Original Article

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The Cytoprotective Effects of *Allium cepa* Methanolic Extract in Freshly Isolated Hepatocytes

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Abstract

Acetaminophen (acetyl-para-amino phenol; APAP)-induced hepatotoxicity is the most common form of drug-induced liver injury (DILI) worldwide. APAP is also used as a model drug to assess hepatoprotective strategies against DILI. In the current study, the potential cytoprotective effects of *Allium cepa* (Onion) extract (OE) was investigated in APAP-treated hepatocytes. Isolated hepatocytes were prepared with the collagenase perfusion of rat liver. Isolated hepatocytes (10 mL, 106 cells/mL) were incubated in the Krebs Henseleit buffer (pH=7.4) in continuously rotating 50 mL round bottom flasks, under an atmosphere of carbogen (95% O2 and 5% CO2) in a 37 °C water bath. Cytotoxicity, ROS formation, and mitochondrial membrane potential collapse were assessed as oxidative stress markers. APAP administration to rat hepatocytes (500 µM) was accompanied by cytotoxicity, ROS formation, depletion of cellular glutathione (GSH) reservoirs, and mitochondrial depolarization. It was found that OE administration (100 µL) significantly reduced cell death, ROS formation, and its consequences, such as the decrease in cellular GSH and mitochondrial injury induced by APAP. These results indicate that the crude extract of Allium cepa exhibits hepatoprotective action, probably through antioxidative properties and protecting vital cellular organelles such as mitochondria.

Keywords: Allium cepa, Cytotoxicity, Hepatocytes, Mitochondrial Membrane Potential, Onion Extract.

1. Introduction

Allium cepa (Onion) is a bulbous plant widely cultivated worldwide. Onion is rich in proteins, carbohydrates, sodium, potassium, and phosphorus (1). Antibacterial, antiviral, anti-parasitic, antifungal effects have been attributed to onion (2, 3). The antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory, and antioxidant activity of onion and its derived chemicals also has been repeatedly investigated (4-6). Organosulfur chemicals are founded in the Alliaceae family (e.g., *Allium cepa*) in large quantities (7). It has been shown that organosulfurs had protective effects against xenobiotics-induced cellular damage and oxidative stress in many cases, such as carbon tetrachloride (CCl_4) (8), cyclophosphamide (9), and aflatoxin B1 (10). N-acetylcysteine (NAC), is also an organosulfur compound that is clinically administered as a stan-

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dard treatment against acetaminophen hepatotoxicity.

Acetaminophen (Acetyl-para-amino phenol; APAP) is the most frequent cause of drug-induced liver injury due to unintentional or deliberate overdose (11). APAP is also widely used as a model drug to investigate the mechanisms of drug-induced cytotoxicity and finding therapeutic strategies against drug-induced liver injury (DILI) (12-14). APAP is metabolized through cytochrome p-450 (CYP2E1) to produce the reactive metabolite, N acetyl-p-benzoquinone imine (NAPQI), which covalently binds to critical intracellular targets and consequently causes cellular injury and death (15). In the current study, APAP-induced toxicity toward isolated hepatocyte was used as an in vitro model to evaluate the potential cytoprotective effects of OE. The results could help the development of therapeutic strategies against xenobiotics-induced liver injury.

2. Material and method

2.1. Chemicals

N-acetyl cysteine (NAC), (4-(2-hydroxyethyl)1-piperazine-ethane sulfonic acid (HEPES), trichloroacetic acid (TCA), ethylene glycol-bis (ρ aminoethyl ether)-N, N, N′, N′ tetra acetic acid (EGTA), thiobarbituric acid (TBA), and trypan blue were obtained from Merck (Darmstadt, Germany). Albumin bovine type was purchased from Roche diagnostic corporation (Indianapolis USA). Rhodamine 123, acetaminophen, and collagenase enzyme from clostridium histolyticum were obtained from Sigma Aldrich (St. Louis, USA).

2.2. Hepatocytes isolation procedure

Male Sprague–Dawley rats (250-300 g) were prepared from Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Animals were housed in plastic cages on a wood-chip bedding. The ambient temperature of 23±1 ºC. Rats had free access to a normal rodents' diet (Behparvar®, Tehran, Iran) and tap water. A local ethics committee in Tabriz University of medical sciences, Tabriz, Iran, approved the animal procedures. Collagenase perfusion via portal vein was used as a method to isolate rat hepatocytes (16). This method is based on liver perfusion with collagenase after the removal of calcium ion (Ca^{2+}) with a chelator (EGTA 0.5 mM). The liver was perfused with several buffer solutions throughout the portal vein (17, 18). The isolated hepatocytes (10 mL, 10 6 cells/mL) were incubated in Krebs Henseleit buffer (pH=7.4) under carbogen atmosphere (95% O_2 and 5% CO_2), in 50 mL round bottom flasks which constantly rotating into at 37 ºC in a water bath (18-20). The expression of CYP2E1, the enzyme responsible for acetaminophen-metabolization, is low at in rat liver (21). Therefore, to accelerate acetaminophen-induced toxicity in rat hepatocytes, the CYP2E1 enzyme was induced using β-naphtoflavone (22). Briefly, CYP induced hepatocytes were prepared by pre-treating rats by β naphtoflavone (40 mg/kg, i.p, for three consecutive days) (22), then hepatocytes were isolated and used. The protective role of N-acetylcysteine as a gold-standard treatment for acetaminophen-induced hepatotoxicity (23), was studied and compared with OE supplementation.

2.3. Cell viability

Trypan blue exclusion test was used to evaluate cell death in isolated hepatocytes (24, 25). Hepatocyte viability was determined at scheduled time intervals. Briefly, 100 µL of trypan blue (0.1% w: v) was added to 1 mL of isolated hepatocytes (106 cells/mL). The percentage of dead cells (Blue nucleus) was determined using a light microscope (24, 26). Hepatocytes were at least 80% viable before their use.

2.4. Reactive oxygen species (ROS) formation

The fluorescent probe 2, 7 dichlorofluorescein diacetate (DCFH-DA) was used to assess ROS formation in isolated rat hepatocytes (27). Briefly, 10 µL of DCFDH-DA (Final concentration of 10 µM) was added to isolated hepatocytes (10^6 cells/mL) and incubated for 15 minutes. Then, the fluorescence intensity was measured using a spectrofluorometer with excitation and emission wavelengths of λ=500 and λ=520 nm, respectively (28).

2.5. Lipid peroxidation Measurement

Hepatocyte lipid peroxidation was deter-

No.	Organosulfur compound	Chemical Formula	Concentration (%)	Molecular Weight	Retention Time (min)
1	Propyl thiocyclane sulfoxide	C_5H_8OS	1.2	90	9.231
2	Dipropyl disulfide	$C_6H_{14}S_2$	0.46	150	12.706
3	2,5-Dimethyl thiophene	C_6H_8S	0.71	112	14.627
4	1,2-Dithiolane	$C_3H_6S_2$	1.13	106	23.476
5	Dipropyl trisulfide	$C_6H_{14}S_3$	0.53	182	31.84
6	Propyl propanthiosulfonate	$C_6H_{12}O_2S_2$	0.89	182	33.89
7	1-Ethynylsulfinyl propane	C_5H_8OS	0.77	118	33.372
8	Methyl thiirane	C_3H_6S	0.59	74	34.267

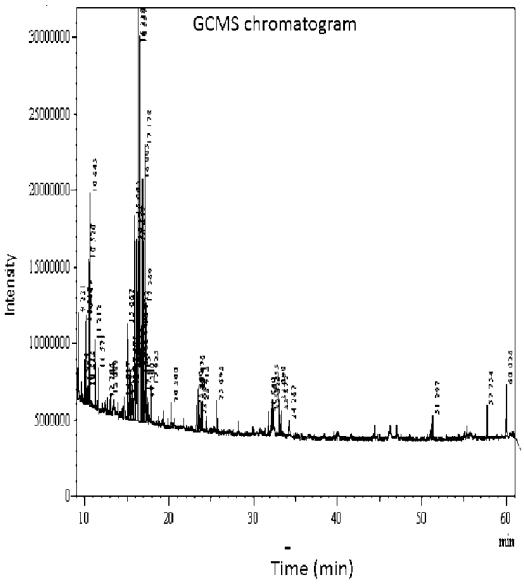

Figure 1. GC-MS spectroscopic analysis of main organosulfur compounds in the onion extract used in the current study.

mined by evaluating the amount of thiobarbituric acid reactive substances (TBARS) formed during the decomposition of lipid hydroperoxides. Briefly, 1mL aliquots of hepatocyte suspension (10^6 cells/mL) was treated with 250 µL of trichloroacetic acid (70% w/v) and 250 µL thiobarbituric acid (0.8% w/v) (29). The mixture was heated (100 ºC, 20 min). Finally, samples were centrifuged (10000 g, 15 min), and the absorbance of appeared color was determined at λ=532 nm (30, 31).

2.6. Mitochondrial membrane potential

Rhodamine 123 was used to estimate the mitochondrial depolarization (32-36). Samples (1 mL) were taken from the cell suspension at arranged time points and centrifuged (1000 rpm, 1 min). The pellet was then resuspended in 2 mL of new incubation containing 1.5 µM rhodamine 123 and gently mixed. Samples were incubated (10 min) at 37 °C. Then, hepatocytes were separated by centrifugation (3000 g, 1 min, 4 °C), and the amount of rhodamine 123 appearing in the supernatant was measured fluorometrically at λ=490 nm excitation and λ=520 nm emission wavelengths (37-39).

2.7. Onion extract preparation

Raw onion (*Allium cepa* L.) was purchased from a retail food store (Tabriz, Iran) and identified by botanists in the herbarium of Tabriz University. Onion bulbs were peeled, weighed, and ground to obtain the juice. Samples were homogenized in methanol (10 mL methanol: 1 g Onion), and the homogenized mixture was filtered. A rotary evaporator was used to remove the methanol content. Afterward, samples were freeze-dried and stored at -70 °C until use. On the day of the experiment, 100 mg of OE was dissolved in 10 ml of the incubation buffer, and 100 µL of the prepared solution was added to isolated hepatocytes. The organosulfur content of OE was characterized by a GC-MS method (Figure1).

2.8. Statistical analysis

Results are given as the mean±SEM for at least three independent experiments. A one-way analysis of variance (ANOVA; followed by Tukey's post hoc test) was used to compare the obtained data. A $P<0.05$ was considered a statistically significant difference.

3. Results

APAP-induced cytotoxicity was evaluated using the trypan blue exclusion test (Table 1). It was found that APAP (500µM) significantly caused cell death at different time intervals (Table 1). On the other hand, OE administration (100 µL) significantly decreased APA-induced cell death

Table 1. Preventing Acetaminophen (APAP)-induced cell death by different concentrations of onion extract (OE).

	Cytotoxicity (% Trypan blue uptake)		
Incubation time (min):	60	120	180
Control (only hepatocytes)	19±1	22±1	27±2
+OE 100µL	22±2	24±2	29±2
+APAP 500 µM	52±4*	84±6*	91±6*
+OE 100 µL	22±3[a]	25±4[a]	31±5[a]
+NAC 100 µM	19±2[a]	26±4[a]	30±4[a]

Isolated rat hepatocytes (106 cells/mL) were incubated at 37 °C in continuously rotating round bottom flasks under carbogen (95 % O_2 and 5 % CO_2) atmosphere in Krebs-Henseleit buffer (pH=7.4). NAC: N-acetyl cysteine. The results shown represent the mean± SE for three independent experiments. *Significantly different from the control group (P<0.05). [a]Significantly different from the APAP-treated group (P<0.05).

(Table 1). NAC (100 µM) treatment also significantly decreased APAP cytotoxicity in isolated rat hepatocytes (Table 1). There was no significant difference between the cytoprotective effects of OE and NAC, as judged by the trypan blue exclusion test (Table 1).

APAP administration (500µM) also caused significant ROS formation in isolated rat hepatocytes. OE treatment alleviated APAP-induced ROS formation (Figure 2). The effects of NAC on APAP-induced ROS formation (Figure 2). On the other hand, there was no significant difference between OE and NAC in preventing ROS formation in APAP-treated hepatocytes (Figure 2).

The TBARS level as an index of lipid peroxidation was significantly higher in APAP-treated rat hepatocytes (Figure 3). It was found that OE and/or NAC treatment significantly mitigated the level of lipid peroxidation in the APAP-treated group (Figure 4). No significant difference between hepatocytes TBARS levels was found between NAC and OE-treated groups (Figure 4).

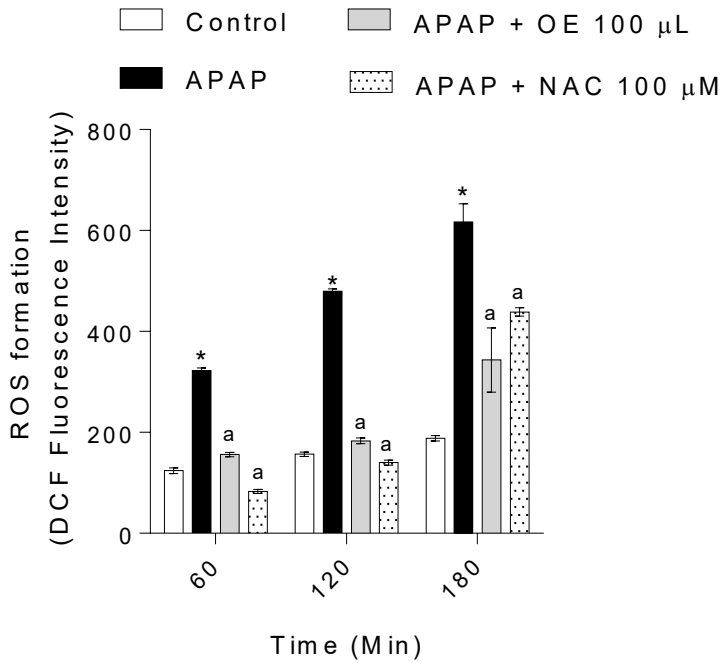

Figure 2. Acetaminophen (APAP)-induced reactive oxygen species (ROS) formation and the role of onion extract (OE) administration. NAC: N-acetyl cysteine. Data are given as mean±SEM for three independent experiments. *Indicates a significant difference as compared with the control hepatocytes (P<0.05).
[a]Indicates significant difference as compared with the acetaminophen-treated cells (P<0.05).

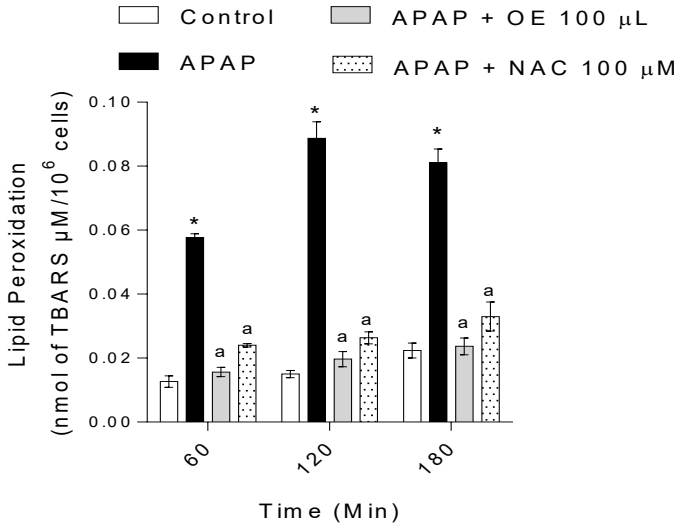

Figure 3. Acetaminophen (APAP)-induced lipid peroxidation in isolated rat hepatocytes. OE: Onion extract, NAC: N-acetyl cysteine. Data are represented as mean±SEM for three independent experiments. *Indicates a significant difference as compared with control hepatocytes (P<0.05). [a]Indicates significant difference as compared with acetaminophen-treated cells (P<0.05).

It was found that the cellular glutathione reservoir (GSH) was decreased after APAP administration (Figure 4). OE significantly prevented cellular GSH depletion induced by APAP (Figure 4). NAC treatment also significantly preserved GSH levels in APAP-treated hepatocytes (Figure 4). There was no significant difference between NAC and OE when their effects on hepatocytes GSH content were compared (Figure 4).

The effect of APAP on cellular mitochondria and the role of OE administration was studied. It was found that APAP caused mitochondrial membrane potential (ΔΨm) collapse (Figure 5). APAP-induced mitochondrial depolarization was significantly alleviated by OE (100 µL) and/or NAC (100 µM) administration (Figure 5). No significant difference was found between the effects of NAC and OE on APAP-induced mitochondrial depolarization in isolated rat hepatocytes (Figure 5).

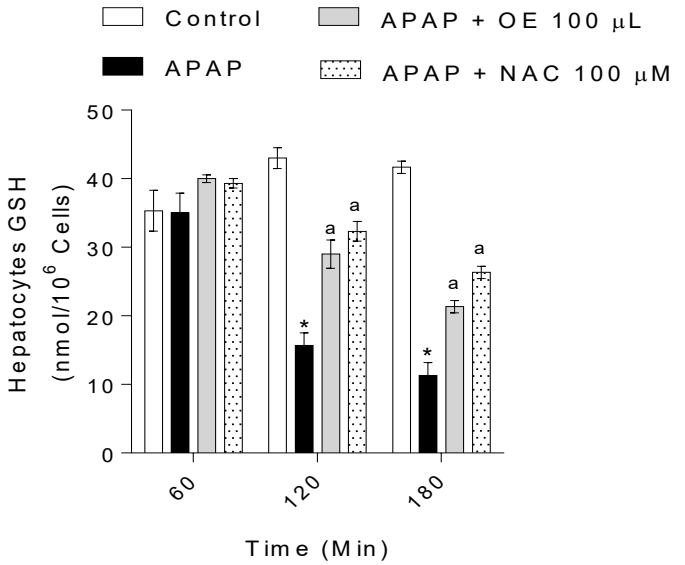

Figure 4. Cellular glutathione levels in acetaminophen (APAP)-treated hepatocytes. OE: Onion Extract, NAC: N-acetyl cysteine. Data are represented as Mean±SEM for at least three separate experiments. *Indicates a significant difference as compared with control hepatocytes (P<0.05). [a]Indicates significant difference as compared with acetaminophen-treated cells (P<0.05).

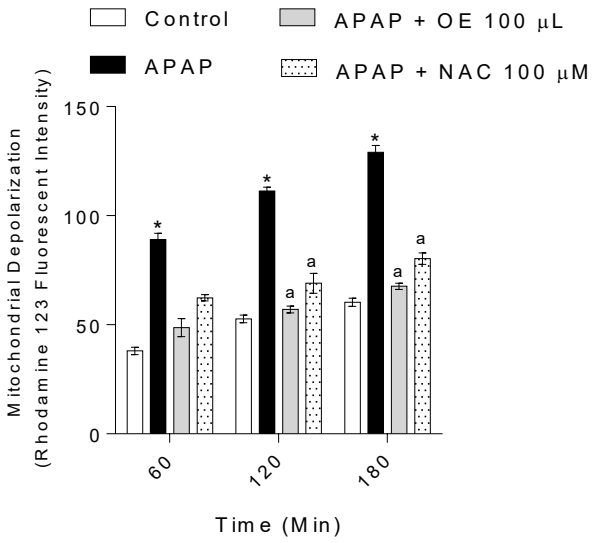

Figure 5. Effect of acetaminophen (APAP) on hepatocytes, mitochondrial depolarization, and the role of onion extract (OE) administration. NAC: N-acetyl cysteine. Data are represented as mean±SEM for at least three separate experiments. *Indicates a significant difference as compared with control hepatocytes (P<0.05). a Indicates significant difference as compared with acetaminophen-treated cells (P<0.05).

4. Discussion

Hepatocytes are continuously exposed to a wide range of xenobiotics. Several chemicals with hepatotoxic properties have been identified (40-42). Therefore, finding compounds with hepatoprotective properties has great clinical value. In the current study, it was found that the OE administration tended to suppress APAP-induced cytotoxicity as judged by lower cell death, ROS formation, increased GSH content, and improved mitochondrial function.

Different extracts from alliacea family have been shown to exhibit a wide range of biological activities, including potent antioxidant properties (5, 43). Most of the researches performed on OE is to determining the bioactive constituents of this extract (43). Many organosulfur compounds are derived from OE in different previous investigations (44, 45). These active ingredients exhibit antioxidative and protective properties in different studies (44, 45) (Figure 1). Oxidative stress and its consequences are the most critical cause of xenobiotics-induced hepatotoxicity, including different drugs (46). Oxidative stress affects a vast range of intracellular targets, including lipids, proteins, cellular mitochondria, and DNA (47). In the current study, APAP administration was accompanied by a significant ROS formation and lipid peroxidation. OE decreased the ROS formation and lipid peroxidation induced by acetaminophen (Figures 2 and 3). The role of onion extract in attenuating oxidative stress might be due to the role of organosulfur compounds in chelating metal ions (45) and/or scavenging reactive species (48). Decreased cellular GSH content (Figure 3) endorses the occurrence of oxidative stress in rat hepatocytes. As shown in Figure 4, the OE administration prevented GSH consumption by APAP. Administration of NAC (100 µM) also increased hepatocytes GSH reservoirs (Figure 4). Based on these data, the antioxidant properties of OE could play a pivotal role in its cytoprotective mechanism.

It has been repeatedly mentioned that APAP could induce mitochondrial injury and respiratory chain dysfunction (49). Mitochondria have a significant role in maintaining cellular homeostasis and play an essential role in xenobiotics-induced cell death, especially in oxidative stress conditions (50). On the other hand, cellular mitochondria are significant sources of ROS (51). Hence, xenobiotics-induced mitochondrial impairment could play a significant role in elevated ROS levels and oxidative stress. In this study, it was found that the mitochondrial membrane potential ($\Delta\Psi m$), as a critical indicator of mitochondrial function, was collapsed in APAP-treated hepato-

cytes (Figure 5). As onion extract administration diminished mitochondrial injury caused by APAP (Figure 5), protecting subcellular organelles could be another mechanism involved in the protective properties of OE. It is noteworthy to mention that the cytoprotective properties of OE and NAC were not significantly different in the current model. These data mention the significant cytoprotective role of OE and its organosulfur compounds.

The data collected in this study indicate significant cytoprotective properties of OE. On the other hand, further studies are required to determine the exact hepatoprotective agent(s) and the hepatoprotective mechanism(s) of OE. Moreover, different *in vitro* and *in vivo* experiments could be carried out in the future on the hepatoprotective effects of OE, especially when the occurrence of oxidative stress is suspected.

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Conflict of Interest

None declared.

5. References

1. Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK, et al. Traditional and modern uses of onion bulb (Allium cepa L.): a systematic review. *Crit Rev Food Sci Nutr.* 2019;59(sup1):S39-S70. doi: 10.1080/10408398.2018.1499074.

2. Santas J, Almajano MP, Carbó R. Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. *Int J Food Sci Tech*. 2010;45;403-409. doi: 10.1111/j.1365-2621.2009.02169.x.

3. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). *Food Sci Technol.* 2004;37;263-268. doi: 10.1016/j.lwt.2003.09.001.

4. Kumari K, Mathew BC, Augusti KT. Antidiabetic and hypolipidemic effects of S-methyl cysteine sulfoxide isolated from Allium cepa Linn. *Indian J Biochem Biophys.* 1995 Feb;32(1):49-54.

5. Nuutila AM, Puupponen-Pimiä R, Aarni M, Oksman-Caldentey K-M. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* 2003;81;485-493. doi: 10.1016/S0308-8146(02)00476-4.

6. Obioha UE, Suru SM, Ola-Mudathir KF, Faremi TY. Hepatoprotective potentials of onion and garlic extracts on cadmium-induced oxidative damage in rats. *Biol Trace Elem Res.* 2009 Summer;129(1-3):143-56. doi: 10.1007/s12011-008-8276-7.

7. Bianchini F, Vainio H. Allium vegetables and organosulfur compounds: do they help prevent cancer? *Environ Health Perspect.* 2001 Sep;109(9):893-902.

8. Fanelli SL, Castro GD, De Toranzo EG, Castro JA, others. Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide; diallyl disulfide; allyl mercaptan and allyl methyl sulfide. *Res Commun Mol Pathol* Pharmacol. 1998 Nov;102(2):163-74.

9. Habs M, Hebebrand J, Schmähl D. Influence of sulfur-containing compounds on the acute toxicity of cyclophosphamide in male Sprague-Dawley rats. *Arzneimittelforschung.* 1984;34(7):792-3.

10. Guyonnet D, Belloir C, Suschetet M, Siess M-H, Le Bon A-M. Mechanisms of protection against aflatoxin B1 genotoxicity in rats treated by organosulfur compounds from garlic. *Carcinogenesis*. 2002 Aug;23(8):1335-41.

11. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med*. 2002 Dec 17;137(12):947-54.

12. Terblanche J, Hickman R. Animal models of fulminant hepatic failure. *Dig Dis Sci.* 1991 Jun;36(6):770-4.

13. Newsome PN, Plevris JN, Nelson LJ, Hayes PC. Animal models of fulminant hepatic failure: A critical evaluation. *Liver Transpl.* 2000 Jan;6(1):21-31.

14. Jaeschke H, Williams CD, McGill MR, Xie Y, Ramachandran A. Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. *Food Chem Toxicol.* 2013 May;55:279-89. doi: 10.1016/j.fct.2012.12.063.

15. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev*. 2012 Feb;44(1):88-106. doi: 10.3109/03602532.2011.602688. Epub 2012 Jan 10.
16. Heidari R, Babaei H, Eghbal M. Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. *Drug Chem Toxicol.* 2013 Oct;36(4):403-11. doi: 10.3109/01480545.2012.749272. Epub 2012 Dec 21.
17. Ommati MM, Farshad O, Mousavi K, Jamshidzadeh A, Azmoon M, Heidari S, et al. Betaine supplementation mitigates intestinal damage and decreases serum bacterial endotoxin in cirrhotic rats. *PharmaNutrition*. 2020;12;In Press. doi: 10.1016/j.phanu.2020.100179
18. Heidari R, Babaei H, Eghbal MA. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. *Res Pharm Sci.* 2014 Mar-Apr;9(2):97-105.
19. Heidari R, Babaei H, Eghbal MA. Cytoprotective Effects of Organosulfur Compounds against Methimazole Induced Toxicity in Isolated Rat Hepatocytes. *Adv Pharm Bull.* 2013;3(1):135-42. doi: 10.5681/apb.2013.023. Epub 2013 Feb 7.
20. Heidari R, Babaei H, Eghbal MA. Cytoprotective effects of taurine against toxicity induced by isoniazid and hydrazine in isolated rat hepatocytes. *Arh Hig Rada Toksikol.* 2013 Jun;64(2):15-24. doi: 10.2478/10004-1254-64-2013-2297.
21. Elbarbry FA, McNamara PJ, Alcorn J. Ontogeny of hepatic CYP1A2 and CYP2E1 expression in rat. *J Biochem Mol Toxicol.* 2007;21(1):41-50.
22. Madan A, Graham RA, Carroll KM, Mudra DR, Burton LA, Krueger LA, et al. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab Dispos.* 2003 Apr;31(4):421-31.
23. Smilkstein MJ, Bronstein AC, Linden C, Augenstein WL, Kulig KW, Rumack BH. Acetaminophen overdose: a 48-hour intravenous N-acetylcysteine treatment protocol. *Ann Emerg Med.* 1991 Oct;20(10):1058-63.
24. Heidari R, Babaei H, Eghbal MA. Ameliorative effects of taurine against methimazole-induced cytotoxicity in isolated rat hepatocytes. *Sci Pharm.* 2012 Oct-Dec;80(4):987-99. doi: 10.3797/scipharm.1205-16. Epub 2012 Aug 6.
25. Niknahad H, Khan S, O'Brien PJ. Hepatocyte injury resulting from the inhibition of mitochondrial respiration at low oxygen concentrations involves reductive stress and oxygen activation. *Chem Biol Interact.* 1995 Oct 20;98(1):27-44.
26. Moridani MY, Scobie H, Jamshidzadeh A, Salehi P, O'Brien PJ. Caffeic acid, chlorogenic acid, and dihydrocaffeic acid metabolism: Glutathione conjugate formation. *Drug Metab Dispos.* 2001 Nov;29(11):1432-9.
27. Ahmadian E, Babaei H, Mohajjel Nayebi A, Eftekhari A, Eghbal MA. Mechanistic approach for toxic effects of bupropion in primary rat hepatocytes. *Drug Res (Stuttg)*. 2017 Apr;67(4):217-222. doi: 10.1055/s-0042-123034. Epub 2017 Jan 24.
28. Seifi K, Rezaei M, Yansari AT, Zamiri MJ, Riazi GH, Heidari R. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. *J Therm Biol.* 2020;98; In Press. doi: 10.1016/j.jtherbio.2020.102520.
29. Heidari R, Niknahad H. The role and study of mitochondrial impairment and oxidative stress in cholestasis. In: Vinken M, editor. Experimental Cholestasis Research. *Methods Mol Biol.* 2019;1981:117-132. doi: 10.1007/978-1-4939-9420-5_8.
30. Heidari R, Babaei H, Eghbal MA. Cytoprotective Effects of Organosulfur Compounds against Methimazole-Induced Toxicity in Isolated Rat Hepatocytes. *Adv Pharm Bull.* 2013;3(1):135-42. doi: 10.5681/apb.2013.023. Epub 2013 Feb 7.
31. Eghbal MA, Tafazoli S, Pennefather P, O'Brien PJ. Peroxidase catalysed formation of cytotoxic prooxidant phenothiazine free radicals at physiological pH. *Chem Biol Interact*. 2004 Dec 30;151(1):43-51.
32. Abdoli N, Heidari R, Azarmi Y, Eghbal MA. Mechanisms of the Statins Cytotoxicity in Freshly Isolated Rat Hepatocytes. *J Biochem Mol Toxicol.* 2013 Jun;27(6):287-94. doi: 10.1002/jbt.21485. Epub 2013 Apr 23.
33. Heidari R, Behnamrad S, Khodami Z, Ommati MM, Azarpira N, Vazin A. The nephroprotective properties of taurine in colistin-treated mice is mediated through the regulation of mito-

chondrial function and mitigation of oxidative stress. *Biomed Pharmacother.* 2019 Jan;109:103-111. doi: 10.1016/j.biopha.2018.10.093. Epub 2018 Nov 2.
34. Akram J, Hossein N, Reza H, Maryam A, Forouzan K, Mohammad Reza A, et al. Propylthiouracil-induced mitochondrial dysfunction in liver and its relevance to drug-induced hepatotoxicity. *Pharm Sci.* 2017;23;95-102. doi: 10.15171/PS.2017.15.
35. Seifi K, Rezaei M, Yansari AT, Riazi GH, Zamiri MJ, Heidari R. Saturated fatty acids may ameliorate environmental heat stress in broiler birds by affecting mitochondrial energetics and related genes. *J Therm Biol.* 2018 Dec;78:1-9. doi: 10.1016/j.jtherbio.2018.08.018. Epub 2018 Aug 29.
36. Ommati MM, Heidari R, Manthari RK, Tikka Chiranjeevi S, Niu R, Sun Z, et al. Paternal exposure to arsenic resulted in oxidative stress, autophagy, and mitochondrial impairments in the HPG axis of pubertal male offspring. *Chemosphere.* 2019 Dec;236:124325. doi: 10.1016/j.chemosphere.2019.07.056. Epub 2019 Jul 15.
37. Abdoli N, Heidari R, Azarmi Y, Eghbal MA. Mechanisms of the Statins Cytotoxicity in Freshly Isolated Rat Hepatocytes. J Biochem Mol Toxicol. 2013 Jun;27(6):287-94. doi: 10.1002/jbt.21485. Epub 2013 Apr 23.
38. Eghbal MA, Pennefather PS, O'Brien PJ. H2S cytotoxicity mechanism involves reactive oxygen species formation and mitochondrial depolarisation. *Toxicology.* 2004 Oct 15;203(1-3):69-76.
39. Ommati MM, Farshad O, Niknahad H, Arabnezhad MR, Azarpira N, Mohammadi HR, et al. Cholestasis-associated reproductive toxicity in male and female rats: The fundamental role of mitochondrial impairment and oxidative stress. *Toxicol Lett.* 2019 Nov;316:60-72. doi: 10.1016/j.toxlet.2019.09.009. Epub 2019 Sep 11.
40. Srivastava A, Maggs JL, Antoine DJ, Williams DP, Smith DA, Park BK. Role of reactive metabolites in drug-induced hepatotoxicity. *Handb Exp Pharmacol.* 2010;(196):165-94. doi: 10.1007/978-3-642-00663-0_7.
41. Heidari R, Niknahad H, Jamshidzadeh A, Eghbal MA, Abdoli N. An overview on the proposed mechanisms of antithyroid drugs-induced liver injury. *Adv Pharm Bull.* 2015 Mar;5(1):1-11. doi: 10.5681/apb.2015.001. Epub 2015 Mar 5.
42. Heidari R, Niknahad H, Jamshidzadeh A, Abdoli N. Factors affecting drug-induced liver injury: antithyroid drugs as instances. *Clin Mol Hepatol.* 2014 Sep;20(3):237-48. doi: 10.3350/cmh.2014.20.3.237. Epub 2014 Sep 25.
43. Block E. The chemistry of garlic and onions. *Sci Am.* 1985 Mar;252(3):114-9.
44. García A, Haza AI, Arranz N, Delgado ME, Rafter J, Morales P. Organosulfur compounds alone or in combination with vitamin C protect towards N-nitrosopiperidine- and N-nitrosodibutylamine-induced oxidative DNA damage in HepG2 cells. *Chem Biol Interact.* 2008 May 9;173(1):9-18. doi: 10.1016/j.cbi.2008.01.011. Epub 2008 Feb 5.
45. Yin M-c, Hwang S-w, Chan K-c. Nonenzymatic Antioxidant Activity of Four Organosulfur Compounds Derived from Garlic. *J Agric Food Chem.* 2002 Oct 9;50(21):6143-7.
46. Amin A, Hamza AA. Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation. *Toxicology*. 2005 Mar 30;208(3):367-75.
47. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of Hepatotoxicity. *Toxicol Sci.* 2002 Feb;65(2):166-76.
48. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr.* 2001 Mar;131(3s):1010S-5S. doi: 10.1093/jn/131.3.1010S.
49. Saito C, Zwingmann C, Jaeschke H. Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology.* 2010 Jan;51(1):246-54. doi: 10.1002/hep.23267.
50. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev.* 2007 Jan;87(1):99-163.
51. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu S-S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol.* 2004 Oct;287(4):C817-33.