Life Sciences 232 (2019) 116607

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Targeting of oxidative stress and inflammation through ROS/NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation

Hamed Haghi Aminjan[a], Seyed Reza Abtahi[a], Ebrahim Hazrati[b], Mohsen Chamanara[a], Maryam Jalili[c], Babak Paknejad[a,*]

[a] Department of Pharmacology and Toxicology, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[b] Department of Anesthesia and Intensive Care, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[c] Department of Clinical Sciences, School of Veterinary, Shiraz University, Shiraz, Iran

ARTICLE INFO

Keywords:
Aluminium phosphide
Histopathology
Inflammation
Oxidative stress
Phosphine

ABSTRACT

Aims: Poisoning with aluminium phosphide (AlP) commonly has a high rate of mortality and morbidities. Phosphine gas is the main cause of AlP poisoning that has deleterious effect on multi-organs especially heart, kidney, and liver. Furthermore, several studies reported that resveratrol has cytoprotective effects through its pleiotropic property. The purpose of this study was to estimate the dose-dependent role of resveratrol on phosphine induced acute hepatic toxicity in rat model.

Main methods: The rats have been exposed to LD_{50} of AlP (12 mg/kg) by gavage, and resveratrol doses (20, 40, and 80 mg/kg) were injected 30 min after intoxication. After 24 h, the serum and liver tissue were collected for present study.

Key findings: The results indicated that phosphine causes an alteration in oxidative stress markers including elevation of ROS, and GSH level, MPO activity, reduction in SOD, catalase and G6PD activity as well as reduction in SOD1 and catalase expression. Furthermore, phosphine significantly induced phosphorylation of IkappaB, NF-kappaB and up-regulation of TNF-α, IL-1β, IL-6, and ICAM-1 expression. Also, phosphine induces markedly reduced hepatocytes lives cell and elevated apoptosis and necrosis. Co-treatment of resveratrol in a dose-dependent manner reversed aforementioned alterations. All in all, histological analysis indicated a deleterious effect of phosphine on the liver, which is mitigated by resveratrol administration.

Significance: The results of the present study suggest targeting ROS/NF-kappaB signalling pathway by resveratrol may have a significant effect on the improvement of hepatic injury induced by phosphine. It also may be a possible candidate for the treatment of phosphine-poisoning.

1. Introduction

Aluminium phosphide (AlP) is an old potent pesticide in forms of tablet, pallet, etc. which is commonly used to protect cereals. AlP is frequently used in some countries because of its low cost, easy usage, high efficacy, and no effect on cereals during transportation or storage [1]. AlP has high toxic effects for non-target species especially human. Self-poisoning of AlP is a common reason of death especially in developing countries due to its availability in the markets [2]. The common way of AlP poisoning in human is due to its ingestion, inhalation and the rare one is absorption through the broken skin. Poisoning by AlP is fatal. As reported amount 30%–70% of individuals poisoned with this pesticide die of multi-organ dysfunction [3].

Most of the patients intoxicated with AlP die in spite of intensive care. Also, there are several ways to manage AlP intoxication, including gastric lavage with oral sodium bicarbonate, potassium permanganate solution, and activated charcoal. Despite many efforts to manage AlP intoxication, it is still one of the greatest challenges in societies. The whole and exact mechanisms of AlP intoxication are not completely understood, but it is demonstrated that phosphine gas is its main poisoning agent. Lethal phosphine gas releases from AlP in reaction with hydrochloric acid or available water in the stomach and is rapidly absorbed through the gastrointestinal tract and can diffuse freely into the cells and induce systemic toxicity [3,4]. Many kinds of literature have been provided with evidence of multi-organ disorders due to exposure to pesticides [5,6]. It is reported that the malfunction of heart, kidney, and liver is the main reason of death by AlP exposure and liver failure is the common reason of death between 24 and 72 h after AlP-intoxication

* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Medicine, AJA University of Medical Sciences, Tehran 14185, Iran.
E-mail address: Drpakb@yahoo.com (B. Paknejad).

https://doi.org/10.1016/j.lfs.2019.116607
Received 13 April 2019; Received in revised form 13 June 2019; Accepted 25 June 2019
Available online 26 June 2019

[7]. It is previously reported that phosphine through interaction with mitochondria respiratory chain as the main source of free radicals, induces reactive oxygen species (ROS) and extensive oxidative stress as well as cell membranes denaturation [8,9]. Moreover, mitochondrial dysfunction induces release of mitochondrial intermembrane space agents such as cytochrome *c* into the cell cytoplasm. These agents are involved in initiation of apoptosis pathway [10]. In addition, free radicals are strongly related to initiating inflammation and apoptosis [11,12].

Furthermore, several numbers of researchers have reported that resveratrol liver-protective role is due to its pleiotropic property. Resveratrol potentially decreases free radicals, apoptosis and inflammation pathways [13, 14]. In addition, resveratrol has a regulatory role in decline apoptosis and inflammatory pathways [15,16]. Moreover, the protective property of resveratrol on liver dysfunction has been previously reported in several pathological situations [17,18]. Although some researches have been carried out for a better understanding of AlP induced liver injury mechanisms, details have not been yet cleared. Also, the results of previous studies on the anti-oxidant, anti-inflammatory, anti-apoptotic, and other cytoprotective effects of resveratrol bring this idea that it may have a potential to neutralize the mechanisms of liver dysfunction induced by AlP. As such, the aim of this study is to demonstrate the positive effects of resveratrol on AlP-induced liver toxicity in rat models. To our best knowledge, this is the first study on the investigation of the resveratrol effect on liver toxicity induced by AlP.

2. Materials and methods

2.1. Ethics

This animal study was approved by the protocols of Animal Care under the ethical committee at AJA University of Medical Science with code number 97058.

2.2. Chemicals

ELISA kits for evaluation of catalase activity were obtained from Abcam (USA). AlP was obtained from Samiran Pesticide Formulating Co. (Iran). Resveratrol, bovine serum albumin (BSA), and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich (GmbH, Munich, Germany). AST and ALT kits were purchased from Pars Azmoon Inc., Iran. ApoFlowEx® fluorescein isothiocyanate (FITC) kit was obtained from Exbio (Vestec, Czech Republic). Antibodies for pIkappaB, pNF-kappaB and β-actin obtained from Santa Cruz Biotechnology (CA, USA). Other materials were obtained from Sigma or Merck.

2.3. Animals

All tests were performed on adult male Albino Wistar rats, weighing 220 ± 20 g housed in standard temperature (20–24 °C) and 50–55% humidity. Animals were kept in 12 h dark/light cycle under conditions in which standard rat diet and ad libitum watering were available. All experimental protocols were done in accordance with guidelines for animal care and it has been approved by the AJA ethics committee.

2.4. Determination of AlP LD_{50}

The 50% lethal dose (LD_{50}) of AlP as previously was reported a range from 8.7 to 12.5 mg/kg [3,7]. For detection exact dose of AlP LD_{50} in this study, we examined the relevant doses of AlP including 9, 10, 11, 12, and 13 mg/kg. First of all, the tablets of AlP were powdered and the doses were dissolved in 1/5 cm^3 of coconut oil and gavaged to rats. The control group just gavaged by coconut oil. In each group, there were 12 rats and followed up 24 h post exposure. All deaths until 24 h were recorded and LD_{50} of AlP was calculated as 12 mg/kg using the probit test.

2.5. Treatment strategy

The protocol of these animal studies was according to the following groups: the 72 rats were equally and randomized into divided into the 6 groups each groups including 12 rats as follows: Group 1 received solely coconut oil by gavage (Control); Group 2 received resveratrol in a dose of 80 mg/kg by intraperitoneal injection (REZ80); Group 3 received AlP by gavage (AlP); Group 4 received AlP + 20 mg/kg of resveratrol 30 min after exposure (AlP + REZ20); Group 5 received AlP + 40 mg/kg of resveratrol 30 min after exposure (AlP + REZ40), and Group 6 received AlP + 80 mg/kg of resveratrol 30 min after exposure (AlP + REZ80). All rats were followed for 24 h. Finally, six alive rats were chosen for analysis.

In treated groups, solvents of resveratrol and AlP were coconut oil. The AlP and resveratrol doses and strategy of rat treatments were chosen according to the literature search and our pilot study [19].

2.6. Serum and liver tissue sampling

Six live treated rats were sacrificed at the end of 24 h. In order to parameters analysis in present study, the blood samples were collected directly from the heart by syringe in heparinized tubes and immediately centrifuged at 3000*g* for 5 min to separate serum. The liver tissue surgically isolated. The liver tissues were washed out by rinsing with cold PBS and divided into several parts. All samples except samples for flow cytometry analysis were immediately frozen at −80 °C and histological samples were fixed in 10% buffered formaldehyde.

2.7. Assessment of hepatic serum enzymes

The levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed with spectrophotometry according to kits protocols. The results are demonstrated as unit per liter.

2.8. G6PD activity

Glucose 6 phosphate dehydrogenase (G6PD) activity was analyzed according to the method of Beutler spectrophotometrically with a microplate reader. The G6PD activity reported as nmol of NADP reduced/min mg protein [20].

2.9. Measurement of enzymatic anti-oxidants

The catalase activities were assessed based on the reaction of a probe with unconverted H_2O_2 using colorimetric assay at OD 570 nm according to the kit protocol. Then catalase activities of the liver sample determined using a standard curve and reported as units per mg of tissue protein. The activity of SOD measured according to as described by Sun et al. [21] based on produce superoxide radicals by xanthine and xanthine oxidase. The activity of SOD was reported as units per mg of tissue protein.

2.10. Measurement of non-enzymatic anti-oxidants

Reactive oxygen species (ROS) analyzed base of oxidation of DCFH-DA into DCF by a method reported previously Chen et al. [22]. The ROS level was calculated using a standard curve and reported as units per mg of tissue protein. The levels of glutathione (GSH) was determined according to the method by Tietze using Elman's reagents (19.8 mg of DTNB in 100 ml of 0.1% sodium nitrate) and absorbance measured at 450 nm [23]. The results reported as μmol per mg of tissue protein.

2.11. Assessment of myeloperoxidase (MPO) activity

Hepatic MPO activity was investigated with spectrophotometry according to the previous method. In brief, liver samples were homogenized in hexadecyltrimethylammonium bromide (HTAB) buffer and were then centrifuged (30,000*g*, 15 min, 4 °C). After that, the MPO activity was measured using spectrophotometer at 460 nm. The unit of MPO activity was alteration in converting 1 μmol of H_2O_2 to H_2O per 1 min at room temperature [7]. The MPO activity was demonstrated as units per mg of tissue protein.

2.12. Determination of protein

Liver protein analysis was determined as reported previously by a spectrophotometer by using Bradford protein assay at 595 nm [24].

2.13. Real-time PCR

To determine changes in gene expression of TNF-α, IL-1β, IL-6, ICAM-1, SOD1, and catalase real-time PCR was perfumed after mRNA extraction and cDNA synthesis. At first, RNA of liver samples was extracted by homogenizing tissues in TRIzol reagent. Then the concentration of RNA was determined using Nano-Drop UV–vis spectrophotometer (Thermo Fisher Scientific, CA) and 1 μg/μl RNA was used to reverse transcribe it to cDNA using iScript cDNA synthesis kit. The specific primers for mentioned genes were designed and purchased from Eurofins genomics (Eurofins genomics, Germany). The SYBR green master mix was used for the real-time PCR reaction. Cycle number (Ct) of each reaction was achieved from Light Cycler 96 (Roche Applied Sciences, USA). The values were normalized to GAPDH mRNA and the relative gene expression level was represented as $2^{-\Delta\Delta Ct}$. The following primers were used in this study:

TNF-α primer forward: AGGATTCCAATGGGCTTTCG
TNF-α primer reverse: ATCTTCAGCAGCCTTGTGAG
IL-6 primer forward: GATACCACCCACAACAGACC
IL-6 primer reverse: TCAGAATTGCCATTGCACAAC
ICAM-1 primer forward: CGGACACAGCTCTCAGTAGT
ICAM-1 primer reverse: GAGAAATTGGCTCCGTGGTC
SOD1 primer forward: TGGGGACAATACACAAGGCT
SOD1 primer reverse: GGTCTCCAACATGCCTCTCT
Catalase primer forward: ACTTTGAGGTCACCCACGAT
Catalase primer reverse: TCCCACAAGGTCCCAGTTAC
GAPDH primer forward: AGTCTACTGGCGTCTTCACC
GAPDH primer reverse: CCACGATGCCAAAGTTGTCA

2.14. Western blot

Total protein concentrations were determined using Bradford reagent and proteins were separated by 10% SDS-PAGE. Overall, 100 μg protein were resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then it is transferred to polyvinylidene fluoride (PVDF) membranes via electrophoretic transfer system prior to Western blot analysis. The membranes were blocked with 5% blocking agent. After 2 h, the blotted papers were coated overnight at 4 °C, by blocking agent containing specific primary antibodies including pNF-kappaB (1:200), pIkappaB (1:200), and monoclonal β-actin (1:1000) antibodies. The membranes were thoroughly washed with TBS, 0.05% Tween-20 (TBS-T) and incubated with goat anti-mouse IgG-HRP conjugated secondary antibodies (1:1000) for 1 h at room temperature. The blots were exposed to chemiluminescent detection reagents for the detection of target antigens. The bands were quantified with Image J software and normalized to corresponding β-actin band intensity [25].

2.15. Investigating apoptosis and necrosis

Fresh liver was used for investigation of apoptosis and necrosis according to the method described by Shi et al. [26]. Then single hepatocytes were stained with annexin V-FITC for determining phosphatidylserine in apoptotic cells, and with propidium iodide (PI) for late apoptotic and necrotic cells according to the kit instruction. Analysis was carried out using flow cytometry (Mindray, Shenzhen, China).

2.16. Histological assay

Liver histology analysis was done according to previously described [27]. Briefly, the liver tissue was fixed in 10% neutral buffered formalin and Four-micrometer serial sections prepared from the paraffin blocks. The blinded sections stained with hematoxylin and eosin (H&E) and observed under a light microscope by an investigator. In each slid five randomly selected fields were chosen and analyzed according to the Suzike's criteria as follows: congestion, vacuolization, and necrosis were assessed using a scale of 0–5 as follows. 0, none; 1, minimal (< 10% affected); 2, mild (11–30% affected); 3, moderate (31–60% affected); 6, severe (> 60% affected).

2.17. Statistical analysis

All the tests were performed three times with 6 rats in each group. Data were presented as Mean ± SEM. Statistically, the analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) with one-way ANOVA test followed by Tukey's multi-comparison.

3. Results

3.1. Levels of AST and ALT in serum

AST and ALT are fundamental biomarkers of liver. It is demonstrated phosphine markedly induced elevation in the levels of these markers compared to control group. Co-treatment of resveratrol with increasing dose significantly dampened the elevation of these markers

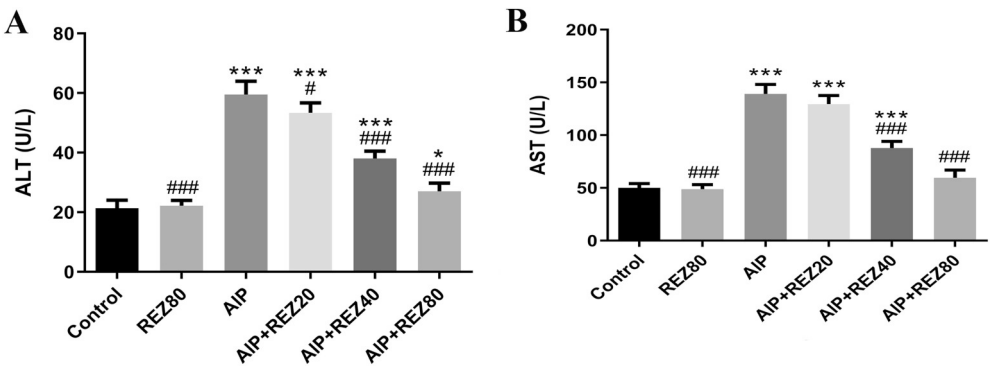

Fig. 1. Data are mean ± SEM of six animals in each group. Control group received coconut oil; REZ80 group received resveratrol (80 mg/kg); AlP group received AlP (12 mg/kg); AlP + REZ20 group received AlP + resveratrol (20 mg/kg); AlP + REZ40 group received AlP + resveratrol (40 mg/kg), AlP + REZ80 group received AlP + resveratrol (80 mg/kg). Serum AST (A) and ALT levels (B). $^{*}P < 0.05$ compared to control group, $^{**}P < 0.01$ compared to control group, $^{***}P < 0.001$ compared to control group, $\#P < 0.05$ compared to AlP group, $\#\#P < 0.01$ compared to AlP group, $\#\#\#P < 0.01$ compared to AlP group.

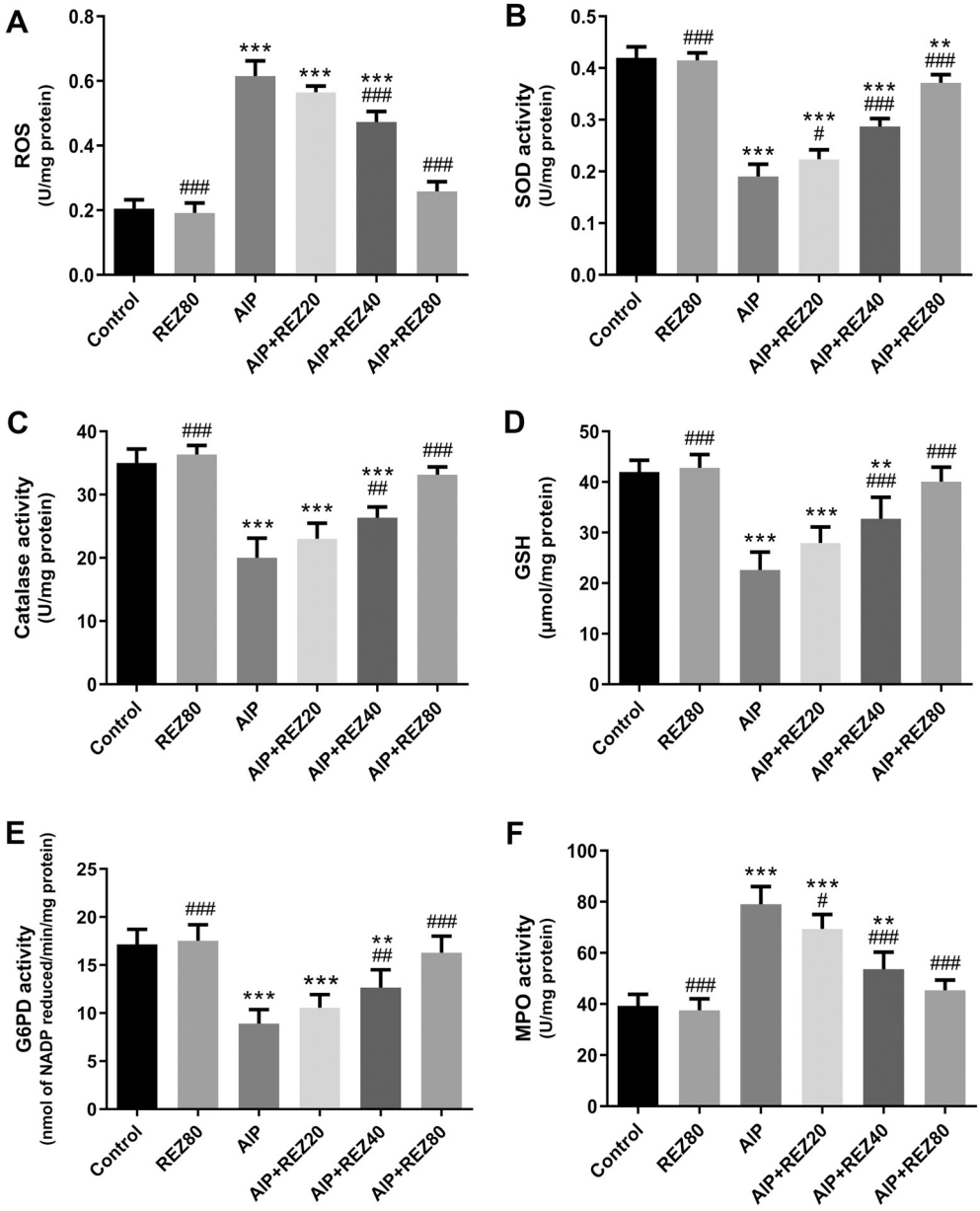

Fig. 2. Data are mean ± SEM of six animals in each group. Control group received coconut oil; REZ80 group received resveratrol (80 mg/kg); AlP group received AlP (12 mg/kg); AlP + REZ20 group received AlP + resveratrol (20 mg/kg); AlP + REZ40 group received AlP + resveratrol (40 mg/kg), AlP + REZ80 group received AlP + resveratrol (80 mg/kg). ROS levels (A), SOD activity (B), catalase activity (C), G6PD activity (E), and MPO levels (F). * $P < 0.05$ compared to control group, ** $P < 0.01$ compared to control group, *** $P < 0.001$ compared to control group, # $P < 0.05$ compared to AlP group, ## $P < 0.01$ compared to AlP group, ### $P < 0.01$ compared to AlP group.

in serum. Moreover, the levels of these markers in only resveratrol-treated animals demonstrated relatively similar to control group (Fig. 1A & B).

3.2. Oxidative stress parameters in liver tissue

ROS was assessed as an important indicator of oxidative stress increased in liver injury induced by phosphine. As illustrated in Fig. 2(A) ROS were increased by phosphine compared to the control group. On the other hand, co-treatment of resveratrol reduced ROS levels whereas dose of 80 mg/kg did not cause a significant difference in comparison to the control group. SOD enzyme is the first defense counteracting oxygen free radicals. It is demonstrated in Fig. 2(B) activity of SOD induced by phosphine was inhibited by resveratrol, but this was not the same as control group. Catalase enzyme activity, as indicated in Fig. 2(C), was significantly reduced by phosphine in comparison to control group. It was also inhibited by resveratrol dose-dependently. As demonstrated in Fig. 2(D), phosphine caused a significant reduction in GSH, as the main anti-oxidant molecules, in rat's liver compared to their control counterparts. Co-treatment with resveratrol restored GSH content compared to AlP group. **G6PD** enzyme as it is shown in Fig. 2(E) was significantly reduced by phosphine in comparison to the control group. This reduction was reduced by resveratrol co-treatment and was as same as the control group in dose of 80 mg/kg. Also, Fig. 2(F) demonstrates that hepatic MPO activity was noticeably increased by phosphine compared to the control groups. Resveratrol co-administration reduced the activity of MPO dose-dependently. This reduction was not significant in co-treatment with the dose of 80 mg/kg compared to control rats.

3.3. mRNA expressions of inflammation and oxidative stress markers

For evaluation of inflammation markers including TNF-α, IL-1β, IL-6, and ICAM-1 as well as oxidative stress markers such as SOD1 and catalase mRNA levels, real-time PCR was assessed. The aforementioned inflammation markers are the important inflammatory cytokines which are increased by NF-kappaB activation. AlP induced an approximately 7-fold increase in TNF-α mRNA expression 24 h after exposure. Moreover, treatment with different doses of resveratrol significantly alleviated this elevation towards control levels (Fig. 3A). In similar, in

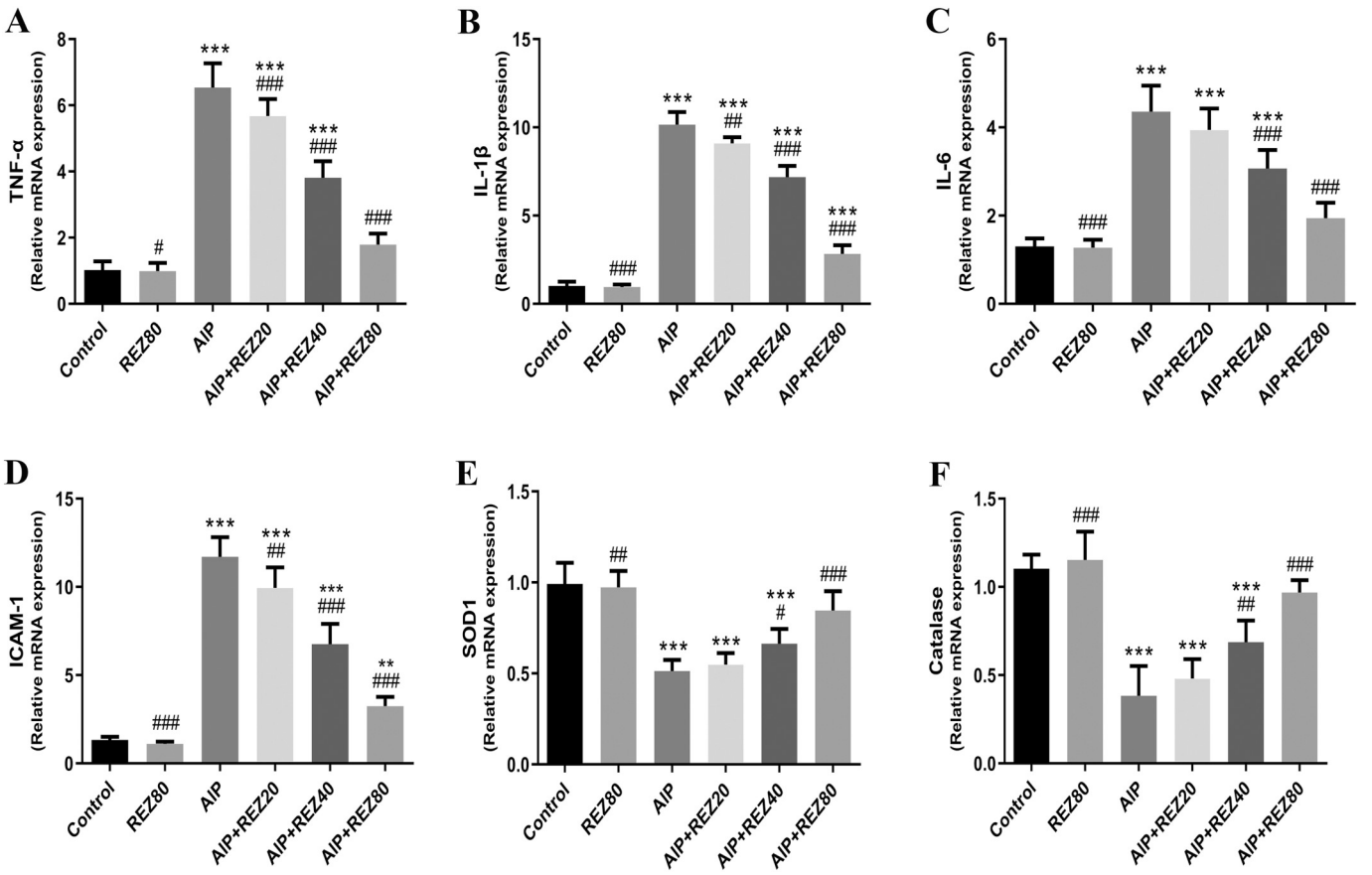

Fig. 3. Data are mean ± SEM of six animals in each group. The mRNA expression of TNF-α (A), IL-1β (B), IL-6 (C), ICAM-1 (D), SOD1 (E) and catalase (F) in treated rats. The control group received coconut oil; REZ80 group received resveratrol (80 mg/kg); AlP group received AlP (12 mg/kg); AlP + REZ20 group received AlP + resveratrol (20 mg/kg); AlP + REZ40 group received AlP + resveratrol (40 mg/kg). AlP + REZ80 group received AlP + resveratrol (80 mg/kg). $^{*}P < 0.05$ compared to control group, $^{**}P < 0.01$ compared to control group, $^{***}P < 0.001$ compared to Control group, $\#P < 0.05$ compared to AlP group, $\#\#P < 0.01$ compared to AlP group, $\#\#\#P < 0.01$ compared to AlP group.

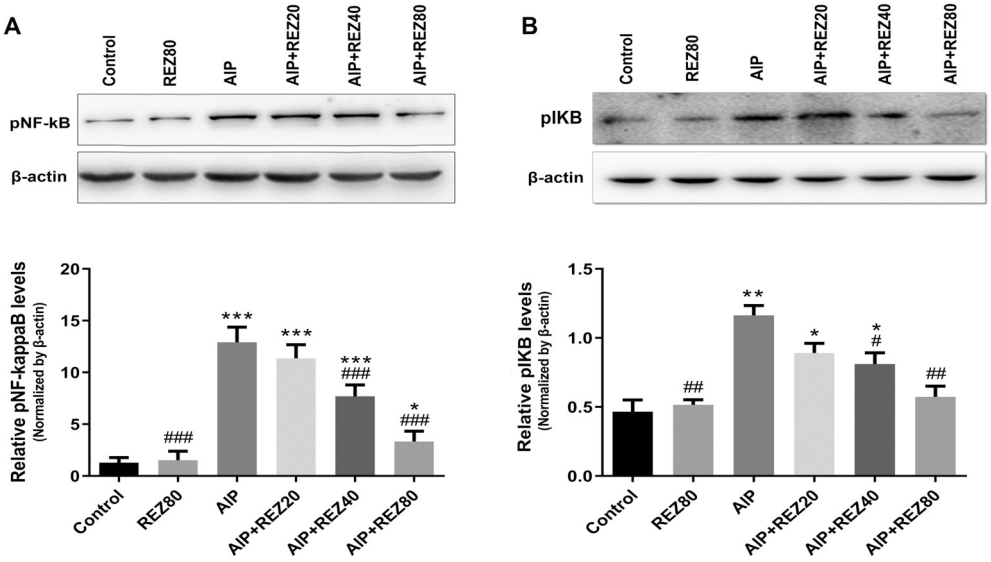

Fig. 4. Data are mean ± SEM of six animals in each group. Effect of resveratrol on pNF-kappaB (A) and pIkappaB (B) in rats. The control group received coconut oil; REZ80 group received resveratrol (80 mg/kg); AlP group received AlP (12 mg/kg); AlP + REZ20 group received AlP + resveratrol (20 mg/kg); AlP + REZ40 group received AlP + resveratrol (40 mg/kg). AlP + REZ80 group received AlP + resveratrol (80 mg/kg). $^{*}P < 0.05$ compared to control group, $^{**}P < 0.01$ compared to control group, $^{***}P < 0.001$ compared to control group, $\#P < 0.05$ compared to AlP group, $\#\#P < 0.01$ compared to AlP group, $\#\#\#P < 0.01$ compared to AlP group.

AlP group, mRNA levels of IL-1β, IL-6, and ICAM-1 were elevated approximately 11-fold, 5-fold, and 12-fold in comparison to control group, respectively (Fig. 3B, C, & D). Groups which received AlP plus administration of different doses of resveratrol had a dramatic reduction in these levels. It has to be mentioned that this reduction was not significant in dose 80 mg/kg for IL-6 and ICAM-1 compared to control group. Also, phosphine markedly induced mRNA expression reduction of SOD1 and catalase 24 h after exposure dose-dependently. Co-treatment of resveratrol markedly inhibited of these reductions so in dose of 80 mg/kg were not significant compared to control group (Fig. 4E & F).

3.4. The protein level of phosphorylated IkappaB, and NF-kappaB in the liver

Phosphorylated levels of IkappaB and NF-kappaB protein were meaningfully elevated in AlP group compared to the control group 24 h after the exposure. The level of pIkappaB, and pNF-kappaB in rats treated with different doses of resveratrol had a drastic reduction compared to the AlP group (Fig. 4A & B). The reduction of pIkappaB was not significantly different in comparison to control group.

3.5. Live, late apoptotic and necrotic cells in the liver

Flow cytometry data analysis indicated in control group live cells (annexin V−/PI−), late apoptotic cells (annexin V+/PI+), and necrotic cells (annexin V−/PI+) cells are 87.61%, 5.59%, and 4.39%, respectively. On the other hand, phosphine meaningfully reduced hepatocytes viability to 60.54% and elevated rate of late apoptosis and necrosis to 24.23% and 13.13%, respectively. Co-administration of resveratrol dose-dependently modified the beforementioned alteration. Surprisingly, among tree dose of resveratrol co-treatment of 80 mg/kg of resveratrol increased to live cells, late apoptotic cells, and necrotic cells to 80.81%, 8.88%, and 8.7%, respectively (Fig. 5A & B). In the groups early apoptosis were not significant (data not shown).

3.6. Histological analysis

Histopathological examination was done according to Suzuki scoring. Rat's liver exposed to AlP for 24 h, demonstrated significant changes compared to the control group. Interestingly, the qualitative analysis revealed that rats receiving AlP with co-treatment of different doses of resveratrol displayed mitigation of hepatic injury. There were no significant changes in rats receiving 80 mg/kg of resveratrol in comparison to control group (Fig. 6A & B).

4. Discussion

As mentioned in a literature review, hepatic injury is one of the possible important causes of death in phosphine intoxication [28]. Phosphine is a very high toxic gas which is released from metal phosphide including AlP, zinc phosphide, etc. [29]. In this study, we used AlP as a source of this toxic gas because AlP-poisoning is a common reason for death worldwide, especially in developing countries [30]. Phosphine toxicity has been widely investigated for its effect on cardiovascular acute injuries while few studies have investigated the toxic effect of phosphine on the liver. This is why there is little information about the mechanism of its hepatic toxicity. The present study focuses on some new mechanisms of phosphine-induced hepatotoxicity and the role of resveratrol in rats model. The mechanisms of hepatocyte phosphine-induced cytotoxicity in present study are summarized in Fig. 7.

The results of this study demonstrated that the AlP administration induced hepatotoxicity through induction of oxidative stress, inflammation and apoptosis.

Oxidative stress is defined as a reduction in anti-oxidant agents that neutralize free radicals, and un-controlled elevation in the cells free radicals [31,32]. Most of the free radicals are known to be the main mechanisms of hepatotoxicity [33].

The results of the present study indicated that phosphine induced an elevation in the level of ROS while reduced catalase, and SOD activity and GSH content as oxidative stress markers in hepatic tissue. Furthermore, phosphine induced down-regulation of SOD1 and catalase expression. Also, it is indicated that the aforementioned alterations were modified by resveratrol. Previously it has been reported that oxidative stress has an important role in phosphine-induced toxicity in several tissues [34]. Free radicals can react with cell molecules and consequently cause malfunctions in cells and organelles such as mitochondria, and therefore can cause initiation of some pathways such as inflammation and apoptosis [35].

Normally, different types of oxygen free radicals are produced in cells especially during oxygen consumption and metabolism in mitochondria [36–38]. ROS production in mitochondria occurs through the electron transport during oxidative phosphorylation. In the mitochondrial respiratory chain complex I (oxidoreductase NADH: ubiquinone) and III (oxidoreductase ubiquinol: cytochrome *c* reductase) are responsible for the production of ROS. Uncoupling of electron transport elevates ROS production [39,40]. It is previously demonstrated that one of the mechanisms for ROS elevation induced by phosphine is through a malfunction in some of the mitochondrial respiratory chain complexes [2].

SOD enzyme is the first and powerful anti-oxidant defense system which converts superoxide free radicals (O_2^-) into either ordinary molecular oxygen or hydrogen peroxide and decreases the extra O_2^- in cells [41]. As indicated in the present study, phosphine reduces SOD activity in liver tissue and it is blocked by resveratrol. This result could be due to the induction of free radicals' high production in hepatocyte.

Catalase enzyme, which is commonly found in almost all living organisms and tissues, is responsible for decomposition of hydrogen peroxide (H_2O_2) to water and oxygen [42]. As previously reported, this enzyme is very efficient and can degrade millions of H_2O_2 molecules in 1 s [43]. The current study found that in hepatic tissue, catalase activity was decreased 24 h after exposure to phosphine. This reduction was inhibited dose-dependently by resveratrol. These results are in an agreement with the previous report, claiming that phosphine reduces catalase activity [44]. The possible mechanism is through interaction between phosphine and metal ion co-factors at the active site of catalase enzyme [28].

In addition, many literatures previously demonstrated that resveratrol inhibited catalase activity reduction [45–47].

GSH is one of the main intracellular defenses during oxidative stress and has a pivotal role with other anti-oxidant molecule in neutralizing free radicals and preserving cell integrity [48]. GSH through its sulfur atom of thiol groups donates an electron to free radicals and then two molecules of GSH are oxidized to glutathione disulfide (GSSG). GSSG is reduced by glutathione reductase (GR) enzyme to GSH by oxidizing NADPH to $NADP^+$ [35]. In the present study, the GSH level was significantly reduced in liver tissues after exposure to phosphine. This reduction was dose-dependently inhibited by resveratrol. A previous study also reported that phosphine decreased liver GSH level [49]. Moreover, there are many studies demonstrating that resveratrol with its anti-oxidant property prevents reduction of GSH level [50,51]. The possible role of resveratrol is increasing GSH cellular content through its direct anti-oxidant property, up-regulation of GSH expression and synergistic effects with other anti-oxidants and stimulating of g-glutamylcysteine syntheses [52,53].

G6PD enzyme is an enzyme responsible for reduction of GSSG to GSH, which is done by GR enzyme using NADPH as co-factor which converts to $NADP^+$ [54]. NADPH is recycled by G6PD enzyme through pentose phosphate pathway [55]. In the present study, it is demonstrated that the activity of G6PD is decreased during phosphine intoxication in liver. Surprisingly, resveratrol co-administration increased the activity of G6PD.

Altogether, phosphine increases oxidative stress in liver tissues through multiple mechanisms and this elevation is reversed by resveratrol via its direct and in-direct anti-oxidant mechanisms.

Inflammation is a biological procedure in response to harmful stimulations and is demonstrated via various alteration including up-regulation of pro-inflammatory agents, down-regulation of anti-inflammatory agents, leukocyte chemotaxis and local or systemic regulation of leukocyte reactions [56–58]. The results of the present study demonstrated phosphine induced elevation of inflammation markers and resveratrol decreases these markers.

In this content, resveratrol possibly exerts its anti-inflammatory property through its modulatory effects on inflammation pathway

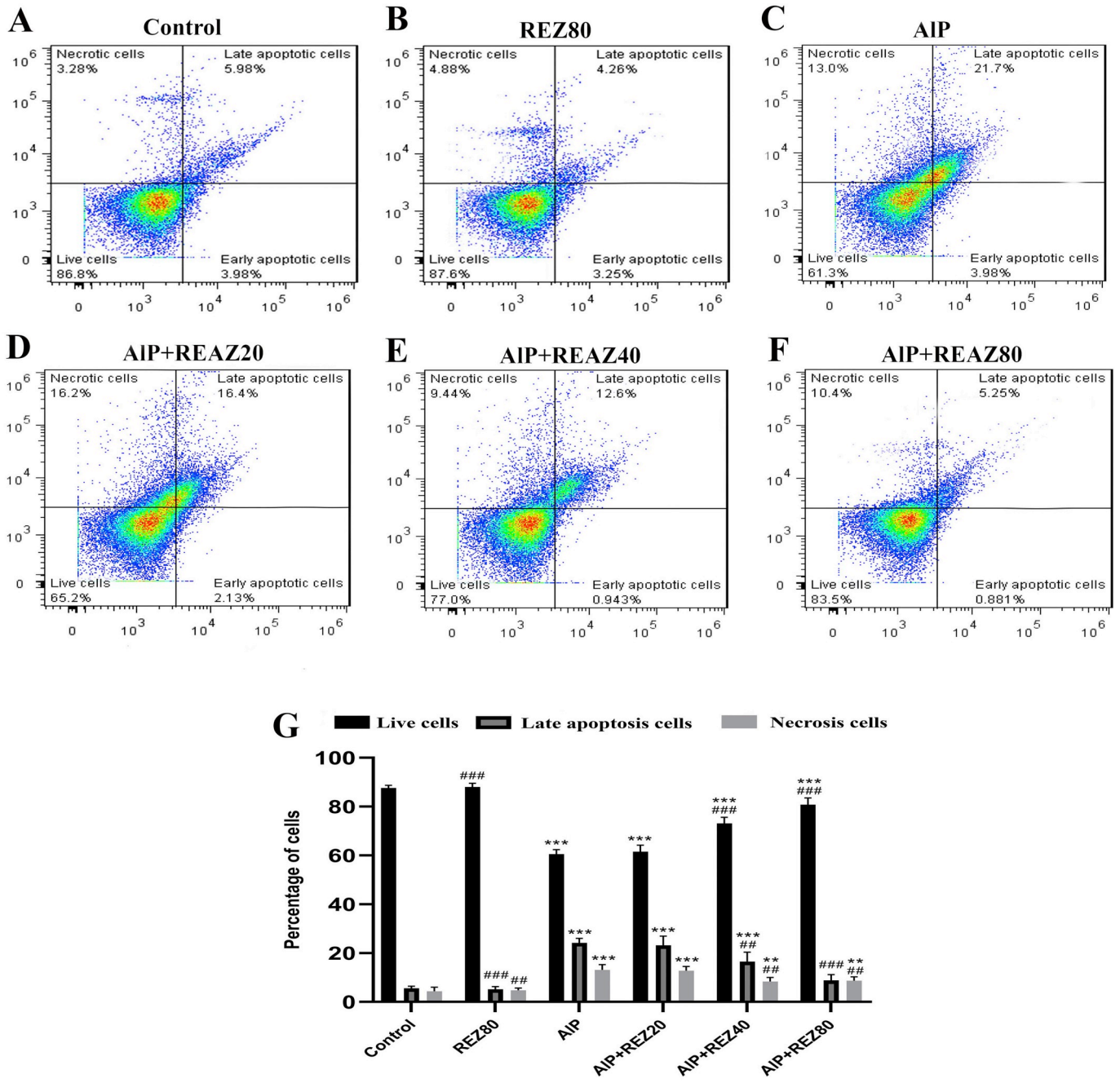

Fig. 5. Analysis of the cell viability with annexin-V/PI staining using by flow cytometry (A). The control group received coconut oil; REZ80 group received resveratrol (80 mg/kg); AlP group received AlP (12 mg/kg); AlP + REZ20 group received AlP + resveratrol (20 mg/kg); AlP + REZ40 group received AlP + resveratrol (40 mg/kg). AlP + REZ80 group received AlP + resveratrol (80 mg/kg). Data are mean ± SEM of six animals in each group (B). $^{*}P < 0.05$ compared to control group, $^{**}P < 0.01$ compared to control group, $^{***}P < 0.001$ compared to control group, $\#P < 0.05$ compared to AlP group, $\#\#P < 0.01$ compared to AlP group, $\#\#\#P < 0.01$ compared to AlP group.

agents and its anti-oxidant free radicals.

In normal condition, NF-kappaB's nucleus translocation is prevented through binding to inhibitory proteins of kB family (IkappaB) [59]. Also, it is well known that ROS induce phosphorylation of IkappaB B and NF-kappaB via IkappaB kinase (IKK) complex activation. IkappaB is dormant. Due to phosphorylation by IKK, and is then degenerated, so NF-kappaB is sequestered from IkappaB B and is liberates in the cell cytoplasm. The phosphorylated NF-kappaB is translocated to the nucleus and then binds to the promoter regions of genes that encode several inflammatory mediators. As demonstrated in the present study phosphorylated IkappaB and NF-kappaB is elevated in the liver of animals' exposed to phosphine. Resveratrol reduced IkappaB and NF-kappaB phosphorylation. The possible mechanism is through the anti-oxidative property of resveratrol [60]. Another possible mechanism might be in the inhibition of the IKK complex activation by resveratrol [61]. This result is in agreement with the previous study which reported that resveratrol reduces NF-kappaB activation [62]. In this experiment, we analyzed the expression of TNF-α, IL-1β, IL-6, and ICAM-1 by real-time PCR and noticed that the expression of these inflammatory markers increased several folds after exposures to phosphine compared to control groups. These elevations have been reduced dose decently by resveratrol.

ICAM-1 is a cell adhesion molecule and a surface protein which has critical implications in the recruitment and infiltration of leucocytes

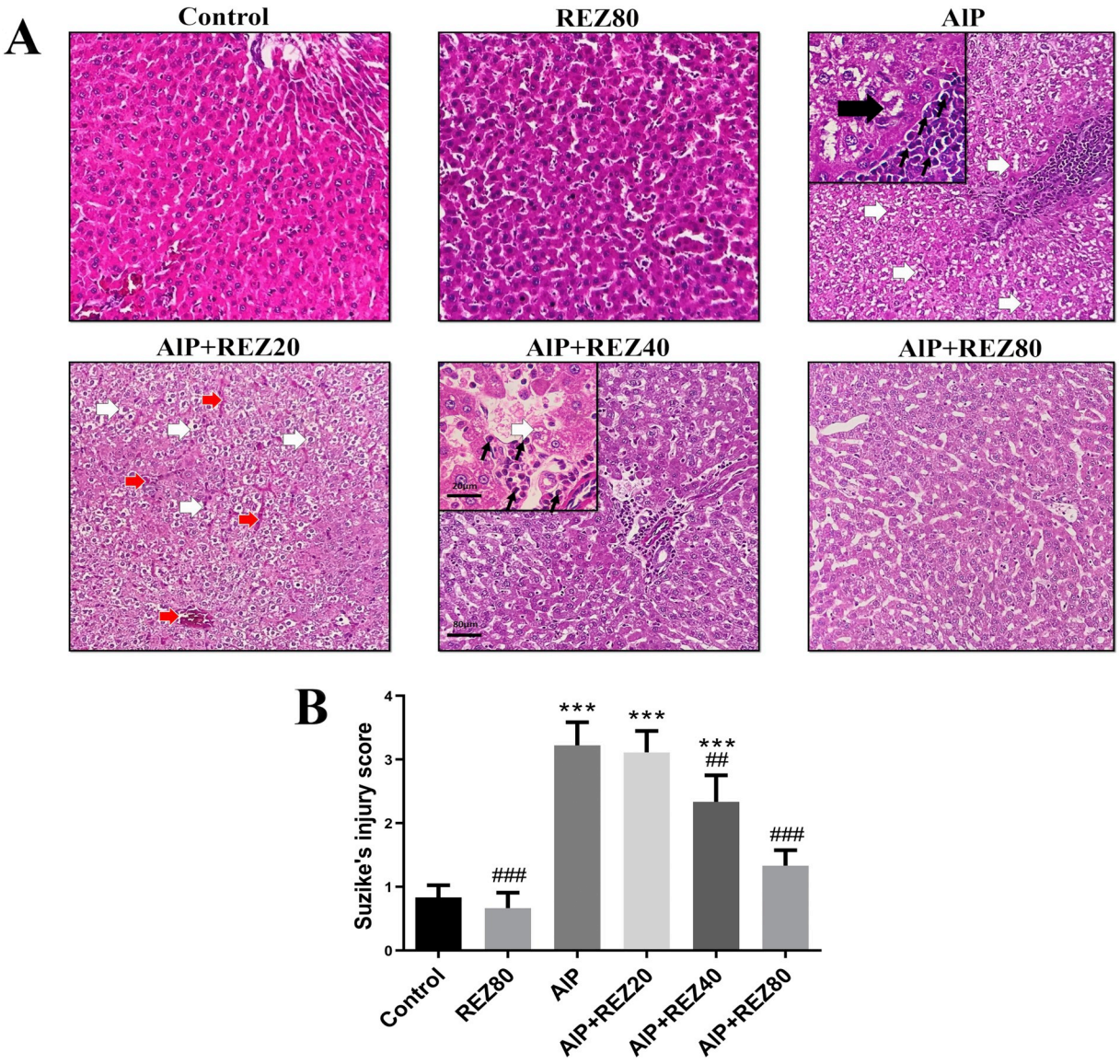

Fig. 6. Histopathological study (A) (stained with H & E, magnifications ×200 and ×800) in rats 24 h after the treatments. The bold dark arrow shows necrosis, the narrow dark arrow shows inflammation cells, the bold white arrow shows vacuolization, the bold red arrow shows congestion. Interpretation of the histological according to Suzike's score (B). Data are mean ± SEM of six animals in each group. $^{*}P < 0.05$ compared to control group, $^{**}P < 0.01$ compared to control group, $^{***}P < 0.001$ compared to control group, $\#P < 0.05$ compared to AlP group, $\#\#P < 0.01$ compared to AlP group, $\#\#\#P < 0.01$ compared to AlP group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

including neutrophils to injured tissues [63]. As demonstrated in this study, the expression of ICAM-1 was elevated during exposure to phosphine. Also, MPO enzyme which is mostly available in neutrophils azurophilic granules is an infiltration marker of neutrophils to damaged sites and induces conversion of H_2O_2 to H_2O and using chloride anion (Cl^-) to produce hypochlorous acid (HOCl). HOCl is also capable of interacting with macromolecules, increasing oxidative stress and catalytic consumption of nitric oxide [64,65]. The results of the current study demonstrated that phosphine increased MPO activity through increasing migration and activation of neutrophils to injured place. The results of the present study demonstrated that resveratrol reduced ICAM-1 expression and MPO level in hepatic tissue. These results are in agreement with other researches which claimed that resveratrol reduced ICAM-1 expression and MPO activity [66,67].

Apoptosis is a highly regulated cell death which has a critical role in homeostasis and cell survival [68]. Physiologically, this orchestrated cellular process is necessary for organ and tissue shaping and also controlling cell numbers. In the other hand, irregularity in this process could lead to tissue disorders [69,70]. It is demonstrated that phosphine increased apoptosis in hepatocytes which resveratrol reduced apoptosis through its cytoprotective effects. This results in line with other studies which is demonstrated phosphine induced apoptosis in several organs [7,71]. Also, it is demonstrated that resveratrol reduced apoptosis in several disorders. The mechanism can be through its anti-oxidant, anti-apoptosis and its capability to reduce mitochondrial malfunction [72–74].

Altogether, according to aforementioned results of the present study it is suggested that ROS/NF-kappaB signalling pathway is a key role for hepatic injury induced by phosphine poisoning, and resveratrol with its pleiotropic effect can be a treatment.

Furthermore, it is reported that ROS/NF-kappaB pathway is involved in hepatic injury [75]. The results of present and previous studies indicated that oxidative stress was elevated in phosphine-induced hepatic injury [76]. Also, it is reported that phosphine is involved in hepatic injury through elevation of inflammation [76,77]. Furthermore, it is demonstrated that resveratrol reduced oxidative stress,

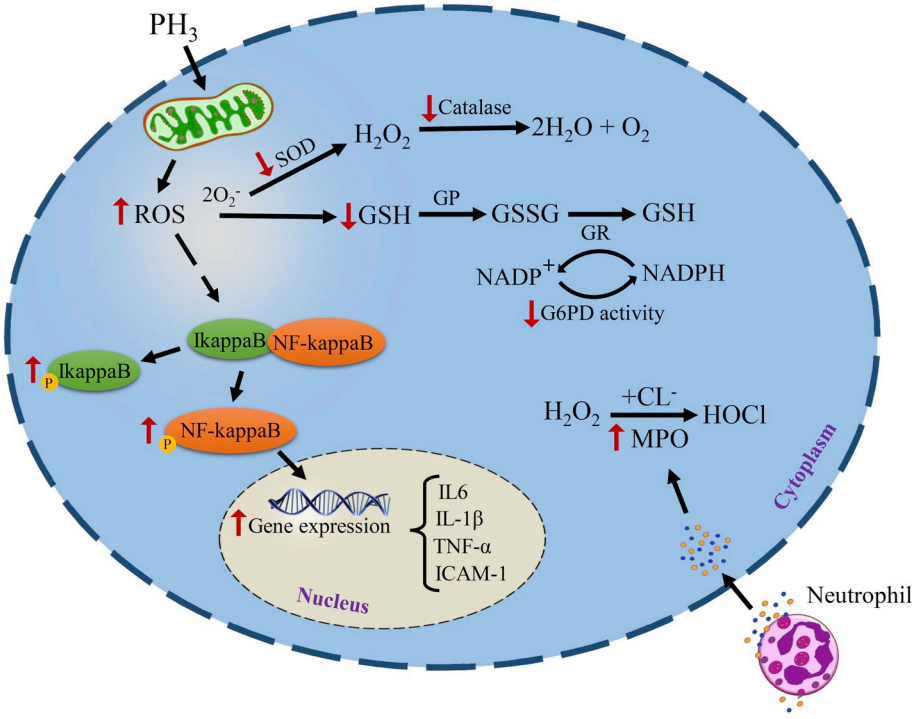

Fig. 7. The molecular mechanisms of hepatocyte phosphine-induced cytotoxicity.
Phosphine induced oxidative stress mainly through mitochondrial dysfunction. Also, phosphine through inhibition of SOD, Catalase, G6PD activity and GSH level induced increasing free radicals. Moreover, elevation of ROS levels induced phosphorylation of IkappaB and NF-kappaB. pNF-kappaB caused an increase in expression of inflammation markers such as TNF-α, IL-6, IL-1β, and ICAM-1. ICAM-1 is involved in chemotaxis of leucocytes like neutrophil which released its azurophil granules containing MPO. MPO induced production of HOCl from H_2O_2 that is responsible for damage elevation.
PH_3; phosphine, ↑ increased by phosphine; ↓ decreased by phosphine; SOD, superoxide dismutase; GSH, glutathione; GSSG; glutathione disulfide, HOCl; hypochlorous acid, GR; glutathione reductase, GP; glutathione peroxidase, MPO; myeloperoxidase, IL; interleukin, ICAM-1; Intercellular Adhesion Molecule 1.

inflammation markers and apoptosis in hepatic injury [73,78,79].

In the present study, treatment with resveratrol blocked oxidative stress markers, declined NF-kappaB activation and inflammatory cytokines expressions such as TNF-α, IL-1β, IL-6, and ICAM-1. It also reduces apoptosis compared to cells that were only exposed to phosphine. Considering all these, it can be implied that phosphine induced hepatic toxicity via the ROS/NF-kappaB pathway. Also, this study underlines this fact that modulation of ROS/NF-kappaB signalling pathway could be the main mechanism of resveratrol protection role.

5. Conclusion

AlP-intoxication is an intricate and multi-faceted condition. Despite the previous studies that have been carried out for clarification of cell damages' mechanisms, more elucidation is needed. As concluded in this study, the activation of ROS/NF-kappaB pathway has an important role in hepatic injury induced by phosphine. Also, it is showed that phosphine induces oxidative stress, inflammation, apoptosis, phosphorylation of IkappaB and activation of NF-kappaB. Taken together, present study indicates that resveratrol might reduce hepatic injury induced by phosphine through mitigating oxidative stress, inflammation, and apoptosis. Also, we concluded that this protection property of resveratrol can be mediated by modifying the activation of ROS/NF-kappaB signalling pathway.

Acknowledgment

This study was supported by a grant from AJA University of Medical Sciences, Tehran, Iran coded 696635.

Author contributions

HHA gave the idea, did the literature search, did the study, drafted the manuscript and statics analysis, SRA was the advisor and helped in the editing of the manuscript; EH and MC participate in animal study and real-time PCR study. MJ participated in the histopathological analysis. BP was the supervisor and supervised the whole study. All authors read and approved the final version.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

References

[1] A.H. Abdolghaffari, A. Baghaei, R. Solgi, M. Gooshe, M. Baeeri, M. Navaei-Nigjeh, et al., Molecular and biochemical evidences on the protective effects of triiodothyronine against phosphine-induced cardiac and mitochondrial toxicity, Life Sci. 139 (2015) 30–39.
[2] M.H. Asghari, M. Moloudizargari, M. Baeeri, A. Baghaei, M. Rahimifard, R. Solgi, et al., On the mechanisms of melatonin in protection of aluminum phosphide cardiotoxicity, Arch. Toxicol. 91 (9) (2017) 3109–3120.
[3] A. Jafari, A. Baghaei, R. Solgi, M. Baeeri, M. Chamanara, S. Hassani, et al., An electrocardiographic, molecular and biochemical approach to explore the cardioprotective effect of vasopressin and milrinone against phosphide toxicity in rats, Food Chem. Toxicol. 80 (2002) 182–192.
[4] A. Baghaei, R. Solgi, A. Jafari, A.H. Abdolghaffari, A. Golaghaei, M.H. Asghari, et al., Molecular and biochemical evidence on the protection of cardiomyocytes from phosphine-induced oxidative stress, mitochondrial dysfunction and apoptosis by acetyl-L-carnitine, Environ. Toxicol. Pharmacol. 42 (2016) 30–37.
[5] B. Lodde, D. Lucas, J.M. Letort, D. Jegaden, R. Pougnet, J.D. Dewitte, Acute phosphine poisoning on board a bulk carrier: analysis of factors leading to a fatal case, J. Occup. Med. Toxicol. 10 (2015) 10.
[6] R. Anand, B.K. Binukumar, K.D. Gill, Aluminum phosphide poisoning: an unsolved riddle, J. Appl. Toxicol. 31 (6) (2011) 499–505.
[7] H. Haghi-Aminjan, M. Baeeri, M. Rahimifard, A. Alizadeh, M. Hodjat, S. Hassani, et al., The role of minocycline in alleviating aluminum phosphide-induced cardiac hemodynamic and renal toxicity, Environ. Toxicol. Pharmacol. 64 (2018) 26–40.
[8] N. Valmas, S. Zuryn, P.R.J.T. Ebert, Mitochondrial uncouplers act synergistically with the fumigant phosphine to disrupt mitochondrial membrane potential and cause cell death, Toxicology 252 (1–3) (2008) 33–39.
[9] H. Kariman, K. Heydari, M. Fakhri, A. Shahrami, A.A. Dolatabadi, H.A. Mohammadi, et al., Aluminium phosphide poisoning and oxidative stress, J. Med. Toxicol. 8 (3) (2012) 281–284.
[10] K. Mortezaee, E. Salehi, H. Mirtavoos-mahyari, E. Motevaseli, M. Najafi, B. Farhood, et al., Mechanisms of apoptosis modulation by curcumin: implications for cancer therapy, J. Cell. Physiol. 234 (8) (2019) 12537–12550.
[11] K. Mortezaee, D. Shabeeb, A.E. Musa, M. Najafi, B. Farhood, Metformin as a radiation modifier; implications to normal tissue protection and tumor sensitization, Curr. Clin. Pharmacol. vol. 14, (1) (2019) 41–53.
[12] M. Najafi, E. Motevaseli, A. Shirazi, G. Geraily, A. Rezaeyan, F. Norouzi, et al., Mechanisms of inflammatory responses to radiation and normal tissues toxicity: clinical implications, Int. J. Radiat. Biol. 94 (4) (2018) 335–356.
[13] R. Yahyapour, D. Shabeeb, M. Cheki, A.E. Musa, B. Farhood, A. Rezaeyan, et al., Radiation protection and mitigation by natural antioxidants and flavonoids: implications to radiotherapy and radiation disasters, Curr. Mol. Pharmacol. 11 (4) (2018) 285–304.

[14] R. Azmoonfar, P. Amini, R. Yahyapour, A. Rezaeyan, A. Tavassoli, E. Motevaseli, et al., Mitigation of radiation-induced pneumonitis and lung fibrosis using alpha-lipoic acid and resveratrol, Antiinflamm. Antiallergy Agents Med. Chem. (2019), https://doi.org/10.2174/1871523018666190319144020.

[15] G. Bureau, F. Longpré, Martinoli MGJJonr, Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation, J. Neurosci. Res. 86 (2) (2008) 403–410.

[16] C.-J. Chen, W. Yu, Y.-C. Fu, X. Wang, J.-L. Li, W.J.B. Wang, et al., Resveratrol protects cardiomyocytes from hypoxia-induced apoptosis through the SIRT1–FoxO1 pathway, Biochem. Biophys. Res. Commun. 378 (3) (2009) 389–393.

[17] H. Rivera, M. Shibayama, V. Tsutsumi, V. Perez-Alvarez, P. Muriel, Resveratrol and trimethylated resveratrol protect from acute liver damage induced by CCl4 in the rat, J. Appl. Toxicol. 28 (2) (2008) 147–155.

[18] H. Sebai, M. Sani, M.T. Yacoubi, E. Aouani, N. Ghanem-Boughanmi, M.J.E. Ben-Attia, et al., Resveratrol, a red wine polyphenol, attenuates lipopolysaccharide-induced oxidative stress in rat liver, Ecotoxicol. Environ. Saf. 73 (5) (2010) 1078–1083.

[19] Y. Han, C. Jiang, J. Tang, C. Wang, P. Wu, G. Zhang, et al., Resveratrol reduces morphine tolerance by inhibiting microglial activation via AMPK signalling, Eur. J. Pain 18 (10) (2014) 1458–1470.

[20] E. Beutler, Active transport of glutathione disulfide from erythrocytes, in: A. Larson, S. Orrenius, A. Holmgren, B. Mannerwik (Eds.), Functions of Glutathione, Biochemical, Physiological, Toxicological and Clinical Aspects, Raven Press, New York, 1983, p. 65.

[21] Y. Sun, L.W. Oberley, Y. Li, A simple method for clinical assay of superoxide dismutase, Clin. Chem. 34 (3) (1988) 497–500.

[22] X. Chen, Z. Zhong, Z. Xu, L. Chen, Y. Wang, 2′,7′-Dichlorodihydrofluorescein as a fluorescent probe for reactive oxygen species measurement: forty years of application and controversy, Free Radic. Res. 44 (6) (2010) 587–604.

[23] F. Tietze, Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues, Anal. Biochem. 27 (3) (1969) 502–522.

[24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[25] A. Shadboorestan, G.A. Tarfiei, H. Montazeri, M.R. Sepand, M. Zangooei, A. Khedri, et al., Invasion and migration of MDA-MB-231 cells are inhibited by block of AhR and NFAT: role of AhR/NFAT1/beta4 integrin signaling, J. Appl. Toxicol. 39 (2) (2019) 375–384.

[26] J. Shi, K. Aisaki, Y. Ikawa, K. Wake, Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride, Am. J. Pathol. 153 (2) (1998) 515–525.

[27] S. Suzuki, L.H. Toledo-Pereyra, F.J. Rodriguez, D. Cejalvo, Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine, Transplantation 55 (6) (1993) 1265–1272.

[28] N.S. Nath, I. Bhattacharya, A.G. Tuck, D.I. Schlipalius, P.R. Ebert, Mechanisms of phosphine toxicity, J. Toxicol. 2011 (2011) 494168.

[29] A.T. Proudfoot, Aluminium and zinc phosphide poisoning, Clin. Toxicol. 47 (2) (2009) 89–100.

[30] A. Etemadi-Aleagha, M. Akhgari, F.S. Iravani, Aluminum phosphide poisoning-related deaths in Tehran, Iran, 2006 to 2013, Medicine 94 (38) (2015) e1637.

[31] A. Ahmadi, A. Shadboorestan, Oxidative stress and cancer; the role of hesperidin, a citrus natural bioflavonoid, as a cancer chemoprotective agent, Nutr. Cancer 68 (1) (2016) 29–39.

[32] M. Shokrzadeh, A. Ahmadi, S. Ramezaninejhad, A. Shadboorestan, Hesperidin, a citrus bioflavonoid, ameliorates genotoxicity-induced by diazinon in human blood lymphocytes, Drug Res. 65 (2) (2015) 57–60.

[33] A.I. Cederbaum, Y. Lu, D. Wu, Role of oxidative stress in alcohol-induced liver injury, Arch. Toxicol. 83 (6) (2009) 519–548.

[34] M. Abdollahi, A. Ranjbar, S. Shadnia, S. Nikfar, A. Rezaiee, Pesticides and oxidative stress: a review, Med. Sci. Monit. 10 (6) (2004) RA141–7.

[35] H. Haghi-Aminjan, B. Farhood, M. Rahimifard, T. Didari, M. Baeeri, S. Hassani, et al., The protective role of melatonin in chemotherapy-induced nephrotoxicity: a systematic review of non-clinical studies, Expert Opin. Drug Metab. Toxicol. 14 (9) (2018) 937–950.

[36] M. Cheki, R. Yahyapour, B. Farhood, A. Rezaeyan, D. Shabeeb, P. Amini, et al., COX-2 in radiotherapy: a potential target for radioprotection and radiosensitization, Curr. Mol. Pharmacol. 11 (3) (2018) 173–183.

[37] K. Mortezaee, N.H. Goradel, P. Amini, D. Shabeeb, A.E. Musa, M. Najafi, B. Farhood, NADPH oxidase as a target for modulation of radiation response; implications to carcinogenesis and radiotherapy, Curr. Mol. Pharmacol. 12 (1) (2019) 50–60.

[38] M. Najafi, M. Cheki, S. Rezapoor, G. Geraily, E. Motevaseli, C. Carnovale, et al., Metformin: prevention of genomic instability and cancer: a review, Mutat. Res. 827 (2018) 1–8.

[39] M. Najafi, E. Salehi, B. Farhood, M.S. Nashtaei, N. Hashemi Goradel, N. Khanlarkhani, et al., Adjuvant chemotherapy with melatonin for targeting human cancers: a review, J. Cell. Physiol. 234 (3) (2019) 2356–2372.

[40] T. Siddiqui, M.K. Zia, S.S. Ali, A.A. Rehman, H. Ahsan, F.H. Khan, Reactive oxygen species and anti-proteinases, Arch. Physiol. Biochem. 122 (1) (2016) 1–7.

[41] T. Fukai, M. Ushio-Fukai, Superoxide dismutases: role in redox signaling, vascular function, and diseases, Antioxid. Redox Signal. 15 (6) (2011) 1583–1606.

[42] M. Zamocky, P.G. Furtmuller, C. Obinger, Evolution of catalases from bacteria to humans, Antioxid. Redox Signal. 10 (9) (2008) 1527–1548.

[43] M.M. Goyal, A. Basak, Human catalase: looking for complete identity, Protein Cell 1 (10) (2010) 888–897.

[44] C.H. Hsu, B.C. Chi, M.Y. Liu, J.H. Li, C.J. Chen, R.Y. Chen, Phosphine-induced oxidative damage in rats: role of glutathione, Toxicology 179 (1–2) (2002) 1–8.

[45] Y. Zheng, Y. Liu, J. Ge, X. Wang, L. Liu, Z. Bu, P. Liu, Resveratrol protects human lens epithelial cells against H_2O_2-induced oxidative stress by increasing catalase, SOD-1, and HO-1 expression, Mol. Vis. 16 (4) (2010) 1467.

[46] S. Sharma, M. Anjaneyulu, S.K. Kulkarni, K. Chopra, Resveratrol, a polyphenolic phytoalexin, attenuates diabetic nephropathy in rats, Pharmacology 76 (2) (2006) 69–75.

[47] E. Gedik, S. Girgin, H. Ozturk, B.D. Obay, H. Ozturk, H. Buyukbayram, Resveratrol attenuates oxidative stress and histological alterations induced by liver ischemia/reperfusion in rats, World J. Gastroenterol. 14 (46) (2008) 7101.

[48] M.L. Circu, T.Y. Aw, Glutathione and modulation of cell apoptosis, Biochim. Biophys. Acta 1823 (10) (2012) 1767–1777.

[49] C. Hsu, B. Han, M. Liu, C. Yeh, J.E. Casida, Phosphine-induced oxidative damage in rats: attenuation by melatonin, Free Radic. Biol. Med. 28 (4) (2000) 636–642.

[50] J. Soeur, J. Eilstein, G. Léreaux, C. Jones, L. Marrot, Skin resistance to oxidative stress induced by resveratrol: from Nrf2 activation to GSH biosynthesis, Free Radic. Biol. Med. 78 (2015) 213–223.

[51] Y. Wang, Y. Jiang, X. Fan, H. Tan, H. Zeng, Y. Wang, P. Chen, M. Huang, H. Bi, Hepato-protective effect of resveratrol against acetaminophen-induced liver injury is associated with inhibition of CYP-mediated bioactivation and regulation of SIRT1–p53 signaling pathways, Toxicol. Lett. 236 (2) (2015) 82–89.

[52] J. Yuan, Z. Zhang, L. Li, W. Song, Resveratrol affects the expression of glutamate cysteine ligase in the kidneys of aged rats, Exp. Ther. Med. 7 (6) (2014) 1762–1766.

[53] D. Albani, L. Polito, A. Signorini, G. Forloni, Neuroprotective properties of resveratrol in different neurodegenerative disorders, Biofactors 36 (5) (2010) 370–376.

[54] M. Ghashghaeinia, D. Giustarini, P. Koralkova, M. Koberle, K. Alzoubi, R. Bissinger, et al., Pharmacological targeting of glucose-6-phosphate dehydrogenase in human erythrocytes by Bay 11-7082, parthenolide and dimethyl fumarate, Sci. Rep. 6 (2016) 28754.

[55] R.C. Stanton, Glucose-6-phosphate dehydrogenase, NADPH, and cell survival, IUBMB Life 64 (5) (2012) 362–369.

[56] M. Najafi, A. Shirazi, E. Motevaseli, A.H. Rezaeyan, A. Salajegheh, S. Rezapoor, Melatonin as an anti-inflammatory agent in radiotherapy, Inflammopharmacology 25 (4) (2017) 403–413.

[57] R. Yahyapour, P. Amini, S. Rezapoor, A. Rezaeyan, B. Farhood, M. Cheki, et al., Targeting of inflammation for radiation protection and mitigation, Curr. Mol. Med. 11 (3) (2018) 203–210.

[58] R. Yahyapour, P. Amini, S. Rezapour, M. Cheki, A. Rezaeyan, B. Farhood, et al., Radiation-induced inflammation and autoimmune diseases, Mil. Med. Res. 5 (1) (2018) 9.

[59] M. Najafi, A. Shirazi, E. Motevaseli, G. Geraily, F. Norouzi, M. Heidari, et al., The melatonin immunomodulatory actions in radiotherapy, Biophys. Rev. 9 (2) (2017) 139.

[60] W. Pan, H. Yu, S. Huang, P. Zhu, Resveratrol protects against TNF-α-induced injury in human umbilical endothelial cells through promoting sirtuin-1-induced repression of NF-KB and p38 MAPK, PLoS One 11 (1) (2016) e0147034.

[61] M. Holmes-McNary, A.S. Baldwin, Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase, Cancer Res. 60 (13) (2000) 3477–3483.

[62] C. Ma, Y. Wang, L. Dong, M. Li, W. Cai, Anti-inflammatory effect of resveratrol through the suppression of NF-kappaB and JAK/STAT signaling pathways, Acta Biochim. Biophys. Sin. Shanghai 47 (3) (2015) 207–213.

[63] F. Olivares-Silva, R. Landaeta, P. Aranguiz, S. Bolivar, C. Humeres, R. Anfossi, et al., Heparan sulfate potentiates leukocyte adhesion on cardiac fibroblast by enhancing Vcam-1 and Icam-1 expression, Biochim. Biophys. Acta Mol. basis Dis. 1864 (3) (2018) 831–842.

[64] E. Malle, P.G. Furtmuller, W. Sattler, C. Obinger, Myeloperoxidase: a target for new drug development? Br. J. Pharmacol. 152 (6) (2007) 838–854.

[65] S.J. Nicholls, S.L. Hazen, Myeloperoxidase and cardiovascular disease, Arterioscler. Thromb. Vasc. Biol. 25 (6) (2005) 1102–1111.

[66] C.W. Liu, H.C. Sung, S.R. Lin, C.W. Wu, C.W. Lee, I.T. Lee, et al., Resveratrol attenuates ICAM-1 expression and monocyte adhesiveness to TNF-alpha-treated endothelial cells: evidence for an anti-inflammatory cascade mediated by the miR-221/222/AMPK/p38/NF-kappaB pathway, Sci. Rep. 7 (2017) 44689.

[67] T. Muller, M. Kirsch, F. Petrat, Attenuation of multiple organ damage by continuous low-dose solvent-free infusions of resveratrol after severe hemorrhagic shock in rats, Nutrients 9 (8) (2017).

[68] P. Shoae-Hagh, M. Rahimifard, M. Navaei-Nigjeh, M. Baeeri, M. Gholami, A. Mohammadirad, et al., Zinc oxide nanoparticles reduce apoptosis and oxidative stress values in isolated rat pancreatic islets, Biol. Trace Elem. Res. 162 (1–3) (2014) 262–269.

[69] H. Haghi-Aminjan, M.H. Asghari, M.H. Goharbari, M. Abdollahi, A systematic review on potential mechanisms of minocycline in kidney diseases, Pharmacol. Rep. 69 (4) (2017) 602–609.

[70] S. Moeini-Nodeh, M. Rahimifard, M. Baeeri, M. Abdollahi, Functional improvement in rats' pancreatic islets using magnesium oxide nanoparticles through antiapoptotic and antioxidant pathways, Biol. Trace Elem. Res. 175 (1) (2017) 146–155.

[71] A. Karimani, A.H. Mohammadpour, M.R. Zirak, R. Rezaee, B. Megarbane, A. Tsatsakis, et al., Antidotes for aluminum phosphide poisoning — an update, Toxicol. Rep. 5 (2018) 1053–1059.

[72] Z. Ungvari, W.E. Sonntag, R. de Cabo, J.A. Baur, A. Csiszar, Mitochondrial protection by resveratrol, Exerc. Sport Sci. Rev. 39 (3) (2011) 128–132.

[73] J.H. Jang, Y.J. Surh, Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells, Mutat. Res. 496 (1–2) (2001) 181–190.

[74] Z. Ungvari, N. Labinskyy, P. Mukhopadhyay, J.T. Pinto, Z. Bagi, P. Ballabh, et al.,

Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells, Am. J. Phys. Heart Circ. Phys. 297 (5) (2009) H1876–H1881.
[75] H. Li, J.J. Sun, G.Y. Chen, W.W. Wang, Z.T. Xie, G.F. Tang, S.D. Wei, Carnosic acid nanoparticles suppress liver ischemia/reperfusion injury by inhibition of ROS, caspases and NF-κB signaling pathway in mice, Biomed. Pharmacother. 82 (2016) 237–246.
[76] S. Saleki, F.A. Ardalan, A. Javidan-Nejad, Liver histopathology of fatal phosphine poisoning, Forensic Sci. Int. 166 (2–3) (2007) 190–193.
[77] S.M. Marashi, M. Arefi, B. Behnoush, M.G. Nasrabad, Z.N. Nasrabadi, Could hydroxyethyl starch be a therapeutic option in management of acute aluminum phosphide toxicity? Med. Hypotheses 76 (4) (2011) 596–598.
[78] A. Kasdallah-Grissa, B. Mornagui, E. Aouani, M. Hammami, M. El May, N. Gharbi, et al., Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver, Life Sci. 80 (11) (2007) 1033–1039.
[79] C.C. Chang, C.Y. Chang, J.P. Huang, L.M. Hung, Effect of resveratrol on oxidative and inflammatory stress in liver and spleen of streptozotocin-induced type 1 diabetic rats, Chin. J. Physiol. 55 (3) (2012) 192–201.