduced. According to Bodaghi-Namileh, Sepand, Omidi, Aghsami, Seyednejad, Kasirzadeh and Sabzevari [31], this decrease could be related to the conversion of As^{5+} into As^{3+} by GSH, the oxidation of GSH by free radicals brought on by arsenic metabolism, or the strong attraction of arsenic for sulfhydryl groups in GSH structure. Additionally, our research showed that the SA-exposed group had a significant suppression of the hepatic antioxidant enzymes SOD, CAT, and GPx, which may indicate that these enzymes were consumed to counteract the over-generation of superoxide anion during arsenic metabolism. Compared to the solo arsenic treatment, the co-treatment with ZN improved the antioxidant response in the liver of rats exposed to arsenic. Significant increases in the GSH content and antioxidant enzyme

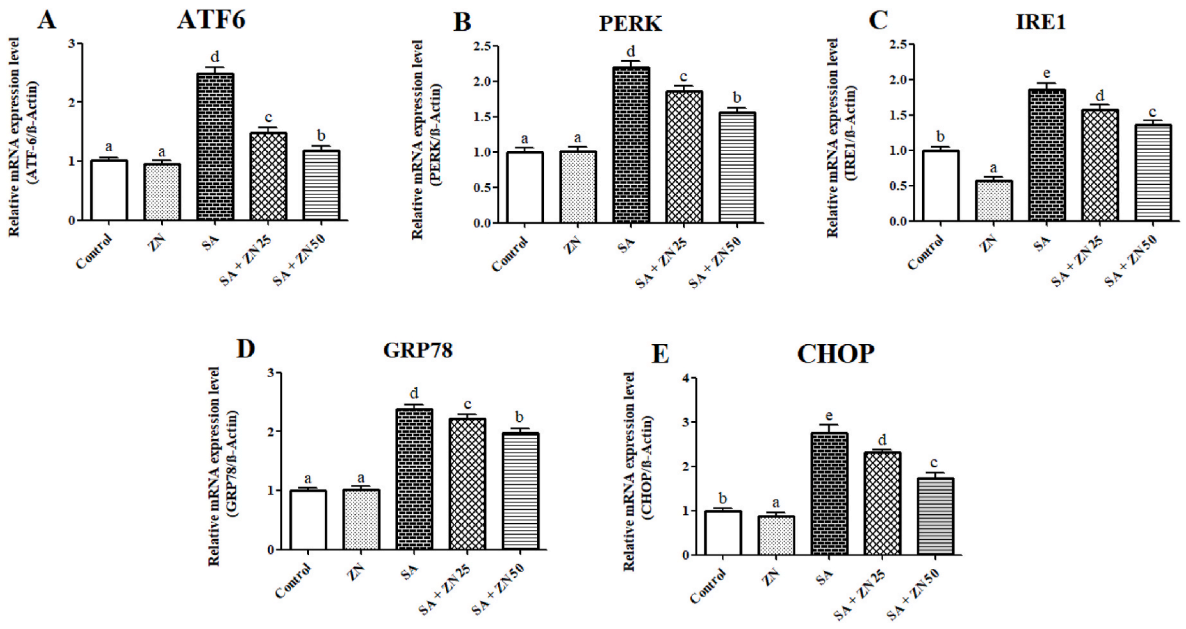

Fig. 5. Effects of ZN and AS treatments on ATF-6, PERK, IRE1, GRP78 and CHOP mRNA transcription levels in liver tissue. A) ATF-6 mRNA transcript levels, B) PERK mRNA transcript level, C) IRE1 mRNA transcript levels, D) GRP78 mRNA transcript levels, E) CHOP mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–e) on the columns show a statistical difference ($p < 0.05$).

activities were observed in the livers of the AS + ZN groups in comparison to the arsenic-exposed group. These elevations were accompanied by notable decreases in the levels of MDA revealing that ZN is a strong radical scavenger with the capacity to reduce lipid peroxidation and ameliorate cellular oxidative stress. Reduced activity of enzymes including CAT, SOD, and GST in kidney and liver tissues has been described as a consequence of acute arsenic exposure. In the kidney and liver tissues of arsenic-induced rats, the study found that non-enzymatic antioxidant levels like GSH and enzymatic antioxidant levels like CAT, SOD, and GPx dramatically dropped. However, these values increased when 100 and 200 mg/kg hesperidin were administered as part of the treatment. The most significant marker of oxidative damage to cells is augmented MDA levels. In the current study MDA was shown to have considerably elevated in the kidney and liver tissues of rats that had been exposed to SA. In a previous study, MDA levels were dose-dependently lowered by hesperidin administered with arsenic [8].

A type of programmed cell death called apoptosis is controlled by a number of variables. Given that the ultrastructural observation results showed abnormalities in the nucleus and cell damage, the changes in the main markers of apoptosis that are mediated through the mitochondrial pathway were examined in greater detail. Bcl-2 and caspase family proteins are essential for the mitochondrial apoptotic pathway [32]. The pro-apoptotic protein Bax is transported to the mitochondrial membrane in response to the generation of the apoptotic signal, and the expression of the anti-apoptotic component Bcl-2 is down-regulated. Increased mitochondrial permeability ensued, releasing cytochrome-c into the cytoplasm and triggering the caspase cascade to start the apoptotic process. Along with previous studies [33–35], this study also demonstrated the apoptotic damage of SA-induced hepatocytes, as indicated by significant increases in Bax, Caspase-3, Apaf-1 P53, Caspase-6 and Caspase-9 and decrease in Bcl-2 in the liver of the SA-treated group compared with that in the control. According to Ding, Ma, Liu, Ni, Lu, Chen, Liu and Zhang [36], arsenic can cause cellular apoptosis by moving cytochrome-c from mitochondria to the cytoplasm, which starts the caspase pathway and causes apoptosomes to develop. The livers of rats treated with SA + ZN exhibited significant reductions in apoptotic markers when compared to the SA-treated group. In another study, co-treatment with ZN significantly down-reguated mRNA transcript levels of caspase-3 and Bax levels and down-regulating Bcl-2, in arsenic induced kidney tissue [37]. These indicate that the potent ROS scavenging ability of the rats' liver and kidney prevented SA-induced hepatic and renal damage.

The generation of proinflammatory cytokines TNF-α, IL-1β, and IL-6 through NF-κB and/or inflammasome NLRP3 is a defining feature of the inflammatory response [38]. These pro-inflammatory variables exacerbate the tissue damage caused by arsenic, mostly by its binding to thiol groups in cellular proteins and formation of ROS [39]. One important mechanism that initiates the transcription of NLRP3 is the NF-κB signalling pathway [40]. It is frequently phosphorylated at Ser536, translocates to the nucleus, and upregulates the production of NLRP3 and IL-1β mRNA. Furthermore, Fann, Lim, Cheng, Lok, Chunduri, Baik, Drummond, Dheen, Sobey, Jo, Chen and Arumugam [41] state that NF-κB is a crucial downstream target of MAPK signalling, which controls several inflammatory cytokines, including TNF-α and IL-1β. The inflammatory cascade is fueled by TNF-α and IL-1β, which both suppress the expression of TNF-α and IL-1β and activate innate immunity and subsequent inflammatory responses. It was reported that arsenic (between 0.1 and 500 μM) activates MAPKs in a time- and dose-dependent way, regulating gene expression, proliferation, mitosis, survival, and death in the cell [25]. Arsenic markedly increased the phosphorylated-JNK (*p*-JNK)/JNK protein levels and the mRNA levels of p38, extracellular signal-regulated kinase (ERK)1, ERK2, and JNK in cardiomyocytes [42]. Arsenic was shown to increase p38 phosphorylation, which caused damage to HT-29 intestinal epithelial cells [43]. Additionally, in order to produce opposing harmful effects that varied depending on time, dose, arsenic species, and cell type, arsenic variably activated MAPK pathways. For instance, in human hepatocytes, chronic arsenic exposure resulted in persistent p38 activation, while acute arsenic exposure activated ERK1/2 and JNK [44]. In our study, it has been highlighted that pathway including ERK, JNK, MAPK, NLRP3, and NF-κB that control the production of several pro-inflammatory and cytotoxic genes may be activated, leading to an increase in inflammatory cytokines brought on by arsenic.

Despite having a robust endoplasmic reticulum homeostasis mechanism, the endoplasmic reticulum can nevertheless become physiologically dysfunctional under some stimulating circumstances, such as oxidative stress [45]. Several independent studies demonstrate that arsenic can trigger the endoplasmic reticulum's unfolded protein

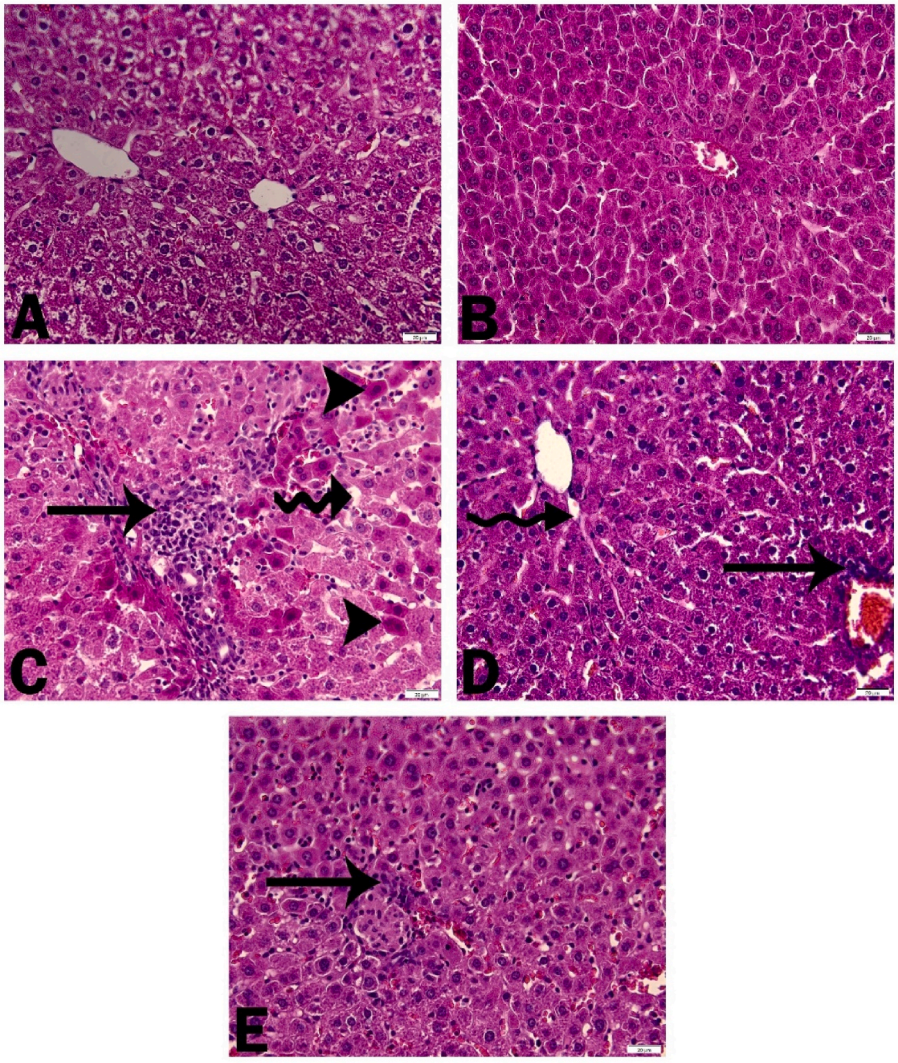

Fig. 6. Photomicrographs showing the effect of ZN applied together with SA on histopathological damage in the liver. A) Normal control group, B) ZN treatment group, C) SA applied group, D) 25 mg/kg ZN applied group after SA application, E) 50 mg/kg ZN applied group after SA application. Optical microscopy: sections were stained with the H&E method, bar: 400 μm, arrow: inflammatory cell infiltration, arrowhead: necrosis, curved arrow: sinusoidal dilatation, arrowhead: acidophilic body, star: congestion in blood vessels.

Table 3
Liver tissue histopathological changes.

	Control	ZN	SA	SA + ZN 25	SA + ZN 50
Sinusoidal dilatation	–	–	++	+	+
Congested blood vessels	+	+	+++	++	+
Necrosis	–	–	+++	+	+
Infiltration of inflammatory cells	–	–	+++	+	+

response by oxidatively stressing the reticulum, which in turn causes endoplasmic reticulum stress [46–48]. Research verified that during endoplasmic reticulum stress, key players in the three signalling pathways—PERK, IRE1, and ATF-6—separated from GRP78. Furthermore, the endoplasmic reticulum lumen's elevated GRP78 can encourage correctly folding proteins that have been unfolded or misfolded [49]. According to earlier research, PERK may alter the conformation of Bax and Bak to cause apoptosis [50,51] and increase the production of the pro-apoptotic transcription factor CHOP [52], which would suppress the level of the anti-apoptotic protein Bcl-2. The results of this experiment indicate that the toxic effect of SA on the rat liver disrupted the endoplasmic reticulum stress homeostasis and heightened the body's unfolded protein response. This is because the expression levels of endoplasmic reticulum-related regulatory factors protein and mRNA increased in the SA group. On the other hand, ZN administration markedly increased the expression of associated factors, suggesting that ZN protects against endoplasmic reticulum stress generated by SA.

In this study, the histopathological examination of the liver tissues confirmed the hepatotoxicity after SA administration and the ameliorative effect of ZN against SA-induced liver damage in rats. The histopathological investigation demonstrated that SA causes liver damage as evidenced by sinusoidal dilatation, congested blood vessels, infiltration of inflammatory cells, and necrosis in the hepatocytes. Similar findings have been observed in experimental studies on the effect of AS toxicity on liver tissues [8,53]. The histopathological results shown within this study demonstrated that ZN treatment reduced the severity of AS-induced liver damage.

5. Conclusion

According to the findings of this study, it could be concluded that ZN effectively reduces the hepatotoxicity caused by SA. The antioxidant,

anti-inflammatory, and anti-apoptotic properties of ZN could play an important role in ameliorating the biochemical, molecular, and histopathological changes caused by SA.

Availability of data and material

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

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CRediT authorship contribution statement

Berna Eriten: Methodology, Formal analysis, Conceptualization. **Cuneyt Caglayan:** Writing – review & editing, Supervision, Methodology. **Cihan Gür:** Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sefa Küçükler:** Visualization, Methodology, Investigation, Conceptualization. **Halit Diril:** Writing – original draft, Software, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare that there are no conflicts of interest.

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