Advances in Bioresearch
Adv. Biores., Vol 8 (1) January 2017: 93-101

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.1.93101

Advances in Bioresearch

ORIGINAL ARTICLE

Heracleum persicum Essential oil administration in CCL_4 treated Rat sustains antioxidant / oxidative stress statue

Kambiz Roshanaei[1*], Abolfazl Dadkhah[2], Faezeh Fatemi[3], Salome Dini[4]
[1] Department of Physiology, Faculty of Science, Qom Branch, Islamic Azad University, Qom, I.R. Iran
[2] Department of Medicine, Faculty of Medicine, Qom Branch, Islamic Azad University, Qom, I.R. Iran
[3] Nuclear Fuel Cycle Research School, Nuclear Science and Technology Research Institute, Tehran, I.R. Iran
[4] Young Researchers and Elite Club, Karaj Branch, Islamic Azad University, Karaj, Iran
[*]Corresponding author:Kambizroshanaei@gmail.com

ABSTRACT
In this study, the efficacy of Heracleum persicum essential oils on the antioxidant/oxidative stress statue against hepatotoxicity induced with CCL_4 was examined. For this purpose, male Wistar rats were divided into 20 groups as untreated negative control, control received CCL_4 (3 ml/kg b.w) via i.p. injection and treatment groups received H. persicum essential oils (100 & 200 mg/kg b.w) i.p. simultaneously with CCL_4 administration. In following, the oxidative and antioxidant parameters were examined in liver homogenate and plasma of rats at different time intervals (2, 4, 8, 16 and 24 h) after CCL_4 administration. It is indicated that H. persicum essential oils could modulate the levels of hepatic LP, GSH and GST concomitant with adapting plasma FRAP, AST and ALT levels disturbed after CCL_4 treatment. It can be concluded that H. persicum essential oils could modulated the oxidative stress/antioxidant disturbance in CCL_4 treated rats. This may be contributed to the essential oils as a potent herbal medicinal compound.
Key word: *H. persicum essential oils, CCL_4, Antioxidant, Oxidative stress*

Received 21/10/2016 Revised 20/11/2016 Accepted 18/12/2016

How to cite this article:
K Roshanaei, A Dadkhah, F Fatemi, S Dini. *Heracleum persicum* Essential oil administration in CCL_4 treated Rat sustains antioxidant / oxidative stress statue. Adv. Biores., Vol 8 [1] January 2017: 93-101

INTRODUCTION

The liver is the major site of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision, reproduction and xenobiotic metabolism [1]. Liver injury or hepatoxicity can be caused partially by free radicals and reactive oxygen species generated by toxic chemicals and drugs [2].

Carbon tetrachloride (CCl_4) is a potent environmental hepatotoxin widely used in animal models to induced acute liver injury [3]. It undergoes hepatic metabolism in phase I by the mixed function cytochrome P_{450} leaded to the generation of its reactive metabolite such as trichloromethyl free radicals (CCl_3) [4]. This reactive metabolite can bind to macromolecules such as proteins, lipids, and DNA, resulting in hepatic injury. Accordingly, blocking or hindering the chain reaction of oxidation by natural antioxidants such as plant extracts and oils could lead to equilibrium in antioxidant status which prevented oxidative stress-induced hepatotoxicity [5].

Heracleum persicum, commonly known as Golpar or Persian Hogweed, is a flowering plant in the family Apiaceae, native to Iran. It was used as a flavouring agent and spice for cooking in many parts of Iran. The fruits and leaves of this genus are also used as antiseptic, carminative, digestive and analgesic in the Iranian folk medicine. Studies indicated different biological properties related to the essential oils of *Heracleum persicum* such as cytotoxic, antioxidant, antibacterial, anti-inflammatory and analgesic properties [6, 7]. Nevertheless, no document has been reported to consider the hepatoprotective

activities of *Heracleum persicum* extracts. Through increasing detection of natural medicine as unconventional forms of health care, this research was going to raise the antioxidant activity of the essential oils derived from *H. persicum* against CCL_4 induced hepatotoxicity in experimental animal model considering capable antioxidant and oxidative stress parameters.

MATERIALS AND METHODS

Oil extraction and analysis

The dried fruits of Iranian *H. persicum* were purchased and characterized by Research in Forests and Rangelands institute, Isfahan, Iran. The oils were isolated by the hydro distillation method with a Clevenger-type apparatus for 2 hours. The essential oil was analyzed using the trace gas chromatograph. The compounds were separated on 60 m × 0.25 mm × 0.25 μm DB-5 column. The column temperature was increased from 45 ºC to 240 ºC at a rate of 4 ºC/min; injector temperature, 280 ºC; injection volume, 0.2μL; split 1:100. The GC/MS unit consisted of Trace DSQ. The mass parameters included a 70-eV electron impact ionization value and a maximum spectral acquisition rate of 500 spectra per second and ionization temperature were set at 220 ºC. Retention indices were calculated using retention times of n-alkenes, injected after the oil, at the same chromatographic condition. The compounds were successfully identified by comparison of the retention indices (RI, HP-5) with those reported in the literature together with comparing of their mass spectra to those in the Adams, NIST and Wiley libraries [8].

Animal treatments

Male Wistar rats were used throughout this study. The animals were obtained from the Pasteur Institute of Iran and maintained in the animal house facilities. Adult animals were 3 months of age and weighing 150±20 g. They were maintained on a commercial pellet food and tap water *ad libitum*. The animals were divided into 20 groups (n=5). In negative control group (NC), the CCL_4 vehicle, i.e., 3 ml/kg b.w olive oil and DMSO were injected. In control group (C), the DMSO simultaneously with carbon tetrachloride (CCL_4) dissolved in olive oil (1:1 ratio) was i.p injected at 3 ml/kg b.w. In the treatment groups, the essential oils prepared from the plant at 100 & 200 mg/kg b.w were diluted in DMSO and injected i.p simultaneously with CCL_4 administration.

Preparation of tissue homogenate and plasma

The heparinated blood samples were collected at different time intervals (2, 4, 8, 16 and 24 h after CCL_4 administration) by heart puncture from all the animals and centrifuged at 3000 × *g* for 10 min to obtain the plasma. Liver samples were immediately transferred to ice-cold containers and homogenized (20%, w/v) in the appropriate buffer using a homogenizer. The homogenates were used to measure the biochemical parameters.

Biochemical assays

Lipid peroxidation: A weighed portion of the liver was homogenized in phosphate buffer (100mM, pH 7.0) and used to measure the level of thiobarbituric acid reactive substances (TBARS) as indices for lipid peroxidation. Lipid peroxides are unstable and decompose to form a complex series of compounds including malondialdehyde (MDA). The concentration of TBARS was measured spectrophotometrically using TBA reagent based on the procedure described by Buege and Aust (1978) [9]. Briefly, one volume of homogenate was mixed with 0.5 volume of trichloroacetic acid (15% w/v) and centrifuged at 200 × *g* for 10 min. One millilitre of the supernatant was mixed with 0.5 ml TBA (0.7% w/v) and boiled for 10 min. After cooling, the absorbance was recorded at 535 by spectrophotometer (Shimadzu UV-3100). MDA concentration was calculated using extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$.

Glutathione (GSH) estimation: GSH level was estimated in liver homogenate according to the procedure of Sedlak and Lindsay (1968) [10]. This procedure based on the non-enzymatic reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) DTNB and GSH leading to the production of a complex measured spectrophotometrically at 412 nm. Finally, the GSH level was calculated by plotting a standard curve of absorbance against different concentrations of GSH standard solution.

Glutathione S-transferase (GST) activity: Liver cytosolic GST was measured spectrophotometrically using CDNB (a general substrate) according to the procedures described by Habig et al. (1974) [11]. Briefly, GST activity leading to make binding between CDNB and GSH leading to the production of a complex measured spectrophotometrically at 340 nm. Finally, the specific activity was calculated based on the nmol/min//mg protein in samples which was measured by Bradford assay [12].

Ferric reducing ability of plasma (FRAP) assay: This assay was performed using TPTZ reagent as described by Benzie and Strain (1996) [13]. This method measures the ability of the antioxidants contained in the sample to reduce ferric-tripiridyltriazine (Fe3+-TPTZ) to a ferrous form (Fe2+) which absorbs light at 593 nm. In this reaction, the Fe3+ binds to a reagent, i.e., TPTZ that is then converted to Fe2+-TPTZ

complex in presence of plasma antioxidant factors. The ferro and ferric-iron form complex with TPTZ reagent are the main products of this reaction. FRAP level was calculated by plotting a standard curve of absorbance against μM/l concentration of Fe (II) standard solution.

Liver function tests: to confirm the liver function and injury, serum alanine transminase (ALT), aspirate transminase (AST) and alkaline phasphatase (ALP) were determined spectrophotometrically according to the procedure described in the kit purchased from the Pars Azmoon, Co, Iran.

Statistical analysis

Data are presented as means ± Standard Error of Mean (SEM) of five samples obtained from five animals in each group. The results were subjected to one-way ANOVA followed by Tukey's HSD using SPSS (version 19.0) software. Significant levels were defined as $P<0.05$. (*) denote significantly different from the respective negative control group ($P<0.05$). (**) denote significantly different from the respective control group ($P< 0.05$).

RESULTS

Essential oil analysis

The qualities and quantities compositions of the essential oil derived from the fruits of *H. persicum* presented in Table 1. Fourteen five compounds are identified in the essential oils with the major chemical components as hexyl butyrate (26.87**%), 2-ethylhexyl acetate** (18.16%) and **heptyl hexanoate** (8.91%) (Fig. 1).

The Effects of *H. persicum* essential oils on the hepatic antioxidant/oxidative stress parameters in rats treated by CCL_4

Administrations of CCL_4 to rats caused a significant increase in LP levels at 8-24 h as compared to the negative control groups ($P<0.05$). The LP levels were restored towards their normal values by treatment with essential oils ($P<0.05$) (Table 2). In addition, the FRAP values as an indicator of total antioxidant capacity of plasma were increased at 16 and 24 h after CCL_4 administration. The essential oil treatments could significantly maintain the FRAP values at normal levels (Table 3).

Hepatic GSH levels were depleted significantly ($P<0.05$) at all time intervals in CCL_4 treated rats as compared to control group. Also, the hepatic GSH levels could significantly reverse to normal levels after applications of the essential oils in compared to the control group ($P<0.05$) (Table 4). In addition, the liver GST activity which was decreased at 24h in control group elevated significantly after oil administrations ($P<0.05$) (Table 5).

The Effects of *H. persicum* essential oils on the hepatic function markers in rats treated by CCL_4

As indicated in Table 6&7, the activities of serum AST and ALT (marker enzymes for liver damage) in CCL_4 treated rats were significantly increased at all time intervals as compared to negative control group. The enzymes were decreased significantly after administration with *H. persicum* essential oils ($P<0.05$). Whereas, the ALP activities maintained in normal values in all groups at 8, 16 and 24 h after CCL_4 treatments ($P>0.05$) (Table 8).

DISCUSSION

Recently, there is a great demand to find a suitable hepatoprotective agent among the natural products especially medicinal plants against toxic chemicals and drugs induced liver damage. So, the present study –for the first time- was designed to evaluate the potentiality of *H. persicum* essential oils in restoration the equilibrium in the oxidative stress/antioxidant system in the liver of rats treated with CCL_4.

CCl_4 is one of the most widely used hepatotoxins in the experimental study of liver diseases [14]. CCl_4 undergoes metabolism in the liver through cytochrome P_{450} system (phase I) to form the reactive metabolic trichloromethyl radical ($\bullet CCl_3$) and trichloromethyl peroxy radical ($\bullet OOCCl_3$). These free radicals can bind with polyunsaturated fatty acids to produce free radicals leading to the generation of lipid peroxides that finally induce hepatic injury [15, 16]. Lipid peroxidation is one of the major characteristics that can be included as an oxidative damage marker. MDA is widely used as marker of lipid peroxidation in the development of CCl_4-mediated hepatotoxicity [17]. In our study, elevations in the level of LP in liver of rats treated with CCl_4 were observed (Table 2) that leads to tissue damage and failure of antioxidant defense mechanisms [18] In addition, the FRAP value as a routine procedure for measuring antioxidant activity according to ferrous ion production raised unusually in the group administrated with CCl_4 (Table 3).

Fig1: Choromotpgram of the compounds of *H. persicum* essential oils.

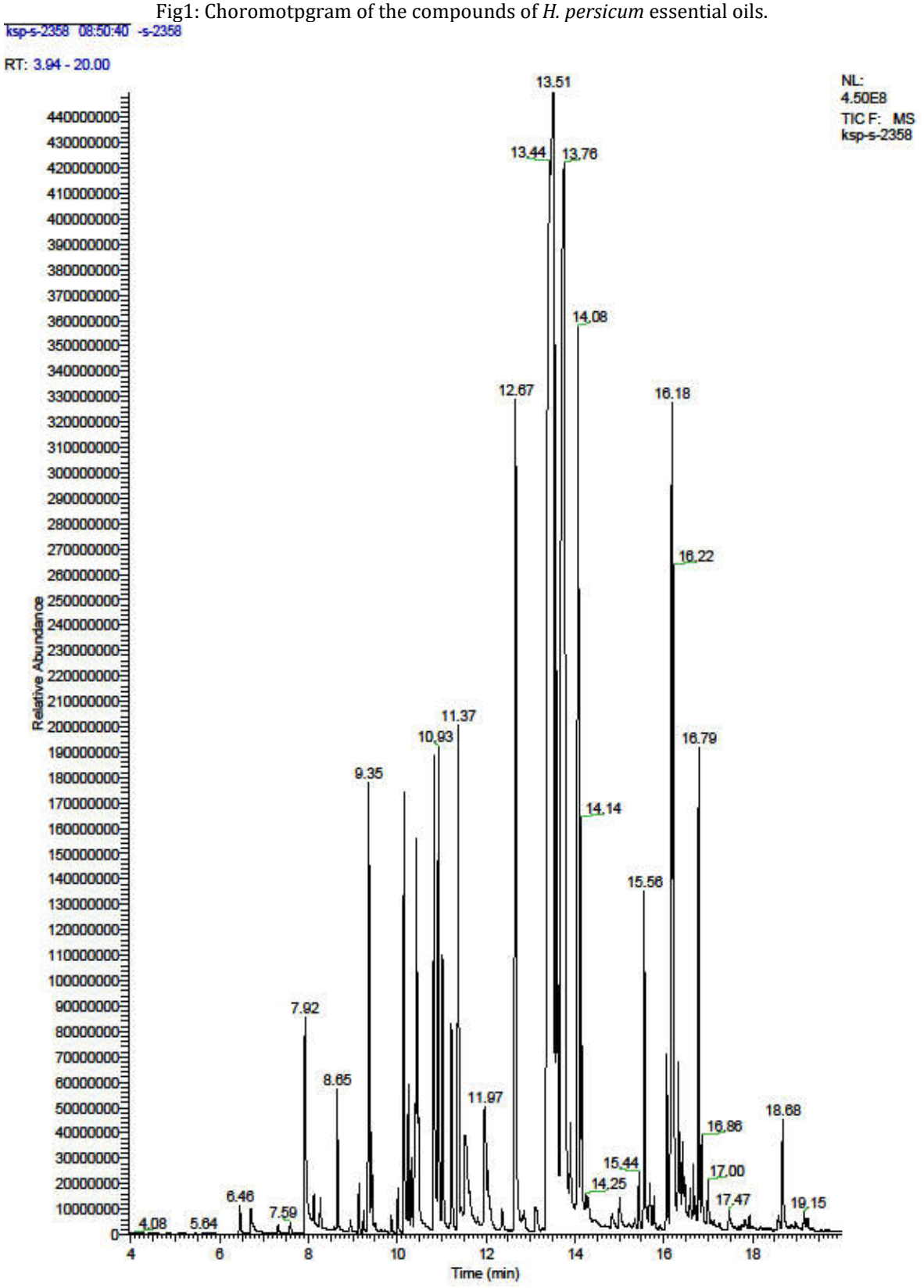

Table 1. Chemical compositions of the *H. persicum* essential oils

No	Synonyms chemical names of compounds	IUPAC names	RI	%
1	isobutyric acid isopropylester	isobutyric acid isopropyl ester	788	0.14
2	hexanal	hexanal	803	0.22
3	butanoic acid 1-methyl ester	methyl butyrate	839	0.05
3	isobutyric acid propylester	propyl isobutyrate	855	0.06
4	hexanol	1-hexanol	875	1.51
5	hexyl 2-methyl butanoate	hexyl 2-methylbutyrate	886	0.12
6	isopropyl isopentanoate	isopropyl isovalerate	895	0.15
7	butyric acid iso butylester	butanoic acid, 2-methylpropyl ester	915	0.66
8	α-thujene	α-thujene	929	0.07
9	α-pinene	α-pinene	939	0.2
10	propyl 2-methylbutyrate	propyl 2-methylbutanoate	943	0.12
11	butyl2-methyl propionate	2-methylbutyl propanoate	949	1.97
12	butanoic acid 2-methyl propyl ester	isobutyl butyrate	952	0.31
13	camphene	camphene	957	0.04
14	sabinene	sabinene	974	0.11
15	β-pinene	β-pinene	981	0.24
16	butanoic acid butyl ester	n-butyl butanoate	988	1.93
17	butyl 2-methyl butyrate	butyl 2-methylbutanoate	993	0.56
18	isovaleric acid isobutyl ester	isovaleic acid isobutyl ester	996	0.27
19	octanal	octanal	1003	3.25
20	p-cymene	p-cymene	1029	2
21	butyl 2-methyl butanoate	n-butyl 2-methylbutyrate	1036	2.22
22	1-butyl isovalerate	butyl isovalerate	1042	1.38
23	3-methyl butyl butanoate	**3-methylbutyl butyrate**	1056	1
24	gamma-terpinene	gamma-terpinene	1066	2.36
25	1-octanol	1-octanol	1077	1.29
26	propionic acid hexyl ester	**hexyl propionate**	1106	0.62
27	3-methyl-butyric acid 3-methyl-butyl ester	isopentyl 3-methyl butanoate	1111	0.28
28	2-butanoic acid , 3methyl -butyl ester	2-butanoic acid , 3methyl -butyl ester	1131	0.21
29	1-hexyl isobutyrate	HEXYL ISOBUTYRATE	1152	5.77
30	acetic acid - decyl ester	acetic acid - decyl ester	1180	0.24
31	butanoic acid hexyl ester	hexyl butyrate	1201	26.87
32	1-ethenyl hexyl butanoate	1-ethenyl hexyl butanoate	1210	2.44
33	β-ethyl hexyl acetate	**2-ethylhexyl acetate**	1223	18.16
34	3-decen-1-ol	3-decen-1-ol	1232	0.31
35	butyric acid 2-methyl hexyl ester	butyric acid 2-methyl hexyl ester	1245	6.05
36	hexyl-3methylbutanoate	hexyl-3methylbutanoate	1249	1.53
37	3-octen-1-ol	3-octen-1-ol	1339	0.24
38	octyl 2-methyl propionate		1347	1.5
39	hexanoic acid hexyl ester	HEXYL HEXANOATE	1356	0.24
40	ethenyl hexyl butanoate	ethenyl hexyl butanoate	1384	0.77
41	hexanoic acid heptyl ester	**heptyl hexanoate**	1391	8.91
42	octyl 2-methyl butanoate	octyl 2-methylbutyrate	1437	2.39
43	isovaleric acid octyl ester	Octyl 3-methylbutyrate	1443	0.24
44	2-ethyl cyclohexanol	**2-ethylcyclohexanol**	1453	0.33
45	hexanoic acid octyl ester	octyl hexanoate	1587	0.59
Total				99.92

Table 2: Effects of *H. persicum* E.O on LP levels (nmol/L/gr tissue) at different time intervals

Groups	4h	8h	16h	24h
NC	13.8±1.9	14.2±1.7	12.9±1.5	13.4±1.9
C	15.6±2.1	20.5±3*	22±2.7*	51±6.2*
E.O 100	13.95±1.7	16.7±1.9**	17.4±1.8**	24.9±2.7**
E.O 200	17.35±1.9	15.8±2.1**	16.9±1.9**	22.3±2.8**
BHT	14.2±1.9	16.9±1.8**	15.3±1.3**	18.7±2.1**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 3: Effects of *H. persicum* E.O on FRAP levels (µmol/L) at different time intervals

Groups	4h	8h	16h	24h
NC	446±45	470±35	480±40	460±50
C	540±50	530±47	622±70*	859±90*
E.O 100	498±41	550±60	497±53**	578±59**
E.O 200	510±57	494±51	511±49**	455±52**
BHT	491±53	421±47**	435±51**	390±42**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 4: Effects of *H. persicum* E.O on GSH levels (µmol/ml/gr tissue) at different time intervals

Groups	4h	8h	16h	24h
NC	20.6±1.7	19.1±1.8	21.2±2.2	19.4±1.5
C	10.9±1.2*	11.1±1.4*	10.9±0.8*	11.62±11*
E.O 100	24.8±2.5**	20.75±2.3**	23.6±2.9**	23.7±1.8**
E.O 200	21.1±2.3**	21.5±2.6**	22.1±1.7**	24.4±1.9**
BHT	20.7±2.3**	24±1.8**	25±2.4**	23.5±2.6**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 5: Effects of *H. persicum* E.O on GST activities (nmol/min/gr tissue) at different time intervals

Groups	4h	8h	16h	24h
NC	1094±170	1120±140	1042±120	1160±170
C	1181±130	1113±160	847±110	516±95*
E.O 100	1035±150	1143±120	1154±160**	985±120**
E.O 200	1135±140	1160±170	1201±160**	1061±130**
BHT	1095±110	1148±130	968±120	1113±130**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 6: Effects of *H. persicum* E.O on AST activities (U/L) at different time intervals

Groups	4h	8h	16h	24h
NC	73.2±11.4	70.1±10	72.3±11.2	73.6±10.8
C	543±13.6*	459±75*	237±4.3*	283±43*
E.O 100	387±23**	300±15**	192±21**	224±17
E.O 200	355±23**	301±38**	175±40**	197±19**
BHT	375±35**	299±23**	179±19**	145±24**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 7: Effects of *H. persicum* E.O on ALT activities (U/L) at different time intervals

Groups	4h	8h	16h	24h
NC	29.5±2.7	31.7±2.9	30.4±3.2	29.1±2.7
C	242±60*	239±40*	126±12*	122±2.2*
E.O 100	64.5±11**	94.2±13.9**	84±10**	95±4.9**
E.O 200	82±14**	106±11**	84±11**	93±21**
BHT	69±13**	107±21**	99±13.2**	94±13**

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Table 8: Effects of *H. persicum* E.O on ALP activities (U/L) at different time intervals

Groups	4h	8h	16h	24h
NC	258±19	249±20	271±23	245±21
C	221±23	273±23	274±26	238±27
E.O 100	257±24	267±29	289±17	242±19
E.O 200	220±23	220±21	223±25	248±24
BHT	245±23	257±21	269±22	269±24

NC: Negative control group; C: Control group; E.O: *H. persicum* essential oil (100 & 200 mg/kg b.w). *P<0.05 is considered significantly between Negative control group (NC) and Control group (C) **P<0.05 is considered significantly between Control group (C) and *H. persicum* essential oil

Free radicals also affect the antioxidant defense mechanisms, reduce the intracellular concentration of GSH that it plays a protective role in tissue by detoxification of xenobiotics and is essential to maintain structural and functions integrity of the cell [19, 20]. GST as a biotransformation enzyme in phase II involved in the detoxification of xenobiotics by conjugating these toxic substances with GSH [21]. A significant reduction of GST activity together with GSH depletion was shown in the groups administrated by CCl_4 as compared to negative control (Tables 4&5). Other study has also reported the similar rise in LP level and fall in the GSH content on CCl_4 intoxication [18]. Furthermore, a remarkable increase of plasma AST and ALT activities due to the damage of hepatic cells (Tables 6&7) in the CCl_4 group in compared to negative control group was indicated. Serums AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases [22]. Because, during hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage [23]. Other studies also showed the disturbed oxidative stress/antioxidant statues as a significant increase in LP level and ALT, AST, and ALP activities by CCl_4 involved in hepatotoxicity [24].

On the other hand, diet antioxidants supplied protective effects against CCl_4-induced liver damage throughout blocking the oxidative chain reaction and suppressing the formation of lipid peroxidant products [5, 25]. In our study, pre-treatments of rats with *H. persicum* oils indicated significant reduction in CCl_4-induced elevations of MDA (Table 2). In addition, increased FRAP level in CCL_4 treated rats-modulated in essential oil treatments- (Table 3), indicating compensatory increased of plasma antioxidants which is leaded to increased resistance and/or decreased susceptibility of the liver to free radical attack. Besides that, GSH is recognized to be an antioxidant substance, playing a significant role against CCl_4-induced injury by covalently binding to its reactive metabolite. This is considered as the initial reactant in the chain reaction of oxidation, and then result in the lipid peroxidation and the cell membrane disruption [26-28]. Pre-treatment with *H. persicum* essential oils (100 and 200 mg/kg.bw) resulted in hepatic GSH and GST elevations (Table 4&5) using to monitor the equilibrium in oxidative stress/antioxidant statue and chemopreventive ability [29]. These results are agreement with previous research found that the treatment of rats with essential oils extracted from Dill (*Anethum graveolens L.*) and Fennel (*Foeniculum vulgare*) could significantly reverse the changes of the biochemical parameters such as AST and ALT activities and MDA level induced by CCl_4-hepatoxcity in rats that it was mentioned the mechanism of hepatoprotection of the oil is due to its antioxidant effect of their active ingredients [30]. Other studies also indicated that the administration of *sonchus asper*, silymarin and tea seed oil significantly lowered the CCl4-induced serum levels of hepatic marker enzymes (AST and ALT) and LP levels that were increased by CCl4. The hepatic contents of glutathione and activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase were compensated by plant treatments [5, 31]. One study also indicated the hepatoprotective and antioxidant

effects of Licorice extract against CCl4-Induced oxidative damage in rats through inhibiting the elevated AST, ALP and ALT activities and malondialdehyde (MDA) level. It also enhanced the liver antioxidant parameters such as glutathione S-transferase (GST) activities and glutathione (GSH) level, reduced malondialdehyde (MDA) level [32].

These results indicated that *H. persicum* essential oils with the major antioxidant components as hexyl butyrate (26.87%), 2-ethylhexyl acetate (18.16%) and heptyl hexanoate (8.91%) (Table 1) may enhance the innate mechanisms of the antioxidant system or provide its antioxidant capacity against CCl_4-induced oxidative stress. Indeed, the biological activities *e.g.* antioxidant, antimicrobial, antifungal and immunomodulatory activities of the oil may associate to the presence of the main compounds [33]. Previous researches also confirmed that the oxidative damage through free radical generation involved in hepatotoxic effect of CCl_4 could be relieved by natural compounds possessed antioxidant activity [34, 35]. Moreover, these compounds protect cell against the damaging effect of reactive oxygen species such as singlet oxygen, superoxide, proxy radicals, hydroxyl radicals and peroxynitrite [36].

In other hands, other study reported hexyl butyrate (56.5%), octyl acetate (16.5%), hexyl 2-methylbutanoate (5.2%), hexyl isobutyrate (3.4%) and n-octyl 2-methylbutyrate (1.5%) as the major constituents of the oil derived from fruits of *H. persicum* collected from the north of Tehran [6]. Generally, hexyl butyrate and octyl acetate were the main constituents of the oils of *H. persicum* fruits [37]. In fact, the differences among chemical compositions of the essential oil depend on many factors, such as type of cultivar, harvest time, extraction methods, geographic variations, storage conditions, environmental conditions and ecological conditions [38].

CONCLUSION

The results indicated that *H. persicum* essential oils could modulated the oxidative stress/antioxidant disturbance in CCL_4 treated rats. This may be contributed to the essential oils as a potent herbal medicinal compound.

DECLARATION OF INTEREST

This research was conducted by the research deputy grant of Qom Branch, Islamic Azad University.

REFERENCES

1. Pandit, A., Sachdeva, T. & Bafna, P. (2012). Drug-Induced Hepatotoxicity: A Review. J Appl Pharm Sci., 2 (5): 233-243.
2. Jemal, A., Siegel, R. & Ward, E. (2007). Cancer statistics. Cancer J Clin., 57: 43–66.
3. Guven, A., Guven, U. & Gulmez, M. (2003). The effect of kefir on the activities of GSH, GSHPX, GST, CAT, GSH and LPO levels in carbon tetrachloride-induced mice tissue. J Vet Med., 50:412–416.
4. Recknagel, R.O., Glende, E.A., Jr. Dolak, J.A. & Waller R.L. (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol Therapeut., 43: 139–154.
5. Lee, C.P. Shih, P.H. Hsu, C.L. Yen, G.C. (2007). Hepatoprotection of tea seed oil (Camellia oleifera Abel.) against CCl4-induced oxidative damage in rats. Food Chem Toxicol., 45: 888–895.
6. Hajhashemi, V., Sajjadi, S.E. & Heshmati, M. (2009). Anti-inflammatory and analgesic properties of *Heracleum persicum* essential oil and hydroalcoholic extract in animal models. J Ethnopharmacol., 124: 475–480.
7. Firuzi, O., Asadollahi, M., Gholami, M. & Javidnia K. (2010). Composition and biological activities of essential oils from four Heracleum species. Food Chem., 122: 117–122
8. Adams R.P. (1995). Identification of Essential Oil Components by Gas Chromato- graphy/ Mass Spectroscopy. Allured Publishing Co. Carol Stream, Illinois.
9. Buege, J.A. & Aust, S.D. (1978). Microsomal lipid peroxidation. In: Packer, L. (Ed.). Method Enzymol., 52: 302–310.
10. Sedlak, J. & Lindsay, R.H. (1968). Estimation of total protein with bound and non- protein sulfhydryl groups in tissues with Ellman's reagent. Anal Biochem., 25: 192–205.
11. Habig, W.H., Pabst, M.J. & Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem., 25:7130–7139.
12. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 72: 248.
13. Benzie, I.F.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem., 239: 70–76.
14. Johnson, D.E. & Kroening, C. (1998). Mechanism of antioxearly carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol. Toxicol., 83: 231-239.
15. Weber, L.W. Boll, M. & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Cr Rev Toxicol., 33:105–136.
16. Ogeturk, M., Kus, I., Colakoglu, N., Zararsiz, I., Ilhan N. & Sarsilmaz, M. (2005). Caffeic acid phenyl ester protects kidney against carbon tetrachloride toxicity in rats. J Ethnopharmacol., 97:273–280.

17. Mansour, M.A. (2000). Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. Life Sci., 66: 2583–2591.
18. Bose, O., Gupta, M., Mazumder, U.K., Kumar, R.S., Sivakumar, H. & Kumar, R.S. (2007). Hepatoprotective and Antioxidant Effects of E*upatorium ayapana* against Carbon Tetrachloride nduced Hepatotoxicity in Rats. Iranian J Pharmacol Ther*.,* 6 (1): 27-33.
19. Sengottuvelu, S., Duraisami, S., Nandhakumar, J., Duraisami, R. & Vasudevan, M. (2008). Hepatoprotective Activity of *Camellia sinensis* and its Possible Mechanism of Action, IJPT., 7 (1), 9-14.
20. Nasiri Bezenjani, S., Pouraboli Malekpour Afshar, *R. &* Mohammadi *G. (2012).* Hepatoprotective Effect of *Otostegia persica* Boiss. Shoot Extract on Carbon Tetrachloride-Induced Acute Liver Damage in Rats. IJPR., 11 (4): 1235-1241.
21. Henderson, C.J., Wolf, C.R., Kitteringham, N., Powell, H., Otto, D. & Park B.K. (2000). Increased resistance to acetaminophen hepatotoxicity in mice lacking glutathione S-transferase Pi. Proc. Natl. Acad. Sci. U.S.A. 97: 12741-5.
22. Pari, L. & Kumar, A.N. (2002). Hepatoprotective activity of *Moringa Oleifera* on antitubercular drug induced liver damage in rats. J. Med., 5: 171-177.
23. Pari, L. & Murugan, P. (2004). Protective role of tetrahydrocurcumin against erythromycin estolate-induced hepatotoxicity. Pharmacol. Res., 49: 481-486.
24. Li, R., Guo, W., Fu, Z., Ding, G., Zou, Y. & Wang, Z. (2011). Hepatoprotective Action of *Radix Paeoniae Rubra* Aqueous Extract against CCl4-Induced Hepatic Damage. Molecules., 16: 8684-8693.
25. Sheweita, S.A., Abd ElGabar, M. & Bastawy, M. (2001). Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: role of antioxidants. Toxicology., 165: 217–224.
26. Szymonik lesiuk, S., Czechowska, G., StryjeckaZimmer, M., Słomka, M., Madro, A., Celinski, K. & Wielosz, M. (2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J Hepato-Biliary-Pancreatic Surg., 10:309–315.
27. Preethi K.C., Kuttan R. (2009). Hepato and reno protective action of Calendula officinalis L. flower extract. *Indian J Exp Biol.* 47:163–168.
28. Brattin, W.J., Glende, E.A.Jr. & Recknagel, R.O. (1985). Pathological mechanisms in carbon tetrachloride hepatotoxicity. Free Radical Bio Med. 1: 27–38.
29. Hatono, S., Jimenez, A. & Wargovich, M.J. (1996). Chemopreventive effect of S-allylcysteine and its relationship to the detoxification enzyme glutathione S-transferase. Carcinogenesis., 17: 1041–1044.
30. Rabeh, N.M. & Aboraya, A.O. (2014). Hepatoprotective Effect of Dill (*Anethum graveolens* L.) and Fennel (*Foeniculum vulgare*) Oil on Hepatotoxic Rats. PJN., 13 (6): 303-309.
31. Khan, R.A., Khan, M.R., Sahreen, S. & Shah, N.A. (2012). Hepatoprotective activity of Sonchus asper against carbon tetrachloride-induced injuries in male rats: a randomized controlled trial. BMC Complement Altern Med., 12:90
32. Huo, H.Z., Wang B., Liang Y.K., Bao Y.Y. & Gu Y. (2011). Hepatoprotective and antioxidant effects of Licorice extract against CCl4-Induced Oxidative Damage in Rats. Int J Mol Sci., 12: 6529-6543;
33. [33]. Hemati, A., Azarnia, M. & Angaji, A. (2010). Medicinal effects of *Heracleum persicum* (Golpar). Middle East J Sci Res., 5(3), 174-176.
34. Lai, J.T., Hsieh, W. T. & Fang, H. L., Lin W. C. (2009). The protective effects of a fermented substance from Saccharomyces cerevisiae on carbon tetrachloride-induced liver damage in rats. Clin. Nutr*., 28*: 338-345.
35. Domitrović, R., Jakovac, H., Romić, Ž., Rahelić, D. & Tadić Ž. (2010). Antifibrotic activity of Taraxacumofficinale root in carbon tetrachloride-induced liver damage in mice. J. Ethnopharmacol., 130: 569-577.
36. Skrzydlewska, E., Ostrowska, J., Farbisxewsk, R. & Michalak. (2002). Protective effect of green tea against lipid peroxidation in the rat liver, blood and brain. Phytomedicine*.,* 9: 232-8.
37. Scheffer JJC., Hiltunen R., Aynehchi Y., Von Schantz M., Baerheim Svendsen A. (1984). Composition of the essential oil of *Heracleum persicum* fruits. *Planta Med.* 50: 56-60.
38. Sefidkon, F., Bahmanzadegan, A. & Assareh, M.H. (2008). The effect of distillation methods and harvesting times on the volatile oil and cineole content of Eucalyptus dealbata. Essent. Oil Bearing Plants., 11(3): 242–251.