The potential hepatoprotective effect of Mentha piperita against formalin-induced liver injury in male rats

Elhadi Araibi[1 (*)]., Basma Aseid[2]., Altayeb Elazomi[1]., Khaled Aburas[3]., Abd Alla A Mohamed[1] and Abdel Abdel-Kareem Mohamed Al-basheer[1]

1- Faculty of Medical Technology, University of Zawia, Libya.

2- Faculty of Sciences, University of Zawia, Libya.

3- Libyan Center for Medical Research, Zawia Libya.

Abstract

Hepatic diseases and injuries remain one of the public health problems and one of the primary causes of morbidity and mortality worldwide. Formalin is an extremely reactive chemical, and generates oxidative free radicals. Detoxifications of toxicants lead to liver cellular stress and hepatoprotective medications could be recommended for this

(*) Email: e.araibi@zu.edu.ly

distractive condition. Plant is known for having several polyphenols that are highly effective antioxidants and are less toxic than the synthetic ones. The study aimed to assess the hepatoprotective effect of Mentha piperita on formalin-induced hepatic injury.

Forty-eight adult male albino Rats were equally divided into four groups; control group, the second, third, and fourth groups were orally formalin treated 10 mg/kg body weight to induce hepatotoxicity. Only the third and fourth groups were also given orally aqueous extract of Mentha piperita at daily doses of 600 and1200 mg/kg BW, respectively for 30 days. At the end of experiment, animals were anesthetized and then scarified by cervical dislocation. Cardiac blood samples were collected and clear serum was separated by centrifugation (15000 rpm/15 min), collected in vials and kept at 4 ºC until further examinations. A portion of each liver was fixed in 10% formalin and kept for histopathological assessments.

The levels of ALT, AST and ALP enzymes significantly increased in the formalin group compared to the control group ($p<0.005$). The mice treated with Mentha piperita extract (ME) showed a significant decrease in ALT ($41.17 \pm 3.13^{\#\#}$) and ALP ($206.33 \pm 9.73^{\#\#}$) compared to the formalin group ($67.50 \pm 9.85^{**}$), ($431.17 \pm 65.24^{**}$) respectively according to table 1 ($p<0.05$). AST also significantly increased in the formalin group ($118.00 \pm 8.32^{**}$) compared to the control group (69.83 ± 6.68) ($p<0.05$). Doses of 1200 mg ME showed the strongest hepatoprotective effect against formalin induced liver injury.

Histopathological observations also support the hepatoprotective potential of Mentha piperita extract against formalin induced hepatic damage. We could demonstrate that aqueous extract of Mentha piperita protects liver from formalin-induced oxidative stress and thus confirm the beneficial effects attributed traditionally to this plant.

Key words: *Hepatic injury, Formalin, Mentha piperita*

Introduction

Liver is the main body organ that plays a vital role in metabolism, detoxification and excretion of toxic materials. Hepatic diseases and injuries remain one of the public health problems and one of the primary causes of morbidity and mortality worldwide (1). It is well known that free radicals released from many environmental sources such as chemical industry can trigger liver cell damage. Formalin is one of the common environmental agents found in tobacco smoke, diesel, gasoline exhaust, and medical and industrial products (2). It is used as an illegal practice to preserve fish, fruits and vegetables which is dangerously affecting the population health. Formalin an extremely reactive chemical, and produces covalently cross-linked complexes with proteins and DNA (3), and generates oxidative free radicals (4). Detoxifications of toxicants lead to liver cellular stress and hepatoprotective medications could be recommended for this distractive condition. Recently, antioxidant agents have attracted researches about hepatoprotective potential of natural sources such as plants. The use of natural products that are rich in bioactive substances is growing along with the demand for plants containing wide range of antioxidant properties and bioactive molecules capable of neutralizing free radicals (5,6). Among the diversity of plants, Mentha piperita (Lamiaceae family) is one of the herbs most widely used worldwide, with a long history of safe use in medicinal preparations (7). This property makes it of great interest to the Food Industry, since the phenolic compounds retard the oxidative degradation of lipids improving the quality and nutritional value of food. (8). Due to an antioxidant activity of Mentha piperita in targeting free radicals and prevent their damaging effects, the present study has been focused on evaluating the

potential hepatoprotective effects of the aqueous extract (ME) of Mentha piperita on formalin induced liver injury in rats.

Material and Methods

Plant material

Leaves of Mentha piperita were collected from pesticide-free gardens. Mint juice was made by mixing mint leaves with 200ml of DW in food mixer. Then filtration paper was used to obtain purified mint extract. The extract (2g) was used in the experiment orally at 0.06 g/kg B.W for 30 days (9).

Animals

Forty-eight adult male albino Rats (180-200 g) obtained from Libyan Medical Research Centre in the city of Zawia were used for this experiment. The animals were maintained on 12 h light and dark cycle, at 25±2 ºC and 60%-70% humidity with standard pellets diet. The experiment was started when animals reached 180 to 200g BW. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication NO. 85–23, revised 1996).

Experimental design

The animals were equally divided into four groups Group I served as a normal control and received drinking water. Group II served as a toxicant control and received only formalin at (10 mg/kg) BW for 30 days. Groups III-VIII received equal dose of formalin at 10 mg/kg BW, and after an hour, were treated with the Mint extract (ME) at doses of 600 and1200 mg/kg BW, respectively daily for 30 days. All samples were

administered orally using gastric tubes. After 24 hours of final dose on the 30th day, the animals were anesthetized and then scarified by cervical dislocation. Cardiac blood samples were collected and clear serum was separated by centrifugation (15000 rpm/15 min), collected in vials and kept at 4 ºC until further examinations. A portion of each liver was fixed in 10% formalin and kept for histopathological assessments.

Determination of biochemical parameters

Biochemical parameters were assessed according to standard methods. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were measured by using the photometric method (DGKC) based of conversion of *p*-nitrophenylphosphate+H2O to phosphate + *p*-nitrophenol. Enzyme levels were obtained using Mindray biosysyem BTS 350-bs200.

Histopathological analysis

The livers were removed, fixed in 10% neutral buffered formalin for 72 h, dehydrated, and embedded in paraffin. Later, 5µm sections were prepared followed by staining with hematoxylin–eosin for histological assessments.

Statistical analysis

All data were expressed as mean ± SD. Multiple comparisons were performed by one-way analysis of variance (ANOVA). Value of $p < 0.05$ was considered to be significant.

Results

The results of the hepatoprotective effect of ME are summarized in table 1. The levels of ALT, AST and ALP enzymes significantly increased in the formalin group compared to the control group (p<0.005). The mice treated with ME showed a significant decrease in ALT (41.17 ± 3.13$^{\#\#}$) and ALP (206.33 ± 9.73$^{\#\#}$) compared to the formalin group (67.50 ± 9.85**), (431.17 ± 65.24**) respectively according to table 1 (p<0.05). AST also significantly increased in the formalin group (118.00 ± 8.32**) compared to the control group (69.83 ± 6.68) (p<0.05). Doses of 1200 mg ME showed the strongest hepatoprotective effect against formalin induced liver injury (table 1).

Table 1. The effects of Mint extracts of on biochemical parameters of formalin-induced liver injury in rats.

Groups / Parameters	Control	Formalin	Formalin + Mint 1	Formalin + Mint 2
ALT	37.67 ± 1.86	67.50 ± 9.85**	48.17 ± 2.48$^{**\#\#}$	41.17 ± 3.13$^{\#\#}$
AST	69.83 ± 6.68	118.00 ± 8.32**	96.17 ± 9.30$^{**\#\#}$	84.50 ± 7.64$^{**\#\#}$
ALP	189.33 ± 13.75	431.17 ± 65.24**	249.50 ± 37.97$^{*\#\#}$	206.33 ± 9.73$^{\#\#}$

*: Significant P<0.05 compared with control group,
**: Significant P<0.01 compared with control group
#: Significant P<0.05 compared with formalin treated group,
##: Significant P<0.01 compared with formalin treated group

The hepatoprotective effect of mint extract against formalin induced liver

Elevated levels of liver enzymes of ALT, AST, and ALP are indicators of hepatocyte damage induced by administration of formalin. Rats treated with Mint extract showed a significant decrease in the enzyme levels of ALT as shown in figure 1.

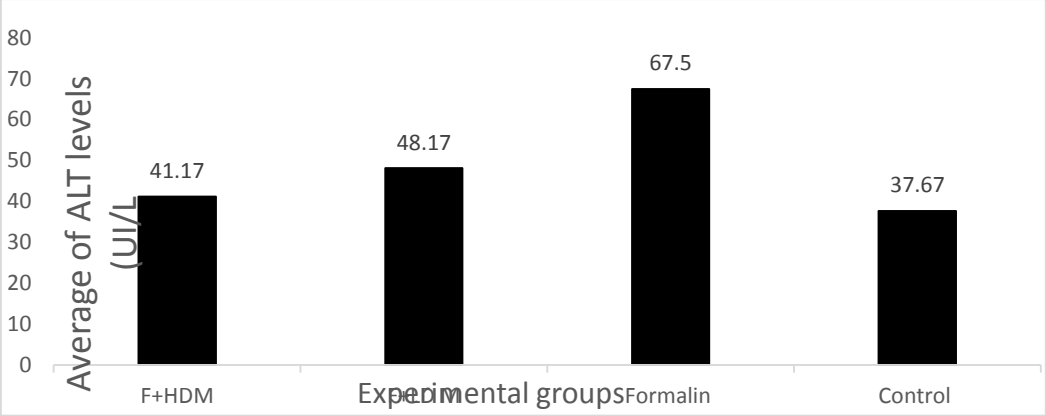

Figure-1: shows the hepatoprotective effect of Mint extract against formalin-induced liver damage. Mean of the enzyme levels for control group is indicated by the first bar from the right; mean of ALT level for formalin group is indicated by the second bar from the right. The third and the fourth bars are indicators of the enzyme's level of third (600 mg of ME/kg of BW) and fourth groups (1200 mg of ME/kg rat BW respectively. Rats treated with double dose of ME (1200 mg/kg of BW) showed more reduction in the enzyme level than the third group treated with only 600 mg of ME/ kg of BW.

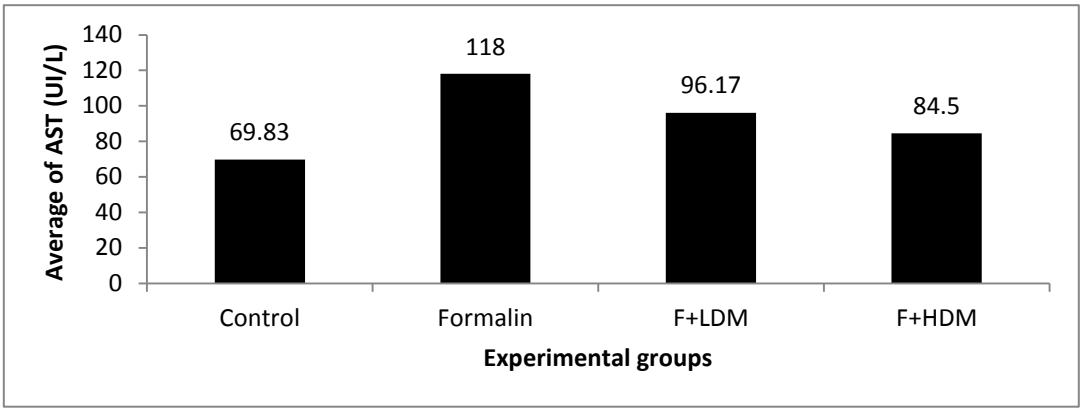

Figure2: shows the hepatoprotective effect of Mint extract against formalin-induced liver damage. Mean of the enzyme level for control group is indicated by the first bar from the left; mean of AST level for formalin group is indicated by the second bar from the left. The third and the fourth bars are indicators of the enzyme's level of third (600 mg of ME/kg of BW) and fourth groups (1200 mg of ME/kg rat BW respectively. Rats treated with double dose of ME (1200 mg/kg of BW) showed more reduction in the enzyme level than the third group treated with only 600 mg of ME/ kg of BW

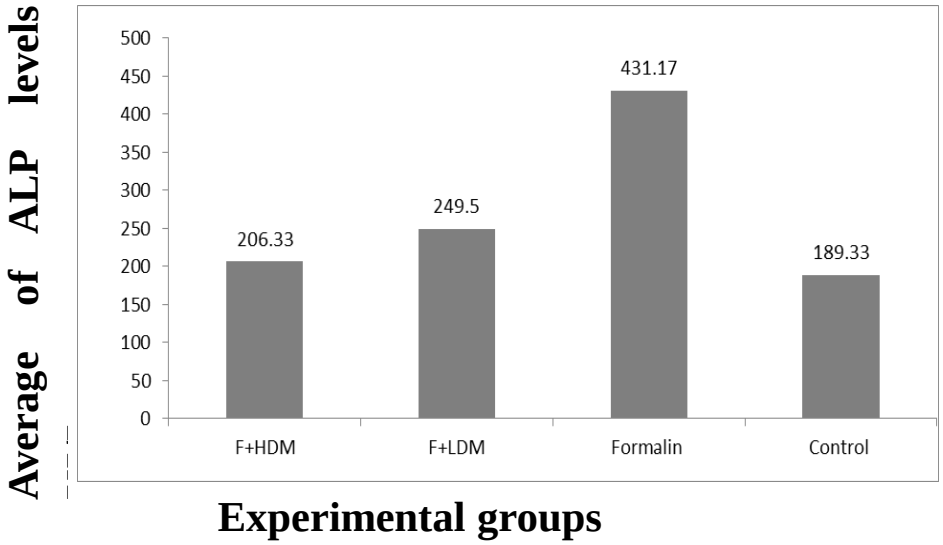

Figure-3: shows the hepatoprotective effect of Mint extract against formalin-induced liver damage. Mean of the enzyme level for control group is indicated by the first bar from the right; mean of ALP level for formalin group is indicated by the second bar from the right. The third and the fourth bars are indicators of the enzyme's level of third (600 mg of ME/kg of BW) and fourth groups (1200 mg of ME/kg rat BW respectively. Rats treated with double dose of ME (1200 mg/kg of BW) showed more reduction in the enzyme level than the third group treated with only 600 mg of ME/ kg of BW.

Histopathological evaluation of the hepatoprotective effect of Mentha piperita extract

Following tissue processing and H&E staining, section glass slides were examined to evaluate the histological changes of the liver using light microscopy connected with digital camera. Images with different magnification were captured, then exported to windows media photo shop for making proper resolution.

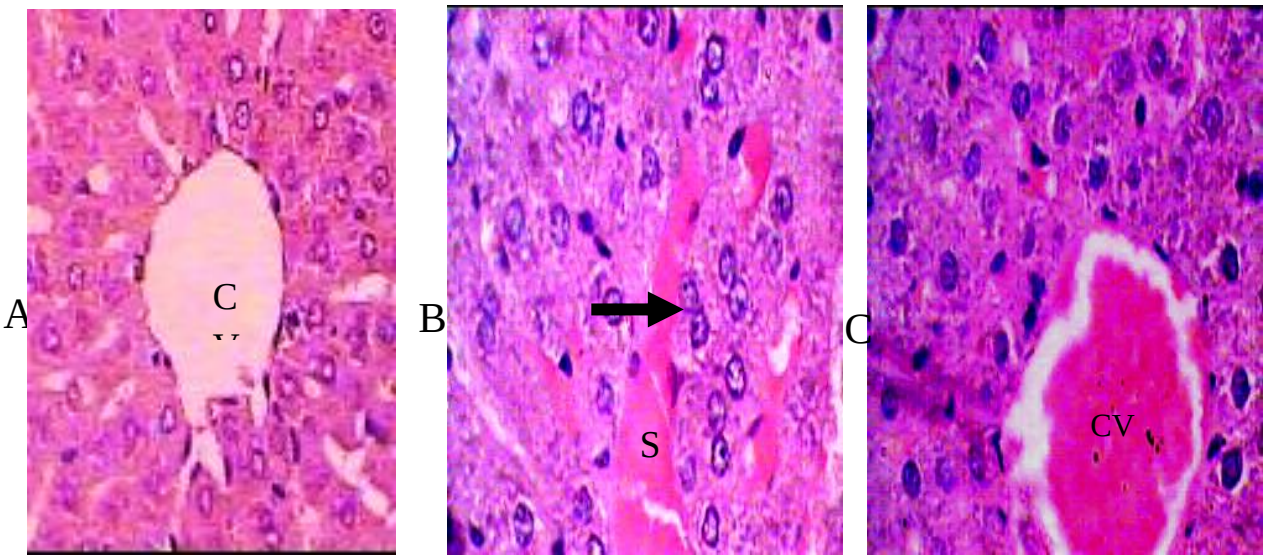

Figure 4: The photomicrographs from liver of formalin-treated rats showed congested blood vessels in sinusoids as shown in section B X1000 (S), increase of Kupffer cells activity indicated by black arrow in section B, congested central vein (CV) as shown in section C compared to liver histology obtained from control group as shown in section A X400.

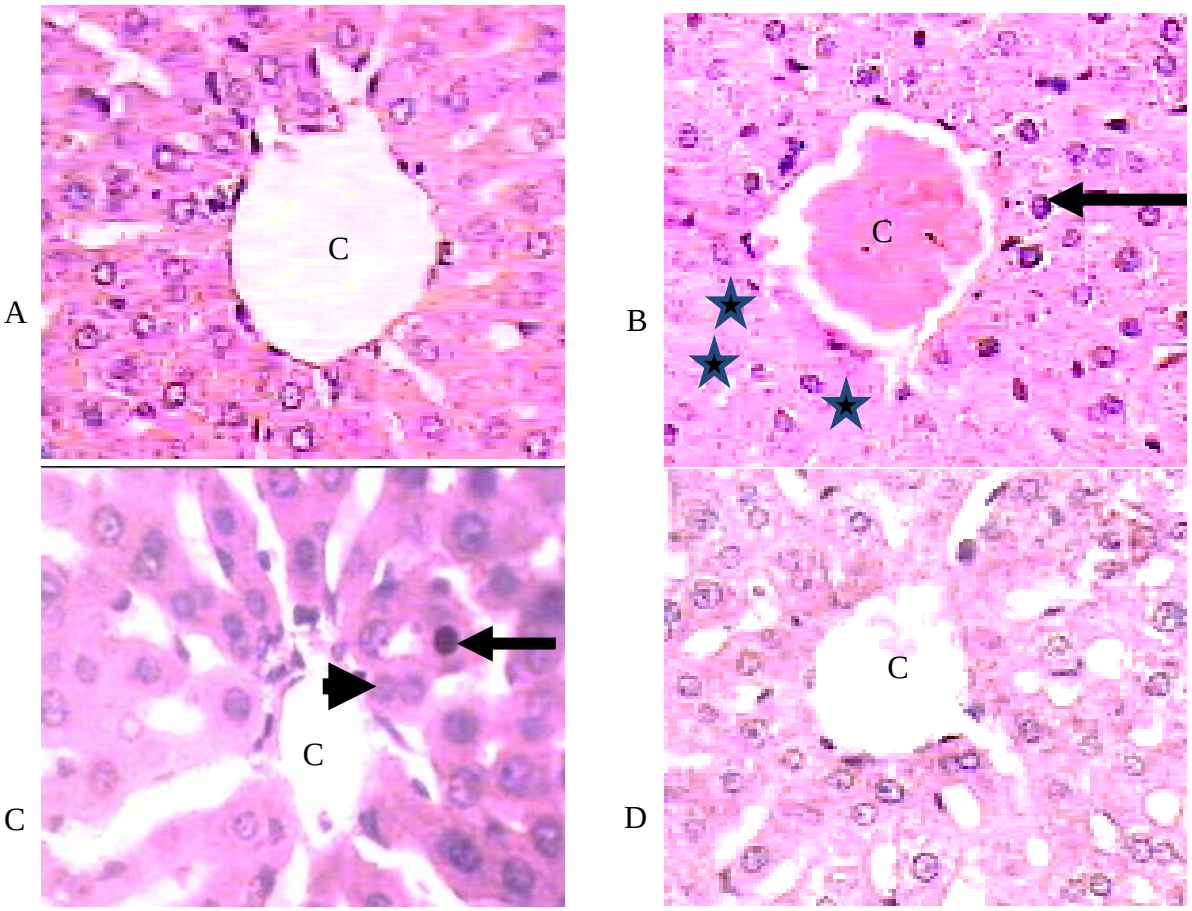

Figure 5: represents centrilobular area of liver, the most susceptible area for toxic induced damage. Section A represents control group and shows normal hepatocytes arrangement around central vein X400. Section B represents formalin administered group and shows congestion within the central vein, presence of dead cells as indicated by pyknotic nuclei (black arrow) and disappearance of nuclei as indicated by stars (X400). Section C represents of third group (given low dose 600 mg/kg BW of Mint extract. The image reveals cell proliferation (mitotic figure; arrow head) and re-arrangement of normal cells around central vein. However, some toxic cellular damage (black arrow) still exist X1000. Section D represents the fourth group (1200 mg/kg BW of Mint extract. There is arrangement of normal cells around central vein in architecture resembling normal liver histology X400.

Discussion

The serum enzyme levels are direct measure of hepatic injury and they reflex the status of the liver. The elevated levels of hepatic enzymes are caused by formalin toxic metabolites, a free radical that binds to lipoprotein and leads to peroxidation of lipids of endoplasmic reticulum. Hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane (10). The increased serum levels of ALT and AST is evidence that these enzymes play an important role in the development of acute and chronic inflammation (11). The formalin hepatotoxic mechanisms may be the result of increase in the production of the reactive species such as ROS and other oxidant intermediates, that cause breakage in DNA structure and protein cross links (12,13,14).Thus, antioxidant activity or inhibiting generation of free radicals' could be important in the protection against formalin induced liver injury. It has been shown that methanol extract of *A. bracteatum* (Girard) has a significant hepatoprotective effect against formaldehyde-induced hepatic damage (15). Another study showed that the significant decrease in AST, and ALT levels was obtained using a higher dose of the hepatoprotective agent. Moreover, a significant reduction in the serum levels of ALT, AST, and ALP in rats co-treated with extract of Mentha piperita and CCl4 compared to the CCl4-only (16) In agreement with the previous studies, our results showed that a significant decrease in serum levels of liver enzymes was obtained following administration of a higher (double) dose of Mint extract as shown in figure 1. Thus, liver enzymes can be useful parameters for monitoring liver condition. Histological observation of H&E liver section showed variable changes between control, formalin-induced damage, and Mint extract administered rats. The present study showed that histological sections of liver obtained from formalin administered rats; photomicrographs reveal loss of normal architecture of hepatocyte arrangement within hepatic lobule, presence of cytoplasmic

vacuoles and active enlarged Kupffer cells. In liver sections obtained from rats treated with Mint extract along with formalin administration showed significant decrease in congestion of sinusoids and central vein, and decrease in Kupffer cell activity. Furthermore, liver sections obtained from rats treated with double dose of mint extract showed an improvement of the liver histological architecture resembling normal architecture of control group. These histological observations are consistent with the previous observations which revealed the hepatoprotective effect of Acantholimon bracteatum on formaldehyde-induced liver injury in adult male mice (17). Additionally, histopathological examination of the liver tissue following treatment of the rats with aqueous extract with Mentha arvensis leaves along with CCL4 administration showed significant restore of normal liver histology compared to the histological picture of rats treated with CCL4 only (18). Thus, the aqueous extract of Mentha piperita probably exerts its protective action against formalin induced liver cell metabolic alterations by the antioxidant effect on formalin induced toxicants.

Conclusion

We could demonstrate that aqueous extract of Mentha piperita protects liver from formalin-induced oxidative stress and thus confirm the beneficial effects attributed traditionally to this plant.

References

1- Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global burden of disease study. *Lancet*. 1997; 349(9061): 1269-1276.

2- Saito, Y., et al. (2005). " Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species." Toxicology 210(2-3): 235-245.

3- Flyvholm, M. A. and P. Andersen (1993). " Identification of formaldehyde releasers and occurrence of formaldehyde and

formaldehyde releasers in registered chemical products." Am J Ind Med 24(5): 533-552.

4- Teng, S., et al. (2001). "The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes." Chem Biol Interact
130-132(1-3): 285-296.

5-Shirani K, Hassani FV, Azar-Khiavi KR, Moghaddam ZS, Karimi G. Determination of
methanol in Iranian herbal distillates. J Complement Integr Med., 2016 Jun 1; 13(2): 123-7

6-Sharafi SM, Rasooli I, Owlia P, Taghizadeh M, Astaneh SD. Protective effects of bioactive phytochemicals from Mentha piperita with multiple health potentials. Pharmacogn. Mag., 2010; 6: 147–153.

7- Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel SW, Herrera-Ruiz D, Torres E. Risks and benefits of commonly used herbal medicines in Mexico. Toxicol Appl Pharmacol., 2008 Fev 15; 227(1): 125-135

8- Mallick B, Sinha S, ROY D. Evaluation of antioxidative potential of field grown and tissue culture derived Mentha piperita L. plants. Int. J. Curr. Microbiol. App. Sci., 2016; 5(3): 382-391

9- Sandra M. Barbalho,1,2 Débora C. Damasceno,3 Ana Paula Machado Spada,4 Vanessa Sellis da Silva,5 Karla Aparecida Martuchi,6 Marie Oshiiwa,2 Flávia M. V. Farinazzi Machado,2 and ClaudemirGregório Mendes[1]. Metabolic Profile of Offspring from Diabetic Wistar Rats Treated with Mentha piperita (Peppermint). Evidence-Based Complementary and Alternative Medicine. Volume 2011, Article ID 430237, 6 pages

10- Zimmerman, H. J. and L. B. Seeff (1970). "Enzymes in hepatic disease." Diagnostic Enzymology. 1, Lea and Febiger, Philadelphia, USA.

11-Anderson, A. J., et al. (1971). "Evidence for the role of lysosomes in the formation of prostaglandins during carrageenan induced inflammation in rat." Pharmacol Res Comm 3: 13-17.

12- Gulec M, Gurel A, Armutcu F. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. *Mol Cell Biochem*. 2006; 290(1): 61-67.

13- Yu P, Zuo D. Formaldehyde produced endogenously via deamination of methylamine. A potential risk factor for initiation of endothelial injury. *Atherosclerosis*. 1996; 120(1-2): 189-197.

14- Sögüt S, Songur A, Özen OA, Özyurt H, Sarsilmaz M. Does the subacute (4-week) exposure to formaldehyde inhalation lead to oxidant/antioxidant imbalance in rat liver?

15- Nasiri1, S. Naserirad2, A. Pasdaran Lashgari3,4*, R. Gazor1, F. Mohammadghasemi5, Z. Atrkar Roushan6. Hepatoprotective effect of *Acantholimon bracteatum* (Girard) Boiss. on formaldehyde-induced liver injury in adult male mice. RJP 3(3), 2016: 55-61

16- Khaled Bellassoued,[1] Anis Ben Hsouna,[2] Khaled Athmouni,[3] Jos van Pelt,[4] Fatma Makni Ayadi,[5] Tarek Rebai,[6] and Abdelfattah Elfeki[1] Protective effects of *Mentha piperita* L. leaf essential oil against CCl_4 induced hepatic oxidative damage and renal failure in rats Lipids Health Dis. 2018; 17: 9

17- E. Nasiri1, S. Naserirad2, A. Pasdaran Lashgari3,4*, R. Gazor1, F. Mohammadghasemi5, Z. Atrkar Roushan6. Hepatoprotective effect of Acantholimon bracteatum (Girard) Boiss. on formaldehyde-induced liver injury in adult male mice. RJP. 3(3), 2016: 55-61

18- Kalpana Patil. Hepatoprotective activity of Mentha arvensis Linn. leaves against CCL4 induced liver damage in rats. Asian Pacific Journal of Tropical Disease 2(Sup 1), 2012: S223–S226