Short Communication

Antioxidant Effects of Amygdalin on Tunicamycin-induced Endoplasmic Reticulum Stress in the Mice Liver: Cross Talk between Endoplasmic Reticulum Stress and Oxidative Stress

Azam Moslehi[1], Tahereh Komeili-movahed[1], Mohammad Moslehi[2]

[1]Cellular and Molecular Research Center, Qom University of Medical Sciences and Health Services, Qom, [2]Department of Pharmaceutics, School of Pharmacy, Shahid Beheshti University of Medical Sciences and Health Services, Tehran, Iran

Abstract

Context: Both endoplasmic reticulum (ER) stress and oxidative stress are involved in pathophysiology of many diseases. Recently, a cross talk between ER stress and oxidative stress has been identified. Amygdalin is an active ingredient in the seeds of apricots, bitter almonds, peaches, and other rosaceous plants. **Aim:** This study was designed to evaluate the antioxidant effects of amygdalin on the liver-induced ER stress. **Materials and Methods:** C57/BL6 inbred male mice were placed in five groups comprising saline, vehicle, and amygdalin as control groups. ER stress was induced by tunicamycin (TM) injection (ER stress group). Amygdalin was administered 1 h before TM challenge (ER stress + amygdalin group). Liver tissue supernatants were prepared and malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were measured. **Results:** The findings showed that ER stress increased MDA level and decreased SOD and CAT activity and GSH level ($P < 0.05$). Pretreatment with amygdalin decreased MDA concentration, whereas it increased SOD and CAT levels ($P < 0.05$). **Conclusion:** This study showed that amygdalin attenuated TM-induced ER stress and has a considerable antioxidant activity in the liver tissue.

Keywords: *Amygdalin, endoplasmic reticulum, liver, oxidative stress, tunicamycin*

Introduction

Endoplasmic reticulum (ER) plays a critical role in lipid synthesis, nascent protein folding, and Ca^{+2} ions storage.[1] In special circumstances, such as pharmacological stimuli, viral infections, and dietary demands, ER homeostasis can disrupt and provide ER stress phenomenon, leading to misfolding or unfolding of proteins.[2] ER stress is closely associated with many diseases such as obesity, atherosclerosis, type 2 diabetes, hepatic cirrhosis, and renal injuries.[3] It has been shown that disruption in ER homeostasis pathway decreases the very low density lipoprotein level, increases the degradation of lipoprotein B100, and changes lipid-related transcription factors.[4] The induction of ER stress is associated with overexpression of lipogenesis transcription factors and enzymes.[5] It could produce inflammation and apoptosis in the hepatic tissue.[6] It has been shown that tunicamycin (TM) challenge, as ER stress inducer agent, could cause pharmacologic ER stress and liver steatosis in *in vitro* and *in vivo* models.[7] Recently, cross talks between ER stress and oxidative stress as two cellular stresses in diseases pathophysiology have been considered.[8,9]

Reactive oxygen species (ROS) are oxygen molecular derivatives and their over-generation induces oxidative stress in body.[10] Environmental factors or mitochondrial dysfunction could produce ROS.[11] Oxidative stress triggers inflammatory molecules, apoptosis cascades, and is involved in the pathogenesis of many diseases such as diabetes, nonalcoholic fatty liver disease, and renal injuries. Several documents have reported that unfolded proteins and disturbances of glutathione (GSH)/GSSH ratio during the ER stress lead to ROS generation in the ER.[12,13] Kim *et al.*[14] have shown that TM-induced ER stress increased lipid peroxidation and decreased GSH concentration in the liver of mice. On the contrary, oxidative stress induction is associated with overexpression of ER stress markers. Oxidative stress activates ER stress cascade and increases activating transcription factor 6 (ATF6), C/EBP homologous protein (CHOP), and activating transcription factor 4 (ATF4) expression.[15,16]

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Address for correspondence:
Prof. Azam Moslehi, Cellular and Molecular Research Center, Qom University of Medical Sciences and Health Services, Qom, Iran.
E-mail: moslehi2000@gmail.com

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Ali *et al.*[17] have also shown that GSH supplement therapy alleviates ER stress chaperones. In another study, selenium, an antioxidant agent, downregulated GRP78, ATF6, and ATF4 gene expressions. It also increased superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) level.[18]

Amygdalin, a cyanide-containing substance, is abundant in the seeds of apricots, bitter almonds, peaches, and other rosaceous plants. Amygdalin had previously used for angiogenesis inhibition, asthma, bronchitis, emphysema, renal fibrosis, diabetes treatment, and pain relief.[19-23] Several studies have also shown the anti-inflammatory and anticancer effects of amygdalin in various cells lines and tissues.[24-26] In the first part of this study, we previously showed that amygdalin attenuated ALT, AST levels, and lipid profile in TM-induced ER stress in the mice liver.[2] Recently, Tang *et al.*[27] have reported that amygdalin decreased malondialdehyde (MDA) and myeloperoxidase (MPO) levels after D-galactosamine liver injury. As the antioxidant effect of amygdalin in the liver induced by ER stress and its relationship between oxidative stress and ER stress has not been investigated, this study was designed to assess the effects of amygdalin on oxidant and antioxidant markers on TM-induced ER stress and its role in relation between ER stress and oxidative stress.

Materials and Methods

Reagents

TM (11089-65-9) and amygdalin (29883-15-6) were purchased from Sigma-Aldrich (USA). Amygdalin was dissolved in saline and TM was dissolved in dimethyl sulfoxide (DMSO). MDA (ZB-MDA-96A), SOD (ZB-SOD-48A), GSH (ZB-GSH-96A), and CAT (ZB-CAT-96A) kits were purchased from ZellBio GmbH, Germany.

Animals

C57/BL6 male mice weighing 23–25 g [Academic Center for Education, Culture and Research (ACECR), Qom, Iran] were used in this study. Animals were kept in a temperature-controlled room and 12:12, light/dark cycle with free access to standard laboratory chow and water. All procedures were in accordance with the Guidelines for Animal Care and Use at the Qom University of Medical Sciences (IR.MUQ.REC.1398.087).

Experimental procedures

The animals were randomly divided into five equal groups ($n = 6$). Control group: received normal saline (0.2 mL i.p.); vehicle group: received DMSO (0.2 mL i.p.); amygdalin group: received amygdalin (3 g/kg i.p.);[28] ER stress group: received single dose of TM (2 mg/kg body weight) to induce ER stress;[7] and ER stress + amygdalin group: received single dose of amygdalin (3 g/kg i.p.) 1 h before TM administration.[26]

Thirty hours post-TM injection,[27] the animals were anesthetized with sodium pentobarbital.[29] The abdomen was excised via midline incision and the liver removed. A part of median lobe sections was then dissected and supernatant immediately prepared. Briefly, 100 mg of the liver tissue was weighted, added 1 mL phosphate buffer, and centrifuged at 3000–4000 rpm for 20 min. Supernatants were then collected, allocated, and kept at –80°C for GSH, MDA, CAT, and SOD measurement according to the manufacturer's instructions. Antioxidant kits measured quantity assay samples based on colorimetric methods that should be read by ELISA reader (for MDA: 535 nm, CAT: 405 nm, GSH: 412 nm, and SOD: 420 nm).

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed by one-way analysis of variances (ANOVA) and Tukey's *post hoc* test using the Statistical Package for the Social Sciences, Italy software version 11.5 for windows. A value of $P < 0.05$ was considered statistically significant.

Results

Effects of amygdalin on MDA concentration in TM-induced ER stress in the mice liver

This study showed that MDA concentration significantly increased after TM-induced ER stress compared with the control group (45.6 ± 1.1 vs. 34.5 ± 0.75 µM/mL, $P < 0.01$), whereas pretreatment with amygdalin significantly decreased MDA concentration compared with the ER stress group (26.4 ± 3.9 vs. 45.6 ± 1.1 µM/mL, $P < 0.001$) [Figure 1].

Effects of amygdalin on CAT enzyme activity in TM-induced ER stress in the mice liver

As noted in Figure 2, in the ER stress group, CAT activity markedly decreased compared with the control group (28.2 ± 0.15 vs. 29.7 ± 0.23 U/mL, $P < 0.001$); however, in ER stress + amygdalin group significantly increased compared with the ER stress group (29.1 ± 0.16 vs. 28.2 ± 0.15, $P < 0.05$).

Effects of amygdalin on SOD enzyme activity in TM-induced ER stress in the mice liver

Our findings indicated a significant decrease of SOD activity in the ER stress group compared with the control group

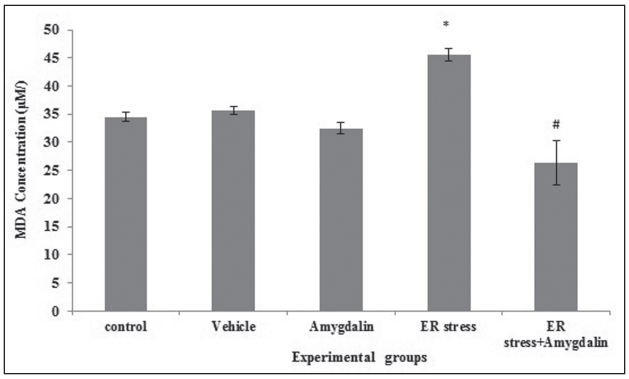

Figure 1: MDA concentrations in different experimental groups ($n = 6$, mean ± SEM). *$P < 0.01$ compared to control group. #$P < 0.001$ compared to ER stress group

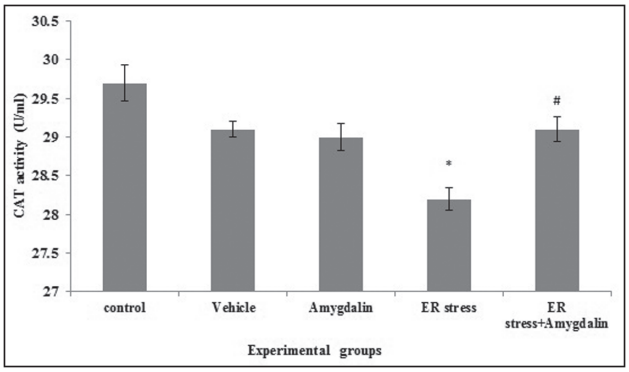

Figure 2: Catalase enzyme activity in different experimental groups (*n* = 6, mean ± SEM). **P* < 0.001 compared to control group. #*P* < 0.05 compared to ER stress group

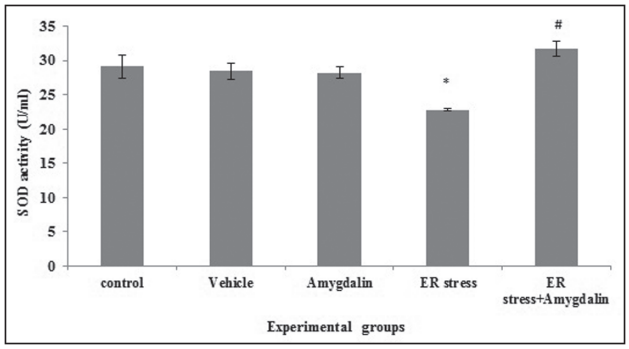

Figure 3: SOD enzyme activity in different experimental groups (*n* = 6, mean ± SEM). **P* < 0.05 compared to control group. #*P* < 0.01 compared to ER stress group

(22.83 ± 0.2 vs. 29.14 ± 1.7 U/mL, $P < 0.05$) and amygdalin administration significantly increased SOD activity compared with the ER stress group (31.78 ± 1.12 vs. 22.83 ± 0.2 U/mL, $P < 0.01$) [Figure 3].

Effects of amygdalin on GSH concentration in TM-induced ER stress in the mice liver

This study revealed that GSH levels, an important antioxidant molecule, significantly lowered in the TM-induced ER stress compared with the control group (0.025 ± 0.004 vs. 0.041 ± 0.006 mM/mL, $P < 0.05$), and pretreatment with amygdalin could partly increase GSH concentration compared with the ER stress group; however, it was not significant [Figure 4].

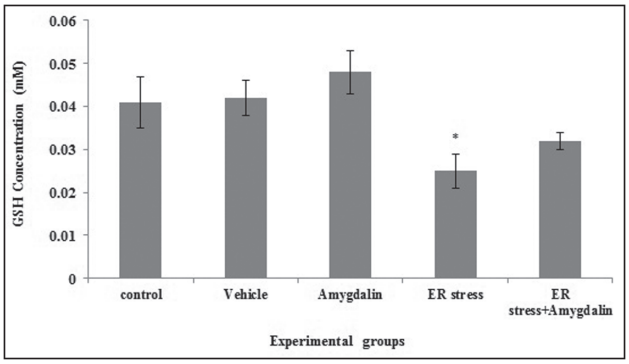

Figure 4: GSH levels in different experimental groups (*n* = 6, mean ± SEM). **P* < 0.05 compared to control group

Discussion

This study showed that amygdalin administration could decrease MDA concentration and increase SOD and CAT enzyme activity. Amygdalin is a cyanogenic glycoside with antidiabetic, anti-atherosclerosis, and anticancer characteristics.[19,21-23] To the best of our knowledge, studies on the antioxidant effects of amygdalin on the ER stress-induced liver injuries are rare and need to more evaluations. Nowadays, accumulating documents show ER stress and oxidative stress are closely linked to provide variety of diseases. During ER stress induction, protein's misfolding leads to ROS generation and GSH depletion.[10] In addition, defects in disulfide bonds formation provide ER stress and produce ROS. TM is a pharmacologic agent, and is frequently used to induce ER stress in cell line and animals.[7,30] In this experiment, TM-induced ER stress increased MDA levels and amygdalin pretreatment significantly decreased it. MDA is produced during peroxidation of the cell membrane and is considered as an indicator of cell injury. Kim *et al.*[30] have shown that TM-induced ER stress increased MDA levels, GRP 78, and CHOP and decreased GSH levels in HepG2 cells. In another study, isoquercetin could ameliorate lactate dehydrogenase (LDH) and MDA after TM-induced ER stress in dorsal root ganglion neurons.[31] Tang *et al.*[27] reported that treatment with amygdalin lowered MDA and MPO levels in the liver injuries. These studies are compatible with our results and indicate antioxidant effects of amygdalin in attenuating of MDA levels.

SOD and CAT are two antioxidant enzymes that catalyze superoxide radicals and rise GSH power. Our findings indicated that ER stress decreased SOD and CAT activity, whereas amygdalin administration could increase their activity. Compatible with our results, Xiao *et al.*[18] have reported that selenium alleviated SOD, CAT, GSH-Px, and GSH as well as GRP78, ATF6, and ATF4 after ER stress induction in chicken spleen lymphocyte. It has also been stated that carvedilol has an alleviating effect on the ATF4, CHOP, and ATF6 as ER stress index that is accompanied with increasing activity of SOD.[32] In two studies, Elsaed.[33]and Hu *et al.*[34] showed that pretreatment with amygdalin could increase SOD and GSH in the autoimmune hepatitis and endometriosis. It seems that decrease of ER stress is associated with decrease of oxidative stress and it is probable that amygdalin could attenuate ER stress and thereby oxidative stress via increment of SOD and CAT activity.

Another important antioxidant in the cell is GSH. Previous studies have shown that decrease of GSH cell content leads to ROS generation in ER stress. Indeed, correct protein-folding need to disulphide bonds formation in the ER and GSH consumption.[35,36] In the ER stress, protein misfolding decreases GSH levels and provides oxidative stress. In this regards, Ali *et al.*[17] have reported that treatment with GSH ameliorated ER stress and ATF6 gene expression in mice

embryos cells. In our study, ER stress decreased GSH level and administration of amygdalin partly increased GSH levels, although it was not significant. It may be due to short time of amygdalin treatment in our study as the change in GSH content is the last part of antioxidant activity chain in the hepatocytes and needs to enough time.

Conclusion

In summary, the findings of this study, for the first time, show that amygdalin could attenuate ER stress, induce oxidative stress, and improve antioxidant enzymes activity. In addition, it could be considered as a notable antioxidant on the therapeutic goals. Molecular studies are needed to clarify more details.

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Conflicts of interest

There are no conflicts of interest.

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