Journal of Pharmacopuncture

ISSN 2093-6966 [Print], ISSN 2234-6856 [Online]
Journal of Pharmacopuncture 2018;21[4]:249-257
DOI: https://doi.org/10.3831/KPI.2018.21.028

Original article

The Preventive Effects of Standardized Extract of *Zataria multiflora* and Carvacrol on Acetaminophen-Induced Hepatotoxicity in Rat

- *Zataria multiflora* and Carvacrol and Hepatotoxicity -

Reza Mohebbati[1], Maryam Paseban[1], Farimah Beheshti[1], Mohammad Soukhtanloo[2], Mohammad Naser Shafei[3], Hasan Rakhshandeh[4], Abolfazl Khajavi Rad[1,3]*

[1] Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran

Key Words

acetaminophen, *Zataria multiflora*, carvacrol, hepatotoxicity

Abstract

Objectives: The hepatotoxicity induced by Acetaminophen (AAP) mostly mediated by effect on oxidative stress parameters. The *Zataria multiflora* (Z.M) is an herbal medicine with well-known antioxidant effect. The aim of this study is investigation of preventive effects of Z.M and Carvacrol (CAR) on AAP-induced hepatotoxicity in rats.

Methods: Rats were randomly divided into four groups including: 1) Control, 2) Acetaminophen (AAP), 3) and 4) CAR. The saline, Z.M (200 mg/kg) and CAR (20 mg/kg) were administrated orally for 6 days, after that AAP (600 mg/kg) was administrated in the 7th day. Blood sampling was performed on the first and last days. Also, the liver tissue was removed for evaluation of Malondyaldehide (MDA), Thiol content, Superoxide dismutase (SOD) and Catalase (CAT). Total Protein (tPro), Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alkaline Phosphatase (ALP) in liver tissue were evaluated. The changes (Δ) of enzymes activities were presented.

Results: The Δ GOT, Δ GPT and Δ ALP in CAR group significantly decreased compared to AAP group ($P < 0.01$ to $P < 0.001$) and Δ GPT in Z.M group was significantly reduced in comparison with AAP group ($P < 0.05$). Also, MDA, Thiol, SOD and CAT levels in treated groups were attenuated compared to AAP group ($P < 0.05$ to $P < 0.001$).

Conclusion: Z.M and CAR have a powerful hepatoprotective effect. CAR is more effective than Z.M. Based on the results. Z.M and CAR could be potent supplementary agents against hepatotoxicity of AAP in patients.

Received: Oct 11, 2018 **Reviewed:** Oct 19, 2018 **Accepted:** Nov 14, 2018

1. Introduction

The liver has an important role in the nutrients metabolism such as proteins, carbohydrates and lipids, and also detoxification of powerful toxic drugs and metabolites, chemicals and environmental pathogens [1]. Drugs and alcohol is one of the most important causes of liver diseases [2]. One of this drugs is Acetaminophen (AAP) (Figure 1) which is commonly used as an antipyretic and analgesic drug for many years,

*Corresponding Author
Abolfazl Khajavi Rad. Associate Professor of Physiology, Department of Physiology, Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad 917794-8564, Iran.
Tel: +98-513-882-8565 Fax: +98-513-882-8564
E-mail: khajavirada@mums.ac.ir

has several side effect especially hepatotoxicity. Epidemiologically, in the USA 400 deaths each year occurs [3] due to AAP usage. Therefore, AAP is now the second leading cause of toxic drug ingestions in the USA [4]. Overdose of this drug in many countries is common [5]. Pharmacologically, AAP is metabolized to N-acetyl-p-benzoquinone imine (NAPQI) via cytochrome P450 enzymes in the liver that is generally produced in little amounts at therapeutic doses of this drug but in overdosing, production of NAPQI vigorously increased [6]. Glutathione depletion is reason of severe damages to the liver when it conjugates with NAPQI. Depletion of glutathione is the main reason of hepatotoxicity and necrosis [7]. The metabolic pathway of AAP is shown in Figure 2. Hepatotoxicity induced by AAP is common in people with alcohol abuse, unintentional overdoses and underlying hepatic disease [8] and N-acetylcysteine treatment was used in clinic for AAP overdose cases [9].

Zataria multiflora (Z.M) which is also known as Avishan Shirazi in Iran, is one of the important herbal plants belonging to the Lamiaceae family [10]. This plant has several components such as multiflorol, multiflotriol, aromatic ester of *p*-hydroxy benzoic acid, dihydroxyaromadendrane, luteolin and α-tocopherolquinone [11]. In addition, the essential oils such as Carvacrol (CAR) (52%), thymol (16%) and p-cymene (10%) are more effective substances of [12, 13]. CAR (2-methyl-5-(1-methylethyl)-phenol) (Figure 3) is a dominant monoterpenic phenol that is present in many essential oils of the Labiatae family including *Zataria* and *Thymus* species [14]. CAR is the effective ingredient that has significant effects on the systemic anti-oxidant and tissue inflammation [12, 15]. Also, the therapeutic effects of Z.M and CAR include antinociceptive [16], antiulcer [17], antimicrobial [18], antiasthma [19, 20], improvement of the chronic obstructive pulmonary disease (COPD) [19, 21], anti-proliferative in cancers [22], anti-diabetic [23], antidepressant [24], etc. Some features of this plant such as antioxidant [25, 26] and anti-inflammatory [27] effects have already been studied. The aim of this study is to investigate the preventive effects of *Zataria multiflora* and Carvacrol and comparative effect of them on AAP-induced hepatotoxicity in rats.

2. Material and Methods

2.1. *Plant material and preparation of the extract*

Zataria multiflora was purchased from local herbal shop in Mashhad, Khorasan province, Iran and identified by botanists in the herbarium of Ferdowsi University of Mashhad (ID:35314).

The aerial parts of *Zataria multiflora* (100 gr) were cleaned, dried, ground, weighed, and homogenized in 70% ethanol at a ratio of 1:10 of plant to ethanol and macerated for 3 days at 37°C with occasional shaking. The mixture was then filtered and the resulting liquid was concentrated under decreased pressure at 45°C in an EYELA rotary evaporator. The concentrated extract was then kept in the incubator at 45°C for 3 days to evaporate the ethanol residue yielding the crude extract. Finally, the extract was dissolved in saline before being gavaged to animals.

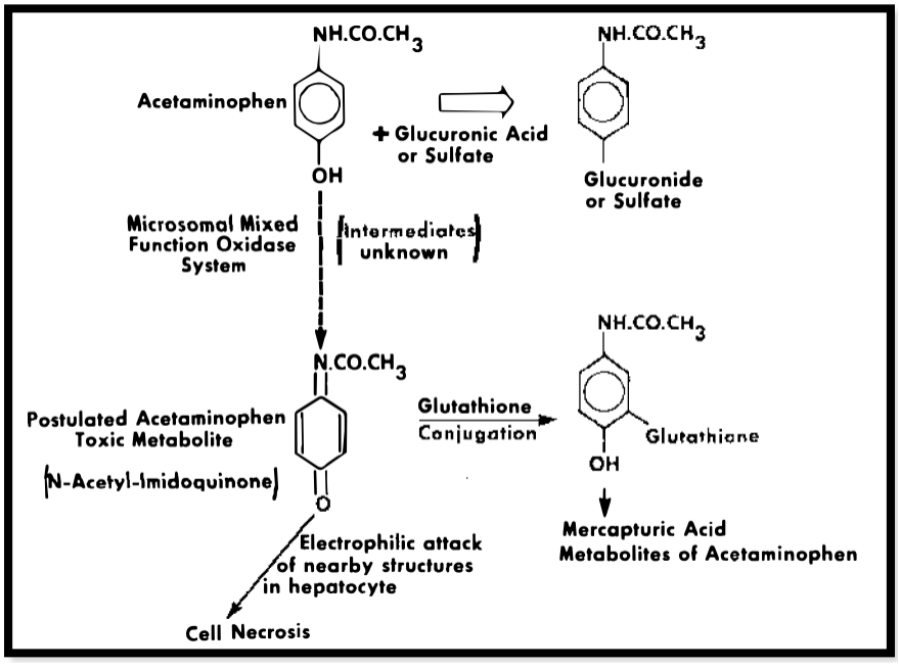

Figure 2 Metabolic and hepatotoxicity pathways of AAP [28]

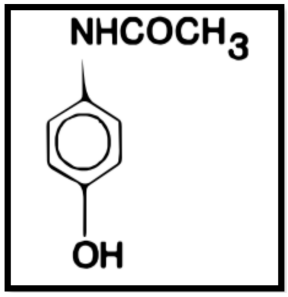

Figure 1 Acetaminophen structure [28]

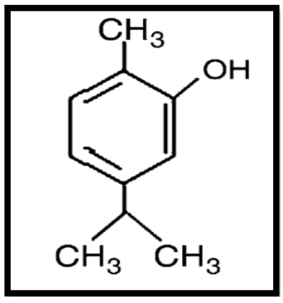

Figure 3 Carvacrol structure [29]

2.2. *Extract standardization*

A The polyphenols content of the ZM extract evaluated based on the Folin–Ciocalteu method in which ZM extract has 37.1 mg gallic acid/g crude extract. Also, the flavonoids content of the ZM extract estimated based on the aluminum chloride colorimetric assay in which ZM extract has 13.7 mg quercetin/g crude extract. Finally, the anthocyanin content of the ZM extract evaluated based on the pH-differential method described by Rodriguez-Saona in which ZM extract has 1.9 mg/g crude extract. The line equations for standard curves of gallic acid and quercetin were Y = 0.0669X + 0.0116 and Y = 0.06632X - 0.01448, respectively.

2.3. *Chemicals and drugs*

The ether and CAR were purchased from Merck and Sigma companies. AAP was obtained from Tehran Daru, Iran. Also, biochemical assay kits were provided by Pars Azmoun, Tehran, Iran.

2.4. *Animals and treatment*

Twenty four male Wistar rats (220 - 240 gr) were housed in a room with standard condition and free access to food and water. The ethical code for current study is IR.MUMS.fm.REC.1396.581.

Rats were randomly divided into four groups including: Control (Co), Acetaminophen (AAP), Z.M and CAR. The saline, Z.M (200 mg/kg) [30] and CAR (20 mg/kg) [31] were administrated orally by gavage for 6 days in different groups, after that AAP (600 mg/kg) [32] was administrated orally by gavage in the 7th day in all groups except Co group. The rats were weighed on the first and last days. Blood samples were collected from the orbital sinus on the first and at the end of the experiment. Also, the liver tissue samples were removed for oxidative markers evaluation.

2.5. *Biochemical assessment*

Blood samples were used to determine the levels of some enzymes including total Protein (tPro), Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Alkaline Phosphatase (ALP) with the relevant kits. Finally, the changes (Δ) of enzymes activities were presented.

Total thiol contents were evaluated using DTNB (2, 2'-dinitro- 5, 5'-dithiodibenzoic acid), a reagent which reacts with the SH groups and produces a yellow colored complex that has a peak absorbance at 412 nm [33]. Generally, 1 ml Tris-EDTA buffer (pH=8.6) was added to 50 µl liver homogenate in 1 ml cuvettes and the absorbance was read at 412 nm versus Tris-EDTA buffer (A1). Then 20 µl DTNB reagents (10 mM in methanol) were added to the mixture and after 15 minutes incubation in room temperature, the absorbance was read again (A2). Also, the absorbance of DTNB reagent was read as a blank (B). A total thiol content (mM) was calculated based on an equation described by Hosseini et al [34, 35].

Malondialdehyde (MDA) concentrations as an index of lipid peroxidation were evaluated in the liver tissue. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) and produces a red colored complex which has a peak absorbance at 535 nm. Briefly, 2 ml TBA/ trichlorooacetic acid (TCA)/ hydrochloric acid (HCL) reagent was added to 1 ml homogenate and the solution was incubated in a boiling water bath for 40 minutes. After cooling, the whole solutions were centrifuged at 1000 g for 10 minutes. The absorbance of supernatant was evaluated at 535 nm. The MDA concentration (C) was calculated as follows [34, 35].

$$C\,(m) = \text{Absorbance} / (1.65 \times 10^5)$$

Catalase (CAT) activity was estimated using the Aebi method [36]. The principle of the assay is based on determination of the rate constant, k, (dimension: s-1, k) of hydrogen peroxide decomposition. By measuring the reduction in the absorbance at 240 nm per minute, the rate constant of the enzyme was evaluated. Activities were expressed as k (rate constant) per liter.

Superoxide dismutase (SOD) activity was evaluated by the procedure explained by Madesh and Balasubramanian [37]. A colorimetric assay involving production of superoxide by pyrogallol auto-oxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to its formazan by SOD was determined at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

2.6. *Data analysis*

The data were expressed as mean ± SEM. The maximal changes (Δ) of data calculated by last data-first data, then Statistical comparisons were done by one-way ANOVA followed by the Tukey's post hoc test. A value of $P < 0.05$ was used to indicate statistical significance.

3. Results

The results showed that ΔGOT, ΔGPT and ΔALP in AAP group significantly increased compared to Co group ($P < 0.05$ to $P < 0.01$; Figure 4) while the tPro did not have any significant effects. Also, the results indicated that ΔGOT, ΔGPT and ΔALP in CAR group significantly decreased compared to AAP group ($P < 0.01$ to $P < 0.001$; Figures 5,6 and 7). The ΔGPT in Z.M group was significantly reduced in comparison with AAP group ($P < 0.05$; Figure 6). The ΔtPro did not have any difference between four groups (Figure 8).

In the liver tissue, MDA level in AAP group was significantly enhanced compared to Co group, ($P < 0.05$). In CAR group, level of MDA was significantly reduced compared to AAP group, ($P < 0.01$) (Figure 9).

Level of Thiol in AAP group was reduced compared to Co group ($P < 0.001$). Thiol level in ZM group significantly increased compared to AAP group ($P < 0.05$) (Figure 10).

The CAT activity in liver of AAP group was not significantly lower than Co group and increased in CAR group in comparison with AAP group ($P < 0.01$) (Figure 11).

Also, the results showed that SOD level in AAP group compared to Co group was significantly decreased ($P < 0.05$) while this level in ZM and CAR groups significantly increased ($P < 0.001$) (Figure 12). Also, significant decrease of MDA concentration ($P < 0.05$) and significant increase of CAT activity ($P < 0.001$) in Z.M group compared to the AAP group was observed.

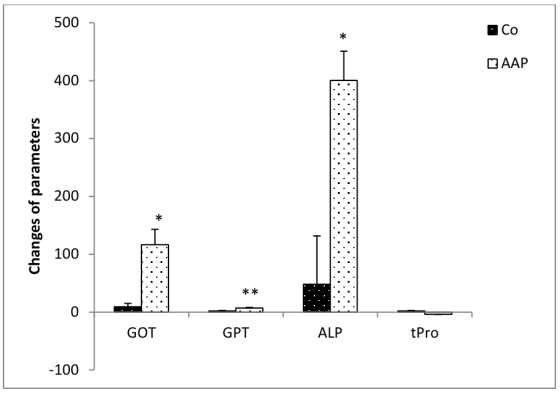

Figure 4 Changes (Δ) of different parameters induced by AAP group in comparison with Co group (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
*:$P < 0.05$, **:$P < 0.01$, compared to Co group.
Co: Control, AAP: Acetaminophen, GOT: Glutamic Oxaloacetic Transaminase, GPT: Glutamic Pyruvic Transaminase, ALP: Alkaline Phosphatase, tPro: total Protein

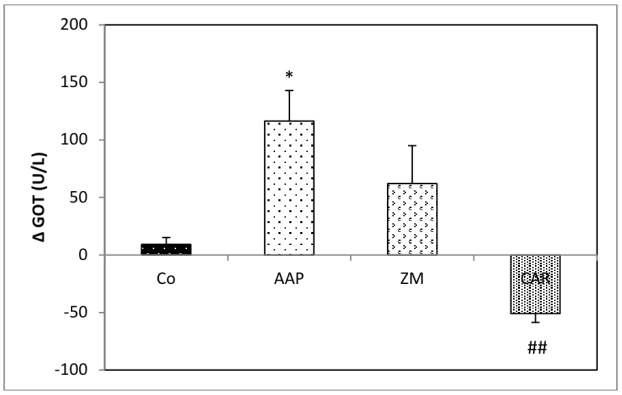

Figure 5 Comparison the changes (Δ) of Glutamic Oxaloacetic Transaminase (GOT) in four experimental groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
*:$P < 0.05$, compared to Co group
##:$P < 0.01$, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

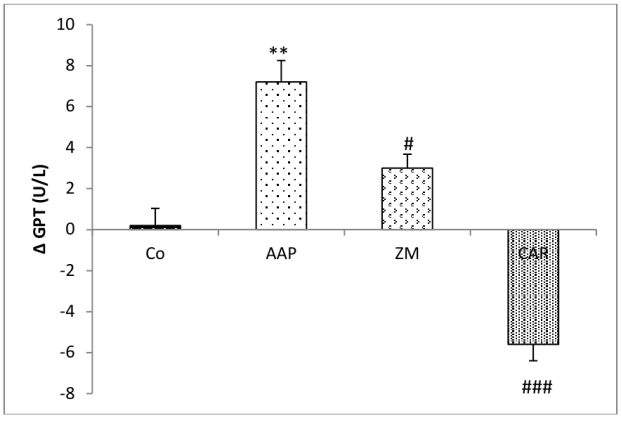

Figure 6 Comparison the changes (Δ) of Glutamic Pyruvic Transaminase (GPT) between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
**:$P < 0.01$, compared to Co group
#:$P < 0.05$, ###:$P < 0.001$, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

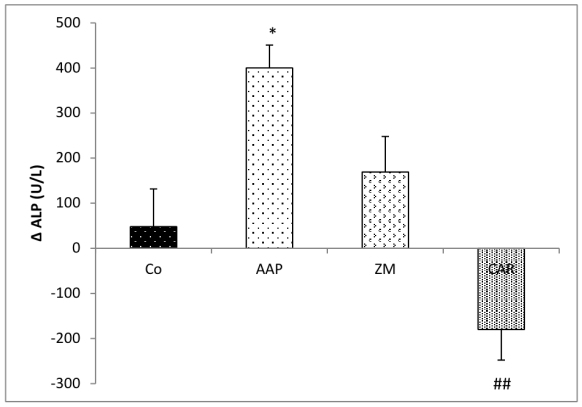

Figure 7 Comparison the changes (Δ) of Alkaline Phosphatase (ALP) concentration between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
*:P < 0.05, compared to Co group
##:P < 0.01, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

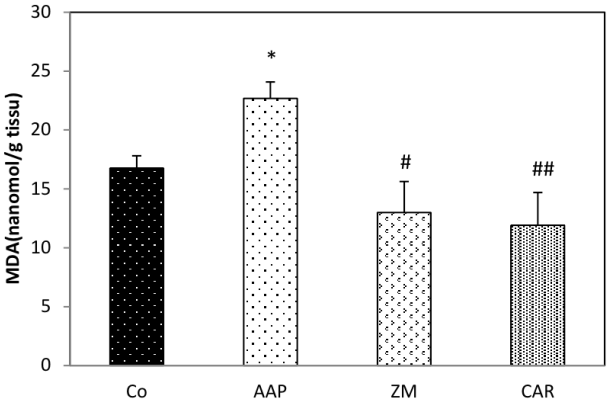

Figure 9 Comparison of Malondyaldehide (MDA) concentration between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
*:P < 0.05, compared to Co group
#:P < 0.05, ##:P < 0.01, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

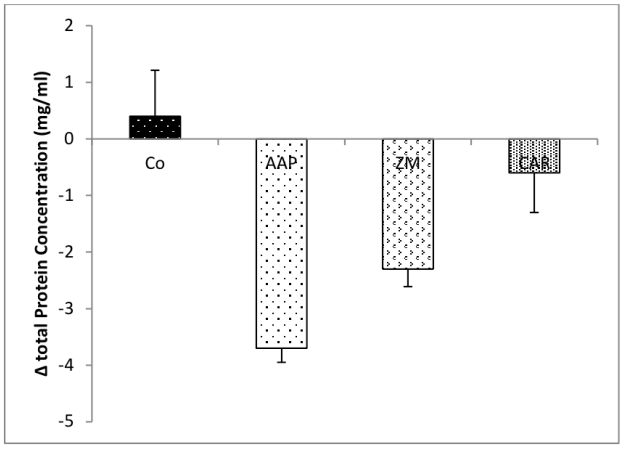

Figure 8 Comparison the changes (Δ) of tPro between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
There is no difference between groups.
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

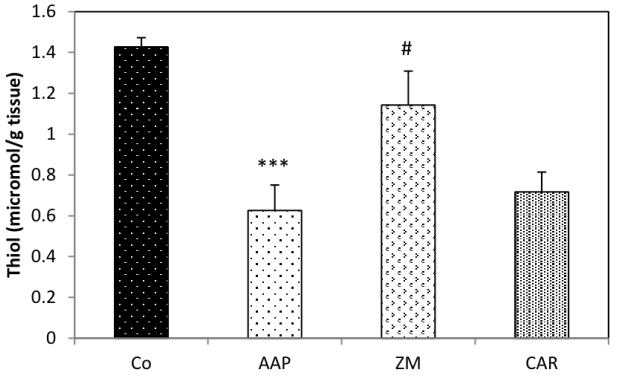

Figure 10 Comparison of Thiol concentration between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
***:P < 0.001, compared to Co group
#:P < 0.05, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

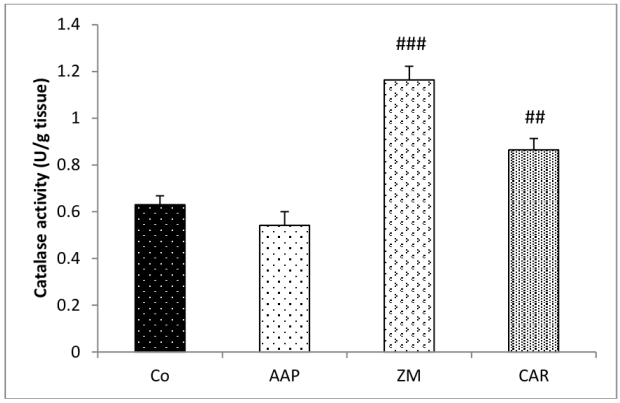

Figure 11 Comparison of CAT activity between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
##:P < 0.01, ###:P < 0.001, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

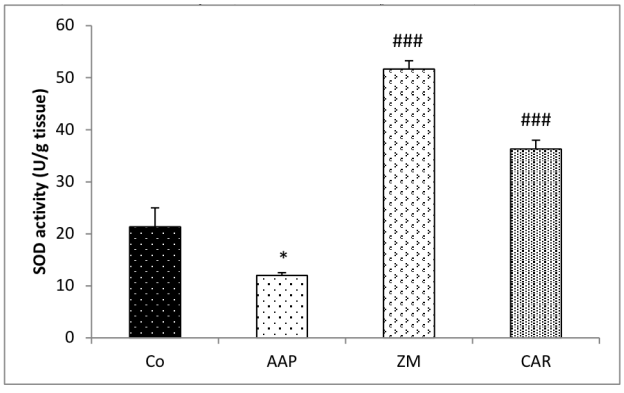

Figure 12 Comparison of Superoxide dismutase (SOD) activity between four groups (n=6 in each group). Values are the Mean ± SEM. The data were analyzed using One-Way ANOVA with tukey post hoc.
*:P < 0.05, compared to Co group
###:P < 0.001, compared to AAP group
Co: Control, AAP: Acetaminophen, Z.M: *Zataria multiflora* + AAP, CAR: Carvacrol + AAP

4. Discussion

Acetaminophen (AAP) which is broadly used as an antipyretic and analgesic drug in higher doses could induce hepatotoxicity in humans and experimental animals [38]. The NAPQI is a toxic metabolite of APP that is produced in the liver by the cytochrome P450 pathway [6]. This agent conjugates with glutathione and excretes in the urine. The AAP overdose causes glutathione depletion that leads to NAPQI accumulation and mitochondrial dysfunction [39]. Glutathione depletion promotes the tumor necrosis factor alpha (TNFα) that leads to production of oxygen free radicals from NADPH oxidase and hepatotoxicity finally [40]. The ample studies have been shown that AAP overdose (600 mg) causes hepatotoxicity in rats [32, 41].

The AAP is a potential trigger of cytochrome P450 that induces the high reactive quinone-imine production. This matches with sulphahydryl groups in proteins and results in rapid depletion of intracellular glutathione [42]. Generally, one part of the potential intracellular antioxidant defensive system is glutathione that consumes the singlet oxygen, superoxide and hydroxyl radicals [43]. Enhancing the intracellular flux of oxygen free radicals resulting from glutathione depletion leads to oxidative stress in hepatocytes [43]. The increased serum levels of GOT, GPT and ALP have been attributed to the structural integrity of hepatic damage [44]. In the liver tissue, the high concentration of GOT and GPT are located in cytosol and mitochondria. In following of liver damage, hepatocyte transport function disrupted and results in the plasma membrane leakage then GOT and GPT were released into blood circulation [45, 46]. In consistent with previous studies [47] in this study also GOT, GPT, ALP and tPro in AAP group, significantly increased compared to Co group. Excretion pathway of ALP is normally via bile. In liver damage due to hepatotoxin, the excretion of bile by the liver is defective that is promoted their serum levels [46]. The tPro is performed as a common test to determinate the toxicity of many chemicals [48]. In liver injury because of reduction in liver function, the protein synthesis decreased and its serum levels reduced. In recent study because of short toxicity induction time, the tPro has a mild fall. Nevertheless, because the half span of albumin is more than two week, we did not expect to change in albumin concentration in serum. Pretreatment of animals with Z.M and specially CAR attenuated the serum GOT, GPT and ALP enzymes that increased by AAP. Probably, these effects of Z.M and CAR refer to functional improvement of hepatocytes. CAR in comparison with Z.M has a better effect on hepatotoxicity induced by AAP because the hepatic enzymes in this group have a significantly reduction. CAR indicated that has a significant antioxidant effect against hepatotoxicity induced by D-Galactosamine [14] and methotrexate [49] via attenuation of liver markers such as ALT, AST and ALP. In one study has been shown that administration of Z.M with four doses orally for a week balanced the liver marker. In this study Z.M with dose 200 as an effective dose, has a significant effect on hepatotoxicity compared to other doses [10]. The pharmacochemistry of Z.M components indicated that it has several phenolic components. Flavonoids and CAR as the effective constituent of Z.M extract have

an important role as a powerful antioxidant. Probably, their positive effect of Z.M comes from their ability to lipid peroxidation inhibition and balancing other procedure involving ROS [11].

Increasing of MDA and reduction of total thiol (tT), SOD and CAT confirm the hepatotoxicity induced by AAP. Main compounds of the enzymatic antioxidant system are three, namely, SOD, CAT and tT which have an important role in detoxifying of H_2O_2 and superoxide anion in cells. Ample of hepatotoxic drugs induces the liver damage by lipid peroxidation indirectly or directly. The proxy radicals are main factors that mediate lipid peroxidation leading to liver injury and kidney damage [50]. MDA as a main reactive aldehyde appears during polyunsaturated fatty acid peroxidation in the biological membranes [51]. The antioxidant effect of CAR has been shown in previous study. For example Nafees et al, reported the protective effect of CAR in doses 25 and 50 mg/kg against thioacetamide (TAA)-induced oxidative stress and inflammation [52]. In other study also, the protective effects of CAR against oxidative stress induced by chronic stress in liver of rat has been shown by Samarghandian et al. suggests that CAR can prevent restraint stress induced oxidative stress in liver [53]. In this studies antioxidant markers such as SOD, CAT, glutathione peroxidase (GPx) and MAD were evaluated and all of these ameliorated by CAR. Our results also are in this direction and confirm other results. Decreased activities of antioxidant enzymes will result in the gathering of high reactive free radicals, leading to worse effects. These effects are loss of cell membrane function and integrity that increases the releasing of GOT and GPT from cell to out [14, 54]. A minor source of oxidant after AAP overdose in liver may be Kupffer cells so that using of Kupffer cells inhibitors completely protects liver against AAP-induced hepatotoxicity [9]. Also, because of glutathione depletion following of AAP overdoses, AAP metabolites bind to membrane and mitochondrial proteins and lead to increasing of cytosolic calcium and reduction of ATP synthesis. This mitochondrial dysfunction incases the superoxide radicals generation [9]. The superoxide radicals can react with nitric oxide and generates the peroxynitrite or increases the lipid peroxidation [9]. CAR has a scavenger role for hydrogen peroxide and superoxide radicals [14]. These antioxidant properties of CAR may reduce the free-radical-mediated inactivation of enzyme proteins and thereby maintaining the enzymatic antioxidants activities [14].

5.Conclusion

Our results indicated that Z.M and CAR have a powerful protective effect against hepatoprotective induced by APP. However, the effect of CAR was higher than Z.M. Therefore, Z.M and CAR could be used as a supplement agent against hepatotoxicity induced by APP.

Acknowledgment

The authors would like to thank the Vice Presidency of Research Center of Mashhad University of Medical Sciences for their financial support.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J hepatol. 2012;56(4):952-64.
2. Lee WM. Drug-induced hepatotoxicity. New England J Med. 2003;349(5):474-85.
3. Shah AD, Wood DM, Dargan PI. Understanding lactic acidosis in paracetamol (acetaminophen) poisoning. Br J clin pharmacol. 2011;71(1):20-8.
4. Gyamlani GG, Parikh CR. Acetaminophen toxicity: suicidal vs accidental. Critical Care. 2002;6(2):155.
5. Gunnell D, Murray V, Hawton K. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. Suicide life threat behav. 2000;30(4):313-26.
6. Tan SC, New LS, Chan ECY. Prevention of acetaminophen (APAP)-induced hepatotoxicity by leflunomide via inhibition of APAP biotransformation to N-acetyl-p-benzoquinone imine. Toxicol let. 2008;180(3):174-81.
7. Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. Adverse Drug Reactions: Springer. 2010;369-405.
8. Myers RP, Shaheen AAM, Li B, Dean S, Quan H. Impact of liver disease, alcohol abuse, and unintentional ingestions on the outcomes of acetaminophen overdose. Clin Gastroenterol Hepatol. 2008;6(8):918-25.
9. Jaeschke H, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxicol let. 2003;144(3):279-88.
10. Shokrzadeh M, Chabra A, Ahmadi A, Naghshvar F, Habibi E, Salehi F, et al. Hepatoprotective effects of *Zataria multiflora* ethanolic extract on liver toxicity induced by cyclophosphamide in mice. Drug res. 2015;65(04):169-75.
11. Ali MS, Saleem M, Ali Z, Ahmad VU. Chemistry of *zataria multiflora* (lamiaceae). Phytochem. 2000;55(8):933-6.
12. Shafiee A, Javidnia K. Composition of essential oil of *Zataria multiflora*. Planta medica. 1997;63(04):371-2.
13. Kavoosi G, Teixeira da Silva JA, Saharkhiz MJ. Inhibitory effects of *Zataria multiflora* essential oil and its main components on nitric oxide and hydrogen peroxide production in lipopolysaccharide-stimulated macrophages. J Pharm Pharmacol. 2012;64(10):1491-500.
14. Aristatile B, Al-Numair KS, Veeramani C, Pugalendi KV. Effect of carvacrol on hepatic marker enzymes and antioxidant status in d-galactosamine-induced hepatotoxicity in rats. Fund clin pharmacol. 2009;23(6):757-65.
15. Mahtaj LG, Feizpour A, Kianmehr M, Soukhtanloo M, Boskabady MH. The effect of carvacrol on systemic inflammation in guinea pigs model of COPD in-

duced by cigarette smoke exposure. Pharmacol Rep. 2015;67(1):140-5.
16. Ramezani M, Hosseinzadeh H, Samizadeh S. Antinociceptive effects of *Zataria multiflora* Boiss fractions in mice. J ethnopharmacol. 2004;91(1):167-70.
17. Minaiyan M, Ghannadi A, Salehi E. Antiulcerogenic effect of *Zataria multiflora* Boiss. on cysteamine induced duodenal ulcer in rats. Iran J pharm sci. 2005;1(4):223-9.
18. Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against Escherichia coli. Let appl microbiol. 2008;47(3):174-9.
19. Boskabady MH, Gholami Mhtaj L. Effect of the *Zataria multiflora* on systemic inflammation of experimental animals model of COPD. BioMed res int. 2014;2014:1-9.
20. Boskabady MH, Kaveh M, Eftekhar N, Nemati A. *Zataria multiflora* Boiss and carvacrol affect β2-adrenoceptors of guinea pig trachea. Evid Based Complement Alternat Med. 2011;2011:1-9.
21. Gholami Mahtaj L, Boskabady MH, Mohamadian Roshan N. The effect of *Zataria multiflora* and its constituent, carvacrol, on tracheal responsiveness and lung pathology in guinea pig model of COPD. Phytother res. 2015;29(5):730-6.
22. Arunasree KM. Anti-proliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB 231. Phytomed. 2010;17(8):581-8.
23. Kamrani YY, Amanlou M, Yazdanyar A, AdliMoghaddam A, Ebrahimi SN. Potential anti-diabetic and anti-oxidant activity of essential oil of *Zataria multiflora* leaves. Planta medica. 2008;74(09):172.
24. Melo FHC, Moura BA, de Sousa DP, de Vasconcelos SMM, Macedo DS, Fonteles MMdF, et al. Antidepressant-like effect of carvacrol (5-Isopropyl-2-methylphenol) in mice: involvement of dopaminergic system. Fund clin pharmacol. 2011;25(3):362-7.
25. Mohebbati R, Jalili-Nik M, Paseban M, Shafei Mn, Khajavi Rad A. Effects of *Zataria multiflora* Extract and Carvacrol on Doxorubicin-Induced Oxidative Stress in Rat Brain. Pharm Sci. 2018;24(3):187-92.
26. Boskabady MH, Mahtaj LG. Lung inflammation changes and oxidative stress induced by cigarette smoke exposure in guinea pigs affected by *Zataria multiflora* and its constituent, carvacrol. BMC complement alternat med. 2015;15(1):39-49.
27. Khazdair MR, Ghorani V, Alavinezhad A, Boskabady MH. Pharmacological effects of *Zataria multiflora* Boiss L. and its constituents focus on their anti-inflammatory, antioxidant, and immunomodulatory effects. Fund clin pharmacol. 2018;32(1):26-50.
28. Black M. Acetaminophen hepatotoxicity. Annual review of medicine. 1984;35(1):577-93.
29. Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N, Chalier P. Antimicrobial activity of carvacrol related to its chemical structure. Let appl microbiol. 2006;43(2):149-54.
30. Hosseinimehr SJ, Ahmadashrafi S, Naghshvar F, Ahmadi A, Ehasnalavi S, Tanha M. Chemoprotective effects of *Zataria multiflora* against genotoxicity induced by cyclophosphamide in mice bone marrow cells. Integ Cancer Ther. 2010;9(2):219-23.
31. Dagli Gul AS, Fadillioglu E, Karabulut I, Yesilyurt A, Delibasi T. The effects of oral carvacrol treatment against H2O2 induced injury on isolated pancreas islet cells of rats. Islets. 2013;5(4):149-55.
32. Omidi A, Riahinia N, Torbati MBM, Behdani M-A. Hepatoprotective effect of Crocus sativus (saffron) petals extract against acetaminophen toxicity in male Wistar rats. Avicenna J Phytomed. 2014;4(5):330.
33. Ellman GL. Tissue sulfhydryl groups. Arch biochem biophys. 1959;82(1):70-7.
34. Hosseini M, Pourganji M, Khodabandehloo F, Soukhtanloo M, Zabihi H. Protective Effect of L-Arginine against Oxidative Damage as a Possible Mechanism of its Beneficial Properties on Spatial Learning in Ovariectomized Rats. Basic Clin Neurosci. 2012;3(5):36-44.
35. Khodabandehloo F, Hosseini M, Rajaei Z, Soukhtanloo M, Farrokhi E, Rezaeipour M. Brain tissue oxidative damage as a possible mechanism for the deleterious effect of a chronic high dose of estradiol on learning and memory in ovariectomized rats. Arq Neuropsiquiatr. 2013;71(5):313-9.
36. Aebi HE. Catalase. In: HU B, editor. Methods in Enzymatic Analysis. New York: Academic Press. 1983;276-86.
37. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J biochem biophys. 1998;35(3):184-8.
38. Prescott LF, Roscoe P, Wright N, Brown SS. Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. Lancet. 1971;297(7698):519-22.
39. Ahmed G, Khandkar MA, Katyare SS. Mitochondrial ATPase: a target for paracetamol-induced hepatotoxicity. Eur J Pharmacol Envir Toxicol Pharmacol. 1995;293(3):225-9.
40. Gupta JW, Kubin M, Hartman L, Cassatella M, Trinchieri G. Induction of expression of genes encoding components of the respiratory burst oxidase during differentiation of human myeloid cell lines induced by tumor necrosis factor and γ-interferon. Cancer res. 1992;52(9):2530-7.
41. Hazelton GA, Hjelle JJ, Klaassen CD. Effects of butylated hydroxyanisole on acetaminophen hepatotoxicity and glucuronidation in vivo. Toxicol appl pharmacol. 1986;83(3):474-85.
42. Jollow DJ, Thorgeirsson SS, Potter WZ, Hashimoto M, Mitchell JR. Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. Pharmacol. 1974;12(4-5):251-71.
43. Miesel R, Sanocka D, Kurpisz M, Kröger H. Antiinflammatory effects of NADPH oxidase inhibitors. Inflammation. 1995;19(3):347-62.
44. Chenoweth MB, Hake CL. The smaller halogenated aliphatic hydrocarbons. Annual Rev Pharmacol. 1962;2(1):363-98.
45. Sherlock S, Dooley J. Drugs and the liver. Sherlock, E Diseases of the Liver and Biliary System. 2002;7:304-33.
46. Sreelatha S, Padma PR, Umadevi M. Protective effects of Coriandrum sativum extracts on carbon tetrachlo-

ride-induced hepatotoxicity in rats. Food Chem Toxicol. 2009;47(4):702-8.
47. Ojo OO, Nadro MS, Tella IO. Protection of rats by extracts of some common Nigerian trees against acetaminophen-induced hepatotoxicity. Afric J Biotechnol. 2006;5(9):755-60.
48. Nevin KG, Vijayammal PL. Effect of Aerva lanata on solid tumor induced by DLA cells in mice. Fitoterapia. 2003;74(6):578-82.
49. Bozkurt M, Bodakci MN, Turkcu G, Kuyumcu M, Akkurt M, Sula B, et al. Protective Effects of Carvacrol Against Methotrexate-induced Liver Toxicity in Rats. Acta Chirurgica Belgica. 2014;114(6):404-9.
50. Recknagel RO, Glende EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol ther. 1989;43(1):139-54.
51. Vaca CE, Wilhelm J, Harms-Ringdahl M. Interaction of lipid peroxidation products with DNA. A review. Mutation Res Rev Gen Toxicol. 1988;195(2):137-49.
52. Nafees S, Ahmad ST, Arjumand W, Rashid S, Ali N, Sultana S. Carvacrol ameliorates thioacetamide-induced hepatotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in liver of Wistar rats. Human exp toxicol. 2013;32(12):1292-304.
53. Samarghandian S, Farkhondeh T, Samini F, Borji A. Protective effects of carvacrol against oxidative stress induced by chronic stress in rat's brain, liver, and kidney. Biochem res int. 2016;2016:1-7.
54. Mohan GK, Pallavi E, Kumar R, Ramesh M, Venkatesh S. Hepatoprotective activity of Ficus carica Linn leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. DARU J Pharm Sci. 2007;15(3):162-6.