Research Article

Tugce Karabas and Gulben Sayilan Ozgun*

Effect of caffeine on acrylamide-induced cellular hepatotoxicity

https://doi.org/10.1515/tjb-2023-0174
Received August 12, 2023; accepted June 3, 2025;
published online June 20, 2025

Abstract

Objectives: This study aims to determine the effects of caffeine on protein oxidation and endoplasmic reticulum (ER) stress in acrylamide (AA)-induced cellular hepatotoxicity.
Methods: First, HepG2 cells were incubated with 10^1, 10^3, and 10^4 µM AA. MTT assay was used to evaluate the cell viability. The western blot method was used to measure protein carbonyl (PC) levels to evaluate protein oxidation, and glucose-regulated protein 78 (GRP78), activating transcription factor 4 (ATF4), and C/EBP homologous (CHOP) levels to evaluate ER stress. Then, according to our results, cells were incubated with 10, 50, and 200 µM caffeine simultaneously with 10^4 µM AA and cell viability, PC, GRP78, ATF4 and CHOP levels were measured with the same methods.
Results: 10^3 and 10^4 µM AA caused a significant decrease in cell viability ($p<0.05$ and $p<0.001$, respectively). 10^4 µM AA caused a significant increase in PC ($p<0.05$), GRP78 ($p<0.01$), ATF4 ($p<0.001$), and CHOP ($p<0.001$) levels. 50 and 200 µM caffeine significantly reduced PC levels in AA-incubated cells ($p<0.05$ and $p<0.01$, respectively). Caffeine concentrations did not significantly change in cell viability, GRP78, ATF4, and CHOP levels ($p>0.05$ for all).
Conclusions: Our study showed that; AA, especially at higher concentrations, caused an increase in cytotoxicity, protein oxidation, and ER stress, and caffeine decreases protein oxidation without changing AA-induced cytotoxicity and ER stress.

Keywords: acrylamide; caffeine; hepG2 cells; protein oxidation; endoplasmic reticulum stress

***Corresponding author: Gulben Sayilan Ozgun**, Department of Medical Biochemistry, Trakya University School of Medicine 22030, Edirne, Türkiye, E-mail: gulbensayilanozgun@gmail.com. https://orcid.org/0000-0001-6990-3484
Tugce Karabas, Department of Medical Biochemistry, Trakya University School of Medicine, Edirne, Türkiye. https://orcid.org/0000-0002-8871-802X

Introduction

Caffeine is a methylxanthine alkaloid, mainly found in coffee, cocoa beans, and tea leaves. Caffeine is the most widely used psychoactive drug in the world [1]. Caffeine is a well-known non-selective adenosine receptor antagonist that blocks adenosine and adenosine receptor binding-induced sleepiness in the central nervous system [2]. Caffeine decreases oxidative stress in many conditions [1]. Caffeine has anti-inflammatory, anti-infection, and anti-fibrosis effects, and its beneficial effects are shown in neurodegenerative disease, cardiovascular disease, obesity, and diabetes. It has also been reported that caffeine has hepatoprotective effects and prevents the progression of liver diseases [3, 4, 5, 6].

Acrylamide (AA) is a synthetic substance primarily used in experimental research and in synthesizing various polymers [7, 8]. AA is also formed in foods at high temperatures during frying and baking processes [9]. Food products such as fried potatoes, bread, biscuits, and coffee contain AA [7]. In addition, AA toxicity can be seen through respiration, water pollution, or skin contact [10] and causes neurotoxicity and genotoxicity [7]. AA metabolism mainly occurs in the liver. Also, AA causes toxicity in the liver [11, 12].

One of the damage mechanisms in AA toxicity is oxidative stress [11]. Reactive oxygen species (ROS) increase affects cellular redox balance and leads to post-translational modification of proteins. Protein carbonyl (PC) is a frequently used marker for protein oxidation and oxidative stress [13].

The endoplasmic reticulum (ER) is a vital organelle for folding and post-translational modifications of proteins. Various stimuli (accumulation of modified proteins, aging, pathological diseases, oxidative stress, etc.) cause ER stress by impairing ER function. Unfolded protein response is activated in the ER to maintain homeostasis, increase folding capacity, or induce cell death as a last option [14]. Glucose-related protein 78 (GRP78), a heat shock protein 70 family member, is an ER chaperone. Under physiological conditions, GRP78 acts as a stress sensor embedded in the ER membrane and is inactive while bound to membrane proteins. When ER stress occurs, GRP78 separates from these proteins, becomes active, and binds to misfolded proteins.

This separation initiates the UPR cascade, including three major pathways named protein kinase R-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) [14, 15].

In previous studies, it was reported that PERK- eukaryotic translation initiation factor 2α (eIF2α)-activating transcription factor 4 (ATF4)-C/EBP-homologous protein (CHOP) signaling was the main pathway in AA-induced apoptotic neuronal cell death, and AA had a slight effect on IRE pathway [16, 17]. In this signaling pathway, PERK activation leads to the phosphorylation of eIF2α, which reduces the amount of protein accumulated in the lumen by inhibiting protein translation and stimulates the transcription of ATF4 [18]. ATF4 is associated with amino acid metabolism and oxidation/reduction, and it activates CHOP, also called the growth arrest and DNA damage-inducible gene 153 (GADD153), which is the most important molecule for the ER stress-induced apoptotic cell death [19, 20, 21, 22].

No study investigated the effects of caffeine on AA toxicity in the liver. Oxidative and ER stresses are tightly associated with AA-induced hepatotoxicity development through complex signaling pathways involving apoptosis, inflammation and autophagy [11]. In this study, we aimed to determine the effects of caffeine on protein oxidation and ER stress in AA-induced cellular hepatotoxicity. The HepG2 cells are immortal human-derived liver hepatoma cell lines frequently preferred in chemical-induced hepatotoxicity models [11, 12]. Depending on daily coffee consumption, plasma caffeine concentrations of adults are usually between 10 and 50 μM, and it has been reported that caffeine has toxic effects at concentrations above 200 μM [23, 24, 25]. In this study, we first determined the effects of AA concentrations in HepG2 cells and then the effects of 10, 50, and 200 μM caffeine in AA-induced protein oxidation and ER stress.

Materials and methods

Experimental design

This study is approved by the ethics committee (TÜTF-BAEK 2018/299). HepG2 cells (ATCC) were incubated at 37 °C and 5 % CO_2 atmosphere. EMEM (Wisent) containing 10 % FBS (Thermo) and 1 % antibiotic and antimycotic (Thermo) was used for the cultivation.

Cells were incubated with 10^1, 10^3, and 10^4 μM AA (Merck) to investigate the effect of AA concentrations for 24 h. The control group was simultaneously incubated with medium without acrylamide in this experiment. For the treatment, cells were incubated with 10, 50, and 200 μM caffeine (Sigma) simultaneously with 10^4 μM AA for 24 h. In this experiment, the control group was simultaneously incubated with a medium that included neither acrylamide nor caffeine. Also, a flask of cells was incubated with 12 μM tunicamycin (Sigma), which is shown to induce ER and oxidative stress, for 24 h to show ER and oxidative stress-induced protein bands for western blot experiments [26, 27].

MTT assay

MTT assay was used to evaluate cell viability [28]. 10^4cells per well seeded into 96 well plates. After removing mediums containing treatments, 5 mg/mL MTT (Sigma) and 100 μL EMEM were added to wells at the end of the incubations. After 4 h at the CO_2 incubator at 37 °C, formazan was dissolved by DMSO and Sorenson buffer, and the optical densities of wells were measured at 570/630 nm [29]. The results were expressed as percentages of the control group.

Western blotting

After incubations, cells were lysed with 10 mM Tris at pH 8.0 with 50 mM EDTA and % 1 SDS (w/v) at 90 °C. The protein content of lysates was measured [30]. For determining PC levels, 5 μg protein containing lysate was incubated with 2,4-dinitrophenylhydrazine (DNPH) (Sigma) [31] as previously described [32]. 20 μg of total protein were loaded into electrophoresis gels to determine GRP78, ATF4, and CHOP levels. Proteins of samples were loaded to electrophoresis gel and blotted to the PVDF membrane (Roche) after separation [33]. After 1 h of blocking with milk powder (Sigma) in TBST (5 %), membranes were incubated with specific antibodies (PC; 1:5,000, GRP78; 1:10,000-, ATF4; 1:2000, CHOP; 1:200, tubulin; 1:10,000) overnight at 4 °C and secondary antibodies for 1 h at laboratory conditions. Specific bands on the membrane were visualized with an electrochemiluminescence imaging system and calculated with Image-J [34]. Results were calculated by dividing specific protein results into the same sample's loading control tubulin result. Then, the results were divided into the result of the control group presented in the same experiment and on the same membrane. All results were expressed as fold change relative to the control.

Statistical analysis

SPSS 20 program (IBM) was used for statistical analysis. Results were calculated as a percentage of control for each MTT assay and as a fold of control for each Western blot.

Data was expressed as the mean ± standard deviation of three results from three independent experiments. The One-Way ANOVA test was used to compare parameters among the groups, and then Tukey's test was used to compare groups. The $p<0.05$ was considered statistically significant.

Results

AA causes cytotoxicity at higher concentrations

The viability percentages were 100 ± 2 % for 10^1 µM, 88 ± 5 % for 10^3 µM, and 57 ± 7 % for 10^4 µM AA-incubated cells. 10^3 µM AA caused significant cytotoxicity compared to control and 10^1 µM AA-incubated cells ($p<0.05$ for both). 10^4 µM AA caused significant cytotoxicity compared to control, 10^1 AA-incubated cells and 10^3 µM AA-incubated cells ($p<0.001$ for all) (Figure 1).

AA increases protein oxidation and ER stress at 10^4 µM

PC levels of 10^1 µM AA-incubated cells were 1.26 ± 0.15 fold, 10^3 µM AA-incubated cells were 1.18 ± 0.20 fold, and 10^4 µM AA-incubated cells were 2.04 ± 0.62 fold of control. 10^4 µM AA caused a significant increase in PC levels compared to the control group ($p<0.05$) (Figure 2).

GRP78 levels of 10^1 µM AA-incubated cells were 1.11 ± 0.16 fold, 10^3 µM AA-incubated cells were 1.16 ± 0.33 fold, and 10^4 µM AA-incubated cells were 1.73 ± 0.17 fold of control. 10^4 µM AA-incubated cells had significantly higher GRP78 levels than control ($p<0.01$), 10^1 AA-incubated cells ($p<0.05$) and 10^3 µM AA-incubated cells ($p<0.05$) (Figure 2).

ATF4 levels of 10^1 µM AA-incubated cells were 1.05 ± 0.05 fold, 10^3 µM AA-incubated cells were 2.92 ± 0.29 fold, and 10^4 µM AA-incubated cells were 20.18 ± 2.34 fold of control. 10^4 µM AA-incubated cells had significantly higher ATF4 levels than the control, 10^1 AA-incubated cells and 10^3 µM AA-incubated cells ($p<0.001$ for all) (Figure 2).

CHOP levels of 10^1 µM AA-incubated cells were 1.19 ± 0.10 fold, 10^3 µM AA-incubated cells were 1.50 ± 0.21 fold, and 10^4 µM AA-incubated cells were 2.26 ± .19 fold of control. 10^3 µM AA-caused a significant increase in CHOP levels ($p<0.05$). 10^4 µM AA-incubated cells had significantly higher CHOP levels than the control ($p<0.001$), 10^1 AA-incubated cells ($p<0.001$) and 10^3 µM AA-incubated cells ($p<0.01$) (Figure 2).

Caffeine concentrations do not change cell viability in AA-incubated cells

According to our results, HepG2 cells were incubated with caffeine concentrations simultaneously with 10^4 µM AA, which induced cytotoxicity, oxidative, and ER stress.

The viability percentages were 58 ± 1 % for 10^4 µM AA-incubated cells, 58 ± 3 % for 10^4 µM AA+ 10 µM caffeine-incubated cells, 63 ± 3 % for 10^4 µM AA+ 50 µM caffeine-incubated cells, and 62 ± 1 % for 10^4 µM AA+ 200 µM caffeine-incubated cells. 10, 50, and 200 µM caffeine did not significantly alter cell viability in 10^4 µM AA-incubated cells ($p>0.05$ for all) (Figure 3).

Caffeine decreases AA-induced protein oxidation at higher concentrations without affecting ER stress

PC levels of 10^4 µM AA-incubated cells were 2.04 ± 0.10 fold, 10^4 µM AA+ 10 µM caffeine-incubated cells were 1.80 ± 0.15 fold, 10^4 µM AA+ 50 µM caffeine-incubated cells were 1.64 ± 0.12 fold, and 10^4 µM AA+ 200 µM caffeine-incubated cells were 1.45 ± 0.13 fold of control. 50 µM caffeine caused a significant decrease in PC levels in 10^4 µM AA-incubated cells ($p<0.05$). 10^4 µM AA+ 200 µM caffeine-incubated cells had

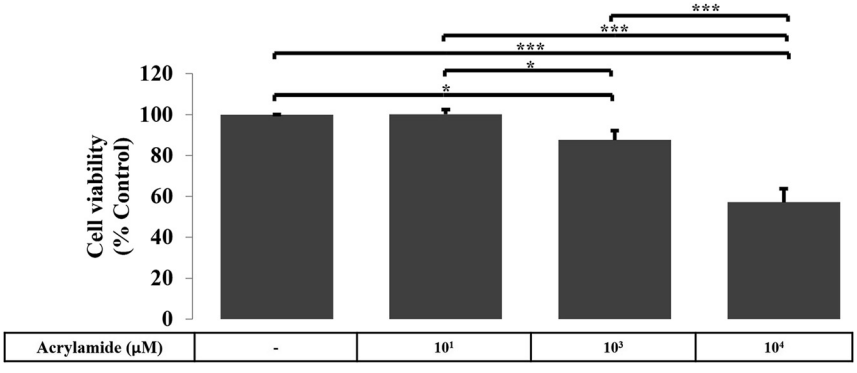

Figure 1: Effects of acrylamide on cell viability in HepG2 cells. Results are expressed as the mean ± standard deviation of three results from three independent experiments. Data was analyzed by one-way ANOVA, and turkey post-hoc test were performed for multiple comparisons. *: $p<0.05$, ***: $p<0.001$.

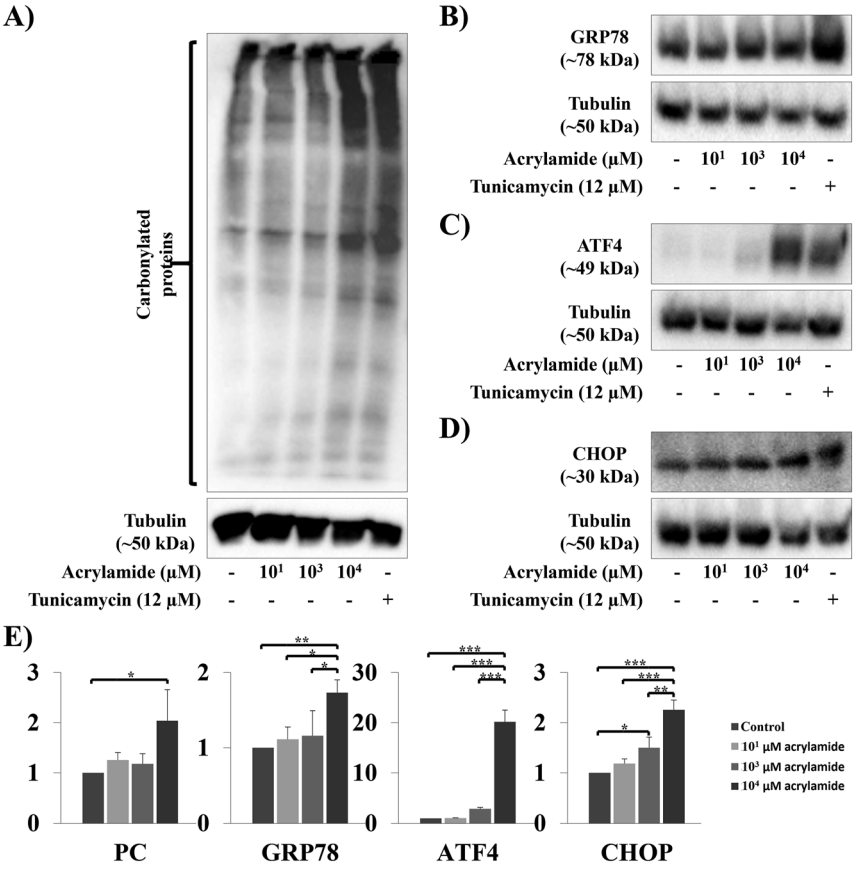

Figure 2: Effects of acrylamide on protein oxidation and ER stress in HepG2 cells. Effect of acrylamide concentrations on (A) PC levels (B) GRP78 levels (C) ATF4, and (D) CHOP levels in HepG2 cells. Tunicamycin-treated cells were used to show ER and oxidative stress-induced protein bands (not calculated). Results are expressed as the mean ± standard deviation of three results from three independent experiments (E). Data was analyzed by one-way ANOVA, and turkey post-hoc test were performed for multiple comparisons. *: p<0.05, **: p<0.01, ***: p<0.001.

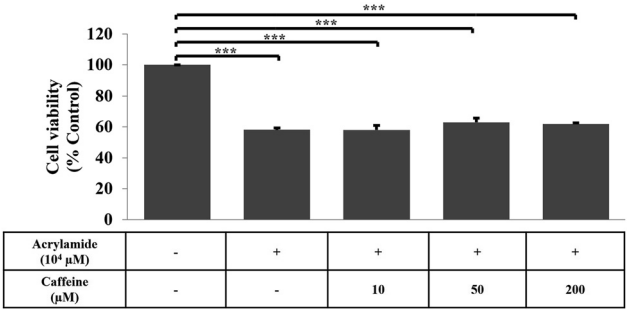

Figure 3: Effects of caffeine on cell viability in acrylamide-incubated HepG2 cells. Results are expressed as the mean ± standard deviation of three results from three independent experiments. Data was analyzed by one-way ANOVA, and turkey post-hoc test were performed for multiple comparisons. ***: p<0.001.

significantly lower PC levels than 10^4 μM AA incubated cells (p<0.01) and 10^4 μM AA+ 10 μM caffeine-incubated cells (p<0.05) (Figure 4).

GRP78 levels of 10^4 μM AA-incubated cells were 1.74 ± 0.14 fold, 10^4 μM AA+ 10 μM caffeine-incubated cells were 1.79 ± 0.22 fold, 10^4 μM AA+ 50 μM caffeine-incubated cells were 1.75 ± 0.19 fold, and 10^4 μM AA+ 200 μM caffeine-incubated cells were 1.94 ± 0.19 fold of control. 10, 50, and 200 μM caffeine treatment did not significantly alter GRP78 levels in 10^4 μM AA-incubated cells (p>0.05 for all) (Figure 4).

ATF4 levels of 10^4 μM AA-incubated cells were 19.64 ± 4.20 fold, 10^4 μM AA+ 10 μM caffeine-incubated cells were 17.84 ± 3.24 fold, 10^4 μM AA+ 50 μM caffeine-incubated cells were 19.21 ± 5.34 fold, and 10^4 μM AA+ 200 μM caffeine-incubated cells were 22.00 ± 4.07 fold of control. 10, 50, and 200 μM caffeine treatment did not significantly alter ATF4 levels in 10^4 μM AA-incubated cells (p>0.05 for all) (Figure 4).

CHOP levels of 10^4 μM AA-incubated cells were 2.06 ± 0.10 fold, 10,000 μM AA+ 10 μM caffeine-incubated cells were 1.60 ± 0.15 fold, 10^4 μM AA+ 50 μM caffeine-incubated cells were 1.95 ± 0.38 fold, and 10^4 μM AA+ 200 μM caffeine-incubated cells were 2.17 ± 0.09 fold of control. 10, 50, and 200 μM caffeine treatment did not significantly alter CHOP levels in 10^4 μM AA-incubated cells (p>0.05 for all). 10^4 μM AA+ 200 μM caffeine-incubated cells had significantly higher CHOP levels than 10^4 μM AA+ 10 μM caffeine-incubated cells (p<0.05) (Figure 4).

Discussion

Caffeine is a plant-based substance in coffee, tea and energy drinks. Thus, it is one of the most frequently consumed

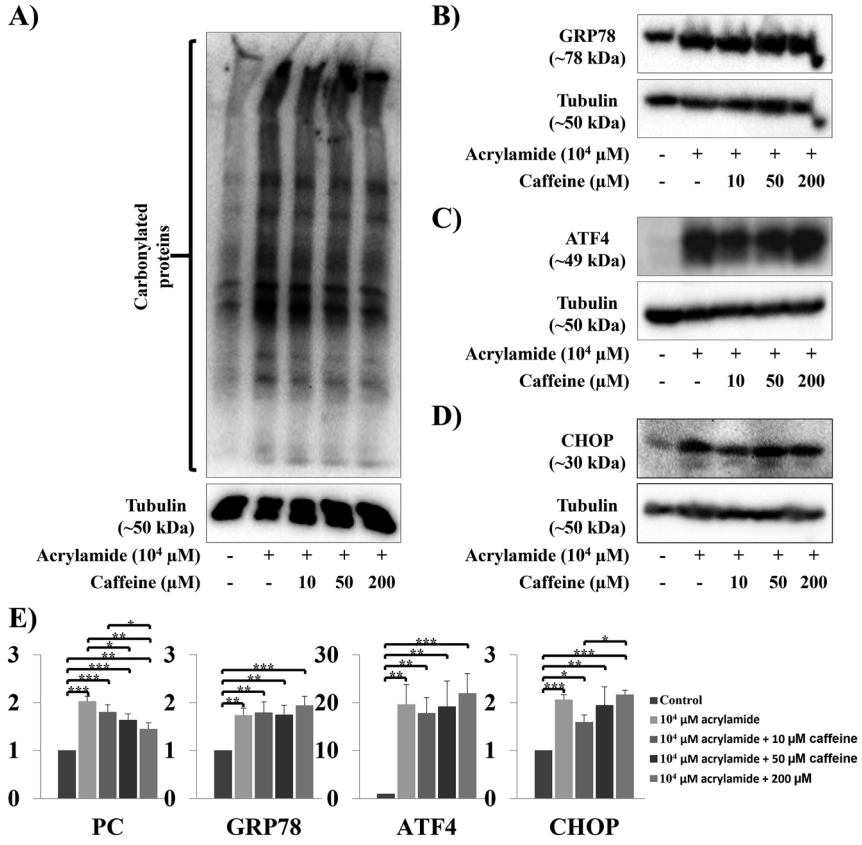

Figure 4: Effects of caffeine on protein oxidation and ER stress in acrylamide-incubated HepG2 cells. Effect of caffeine concentrations on (A) PC levels (B) GRP78 levels (C) ATF4, and (D) CHOP levels in acrylamide-incubated cells. Results are expressed as the mean ± standard deviation of three results from three independent experiments (E). Data was analyzed by one-way ANOVA, and turkey post-hoc test were performed for multiple comparisons. *: p<0.05, **: p<0.01, ***: p<0.001.

chemicals worldwide [1]. AA is a water-soluble substance that people are exposed to occupationally and with food [35]. It's metabolized in the liver and has been shown to have toxic effects on the liver [11, 12, 36]. Previous studies reported the hepatoprotective effects of caffeine in alcoholic liver injury [5] and liver fibrosis [6]. Still, there is no study investigating the effects of caffeine on AA toxicity in the liver. In the present study, we aimed to examine the effects of caffeine on protein oxidation and ER stress in AA-induced cellular hepatotoxicity. Also, researchers pre-treated rat primary astrocytes [37], human astrocytoma [38, 39], neuroblastoma and glioblastoma cells [40] with caffeine as ataxia telangiectasia mutated (ATM)/ATM-Rad3-related (ATR) inhibitor for investigating the mechanism action of AA in their study. However, we could not find any study determining the effect of caffeine together with acrylamide. With the present study, we also determined the effect of caffeine together with acrylamide for the first time.

First, to evaluate the effects of AA concentrations on cytotoxicity, protein oxidation, and ER stress, cells were incubated with 10^1, 10^3, and 10^4 µM AA. In our study, 10^3 and 10^4 µM AA caused cytotoxicity in HepG2 cells. Our results support the studies in the literature, which showed that AA has toxic effects on HepG2 cells at high concentrations [12, 40, 41]. It is known that acrylamide weakens antioxidant defense systems by causing glutathione depletion during its metabolism, and oxidative stress increases acrylamide toxicity [7, 11]. We showed that 10^4 µM AA increased oxidative stress with increased PC levels in HepG2 cells, and similar to our results, the increase of oxidative stress in acrylamide-treated HepG2 cells was shown in previous reports [41, 42]. It was demonstrated that AA has upregulated ER stress pathways [16, 43]. Also, previous studies reported that AA causes an increase in ER stress, including GRP78 and CHOP levels in kupffer cells, rat liver [44] and HepG2 cells [42]. Compatible with the literature, 10^3 µM and 10^4 µM AA caused an increase in ER stress in HepG2 cells.

Based on our first results, we chose 10^4 µM AA to induce cytotoxicity, protein oxidation and ER stress in subsequent experiments to investigate the effects of caffeine concentrations. Plasma caffeine concentrations in adults are usually between 10 and 50 µM, and it has been reported that caffeine has toxic effects at concentrations above 200 µM [23, 24, 25]. Thus, to determine the effects of caffeine on AA-induced cellular hepatotoxicity, the cells were incubated with 10, 50, and 200 µM caffeine simultaneously with 10^4 µM AA.

In our study, caffeine concentrations did not significantly affect the cell viability in AA-treated HepG2 cells. Our findings showed that our caffeine concentrations were ineffective in preventing AA-induced hepatotoxicity in HepG2 cells. On the

other hand, caffeine was reported to be effective at 20 mM concentration against lipopolysaccharide-induced cytotoxicity [45]. We used caffeine concentrations compatible with human plasma caffeine concentrations, and our previous report showed that cell viability decreased to 84 % even when incubated with 200 µM caffeine for 24 h in HepG2 cells [46]. Also, it was shown that higher caffeine concentrations are cytotoxic to HepG2 cells [47]. In light of our previous study and literature, higher caffeine concentrations may do more harm than good in AA-induced hepatotoxicity in HepG2 cells.

We found that 50 and 200 µM caffeine caused a significant decrease in PC levels in AA-incubated HepG2 cells. The antioxidant properties of caffeine are well-known [1], and our results showed that caffeine causes an antioxidant effect against AA-induced protein oxidation. Also, Pasaoglu et al. [48] reported that 30 and 100 mg/kg/day oral caffeine treatment for 14 days causes a decrease in liver advanced oxidation protein products levels in rats, and Amer et al. [49] reported 37.5 mg/kg/day caffeine caused a decrease in liver protein carbonyl levels and malondialdehyde levels in thioacetamide-induced liver injury in rats.

Caffeine concentrations in this study did not significantly change the GRP78, ATF4, and CHOP levels in AA-treated HepG2 cells. Our findings showed that 10, 50, and 200 µM caffeine does not affect AA-induced ER stress in HepG2 cells. We could not encounter any study investigating the effect of caffeine at concentrations similar to our research on ER stress in the liver *in vivo* or *in vitro*. However, at high concentrations, there are contradictory results regarding the effects of caffeine on ER stress in different liver-associated cell lines. Hu et al. [45] reported that caffeine is effective at 20 mM concentration against lipopolysaccharide-induced cytotoxicity and ER stress in L02, human fetal hepatocyte, cell line. On the other hand, Li et al. [50] reported that caffeine induces ER stress and apoptosis at 5 mM concentrations and above in LX-2, immortalized human hepatic stellate cell line.

As a limitation, although caffeine and acrylamide have systemic effects, our *in vitro* study investigated the effects of these chemicals in a cellular liver model using an immortal cell line.

In conclusion, we found that AA, especially at higher concentrations, caused an increase in cytotoxicity, protein oxidation, and ER stress, and caffeine decreases protein oxidation without changing AA-induced cytotoxicity and ER stress. Our results show that caffeine may be beneficial in preventing protein oxidation, but it's insufficient to prevent AA-induced hepatotoxicity.

Acknowledgments: Scientific Research Appropriation of Trakya University-Turkey financially supported this study as the M.Sc. thesis of Tugce Karabas (TÜBAP 2018/266). This study was also presented as an oral presentation at TBS İnternational Biochemistry Congress 2022 33rd National Biochemistry Congress (October 2022, İzmir, Turkey). We thank the Technology Research and Development Center of Trakya University (TÜTAGEM) for providing the ECL detection system.

Research ethics: The ethics committee of Trakya University School of Medicine approved this study (TÜTF-BAEK 2018/299, date of approval: 17.09.2018).

Informed consent: Not applicable.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: None declared.

Conflict of interest: The authors state no conflict of interest.

Research funding: This study was supported by Scientific Research Appropriation of Trakya University–Turkey under grant number: TÜBAP 2018/266.

Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Ősz BE, Jîtcă G, Ștefănescu RE, Pușcaș A, Tero-Vescan A, Vari CE, et al. Caffeine and its antioxidant properties-it is all about dose and source. Int J Mol Sci 2022;23:13074.
2. Song X, Kirtipal N, Lee S, Malý P, Bharadwaj S. Current therapeutic targets and multifaceted physiological impacts of caffeine. Phytother Res 2023;37:5558–98.
3. Saimaiti A, Zhou DD, Li J, Xiong RG, Gan RY, Huang SY, et al. Dietary sources, health benefits, and risks of caffeine. Crit Rev Food Sci Nutr 2023;63:9648–66.
4. Kolahdouzan M, Hamadeh MJ. The neuroprotective effects of caffeine in neurodegenerative diseases. CNS Neurosci Ther 2017;23:272–90.
5. Xiongwen L, Chen Z, Li J, Zhang L, Liu H, Huang C, et al. Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress. Inflamm Res 2010;59:635–45.
6. Modi AA, Feld JJ, Park Y, Kleiner DE, Everhart JE, Liang JT, et al. Increased caffeine consumption is associated with reduced hepatic fibrosis. Hepatology 2010;51:201–9.
7. Friedman M. Chemistry, biochemistry, and safety of acrylamide, a review. J Agric Food Chem 2003;51:4504–26.
8. Semla M, Goc Z, Martiniaková M, Omelka R, Formicki G. Acrylamide: a common food toxin related to physiological functions and health. Physiol Res 2017;66:205–17.
9. Rifai L, Saleh FA. A review on acrylamide in food: occurrence, toxicity, and mitigation strategies. Int J Toxicol 2020;39:93–102.
10. Pedreschi F, Mariotti MS, Granby K. Current issues in dietary acrylamide: formation, mitigation and risk assessment. J Sci Food Agric 2014;94:9–20.
11. Zhang L, Yang L, Luo Y, Dong L, Chen F. Acrylamide-induced hepatotoxicity through oxidative stress: mechanisms and interventions. Antioxidants Redox Signal 2023;38:1122–37.

12. Al-Hajm AYS, Ozgun E. Effects of acrylamide on protein degradation pathways in human liver-derived cells and the efficacy of N-acetylcysteine and curcumin. Drug Chem Toxicol 2022;45:1536–43.
13. Akagawa M. Protein carbonylation: molecular mechanisms, biological implications, and analytical approaches. Free Radic Res 2021;55: 307–20.
14. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 2020;21:421–38.
15. Ibrahim IM, Abdelmalek DH, Elfiky AA. GRP78: a cell's response to stress. Life Sci 2019;226:156–63.
16. Komoike Y, Matsuoka M. Endoplasmic reticulum stress-mediated neuronal apoptosis by acrylamide exposure. Toxicol Appl Pharmacol 2016;310:68–77.
17. Yan D, Wang N, Yao J, Wu X, Yuan J, Yan H, et al. Curcumin attenuates the PERK-eIF2α signaling to relieve acrylamide-induced neurotoxicity in SH-SY5Y neuroblastoma cells. Neurochem Res 2022;47:1037–48.
18. Liu Z, Lv Y, Zhao N, Guan G, Wang J. Protein kinase R-like ER kinase and its role in endoplasmic reticulum stress-decided cell fate. Cell Death Dis 2015;6:e1822.
19. Wortel IMN, van der Meer LT, Kilberg MS, van Leeuwen FN. Surviving stress: modulation of ATF4-mediated stress responses in normal and malignant cells. Trends Endocrinol Metabol 2017;28:794–806.
20. Iurlaro R, Muñoz-Pinedo C. Cell death induced by endoplasmic reticulum stress. FEBS J 2016;283:2640–52.
21. Li Y, Guo Y, Tang J, Jiang J, Chen Z. New insights into the roles of CHOP-induced apoptosis in ER stress. Acta Biochim Biophys Sin 2014;46: 629–40.
22. Hu H, Tian M, Ding C, Yu S. The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection. Front Immunol 2019;9:3083.
23. Kulkarni PB, Dorand RD. Caffeine toxicity in a neonate. Pediatrics 1979; 64:254–5.
24. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999;51:83–133.
25. de Leon J, Diaz FJ, Rogers T, Browne D, Dinsmore L, Ghosheh OH, et al. A pilot study of plasma caffeine concentrations in a US sample of smoker and nonsmoker volunteers. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:165–71.
26. Uppala JK, Gani AR, Ramaiah KVA. Chemical chaperone, TUDCA unlike PBA, mitigates protein aggregation efficiently and resists ER and non-ER stress induced HepG2 cell death. Sci Rep 2017;7:3831.
27. Kim SH, Kwon DY, Kwak JH, Lee S, Lee YH, Yun J, et al. Tunicamycin-induced ER stress is accompanied with oxidative stress via abrogation of sulfur amino acids metabolism in the liver. Int J Mol Sci 2018;19:4114.
28. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
29. Ahmadian S, Barar J, Saei AA, Fakhree MA, Omidi Y. Cellular toxicity of nanogenomedicine in MCF-7 cell line: MTT assay. J Vis Exp 2009;26:1191.
30. Lowry O, Rosebrough H, Farr NJ, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
31. Jain V, Kaiser W, Huber SC. Cytokinin inhibits the proteasome-mediated degradation of carbonylated proteins in Arabidopsis leaves. Plant Cell Physiol 2008;49:843–52.
32. Ozgun GS, Ozgun E. The cytotoxic concentration of rosmarinic acid increases MG132-induced cytotoxicity, proteasome inhibition, autophagy, cellular stresses, and apoptosis in HepG2 cells. Hum Exp Toxicol 2020;39:514–23.
33. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
34. Schneider CA, Rasband WS, Eliceiri KW. NIH image to imageJ: 25 years of image analysis. Nat Methods 2012;9:671–5.
35. Timmermann CAG, Mølck SS, Kadawathagedara M, Bjerregaard AA, Törnqvist M, Brantsæter AL, et al. A review of dietary intake of acrylamide in humans. Toxics 2021;9:155.
36. Er R, Aydın B, Şekeroğlu V, Atlı Şekeroğlu Z. Protective effect of Argan oil on mitochondrial function and oxidative stress against acrylamide-induced liver and kidney injury in rats. Biomarkers 2020;25:458–67.
37. Lee JG, Wang YS, Chou CC. Acrylamide-induced apoptosis in rat primary astrocytes and human astrocytoma cell lines. Toxicol Vitro 2014;28: 562–70.
38. Chen JH, Tsou TC, Chiu IM, Chou CC. Proliferation inhibition, DNA damage, and cell-cycle arrest of human astrocytoma cells after acrylamide exposure. Chem Res Toxicol 2010;23:1449–58.
39. Chen JH, Chou CC. Acrylamide inhibits cellular differentiation of human neuroblastoma and glioblastoma cells. Food Chem Toxicol 2015;82: 27–35.
40. Zhou L, Luo S, Wang X, Zhou Y, Zhang Y, Zhu S, et al. Blumea laciniata protected Hep G2 cells and Caenorhabditis elegans against acrylamide-induced toxicity via insulin/IGF-1 signaling pathway. Food Chem Toxicol 2021;158:112667.
41. Tan X, Zhao T, Wang Y, Wang J, Wang Z, Liu Z, et al. Acrylamide defects the expression pattern of circadian clock and mitochondrial dynamics in C57BL/6J mice liver and HepG2 cells. J Agric Food Chem 2018;66: 10252–66.
42. Bo N, Yilin H, Chaoyue Y, Lu L, Yuan Y. Acrylamide induces NLRP3 inflammasome activation via oxidative stress- and endoplasmic reticulum stress-mediated MAPK pathway in HepG2 cells. Food Chem Toxicol 2020;145:111679.
43. Komoike Y, Matsuoka M. In vitro and in vivo studies of oxidative stress responses against acrylamide toxicity in zebrafish. J Hazard Mater 2019; 365:430–9.
44. Nan B, Yang C, Li L, Ye H, Yan H, Wang M, et al. Allicin alleviated acrylamide-induced NLRP3 inflammasome activation via oxidative stress and endoplasmic reticulum stress in Kupffer cells and SD rats liver. Food Chem Toxicol 2021;148:111937.
45. Hu XW, Li XM, Wang AM, Fu YM, Zhang FJ, Zeng F, et al. Caffeine alleviates acute liver injury by inducing the expression of NEDD4L and deceasing GRP78 level via ubiquitination. Inflamm Res 2022; 71:1213–27.
46. Sayılan OG, Ozgun E, Tabakcioglu K, Suer Gokmen S, Eskiocak S, Cakır E, et al. Caffeine increases apolipoprotein A-1 and paraoxonase-1 but not paraoxonase-3 protein levels in human-derived liver (HepG2) cells. Balkan Med J 2017;34:534–9.
47. Shan L, Zhao N, Wang F, Zhai D, Liu J, Lv X, et al. Caffeine in hepatocellular carcinoma: cellular assays, animal experiments, and epidemiological investigation. J Inflamm Res 2024;17:1589–605.
48. Pasaoglu H, Ofluoglu Demir FE, Yılmaz DC, Hussein A, Pasaoglu OT. The effect of caffeine on oxidative stress in liver and heart tissues of rats. Turk J Med Sci 2011;41:665–71.
49. Amer MG, Mazen NF, Mohamed AM. Caffeine intake decreases oxidative stress and inflammatory biomarkers in experimental liver diseases induced by thioacetamide: biochemical and histological study. Int J Immunopathol Pharmacol 2017;30:13–24.
50. Li Y, Chen Y, Huang H, Shi M, Yang W, Kuang J, et al. Autophagy mediated by endoplasmic reticulum stress enhances the caffeine-induced apoptosis of hepatic stellate cells. Int J Mol Med 2017;40:1405–14.