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Original Article

Composition and Anti-Toxicity Effects of *Cichorium intybus* Distillate on Serum Antioxidant Status in Carbon Tetrachloride-Treated Rats

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Abstract

The role of oxidative stress in female fertility is a compelling area for research. According to traditional medicine, *Cichorium intybus*, known as Kasni, is believed to improve fertility. For this purpose, the effects of *C. intybus* distillate (CI) on blood antioxidant status were assessed in rats with carbon tetrachloride (CCl4)-induced toxicity. The rats were assigned to four experimental groups of Control, CI, CCl4, and CI+CCl410 (n=10 in each group). The level of antioxidant enzymes, such as glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT), as well as lipid peroxidation and reduced glutathione (GSH) level, were measured in serum samples. In the second part of the study, the antioxidant activity and phytochemical composition of the hydrodistillate of *C. intybus* aerial parts were determined by DPPH radical scavenging and gas chromatography-mass spectrometry analysis, respectively. The administration of CCl4 decreased the enzyme activities of GPx, GR, and CAT which were significantly ameliorated after CI administration. The decreased level of serum GSH following CCl4 administration was not considerably elevated in the CI+CCl4 group. Furthermore, the level of malondialdehyde in the serum of CI+CCl4 rats was decreased, compared to the CCl4 group. The main compositions of the essential oil from the *C. intybus* distillate were the antioxidants of Pulegone (8.10%), Piperitenone (7.68%), dihydroactinidiolide (5.0%), and carvone (4.18%). The antioxidant activity of the distillate was obtained at 75µg/l using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test. In general, the results of the present study demonstrated that *C. intybus* distillate, as a safe herbal remedy, can attenuate CCl4-induced oxidative damages via boosting the endogenous antioxidant defense system.

Keywords: Chicory, Catalase, Glutathione, Malondialdehyde, Mass spectrometry

Composition et Effets Anti-Toxicité du Distillat de *Cichorium intybus* sur le Statut Antioxydant Sérique chez les Rats Traités au Tétrachlorure de Carbone

Résumé: Le rôle du stress oxydatif dans la fertilité féminine est un domaine de recherche incontournable. Selon la médecine traditionnelle, *Cichorium intybus*, connu sous le nom de *Kasni*, améliorerait la fertilité. À cette fin, les effets du distillat de *C. intybus* (CI) sur le statut antioxydant du sang ont été évalués chez des rats présentant une toxicité induite par le tétrachlorure de carbone (CCl4). Les rats ont été affectés à quatre groupes expérimentaux de contrôle, CI, CCl4 et CI+CCl410 (n=10 dans chaque groupe). Le niveau d'enzymes antioxydantes, telles que la glutathion peroxydase (GPx), la glutathion réductase (GR) et la catalase (CAT), ainsi que la peroxydation lipidique et le niveau de glutathion réduit (GSH), ont été mesurés dans des échantillons de sérum. Dans la deuxième partie de l'étude, l'activité antioxydante et la composition phytochimique de

l'hydrodistillat des parties aériennes de *C. intybus* ont été déterminées respectivement par piégeage de radicaux DPPH et analyse par chromatographie en phase gazeuse-spectrométrie de masse. L'administration de CCl4 a diminué les activités enzymatiques de GPx, GR et CAT qui ont été considérablement améliorées après l'administration de CI. La diminution du taux sérique de GSH après l'administration de CCl4 n'a pas été considérablement élevée dans le groupe CI+CCl4. De plus, le niveau de malondialdéhyde dans le sérum des rats CI+CCl4 a été diminué par rapport au groupe CCl4. Les principales compositions de l'huile essentielle du distillat de *C. intybus* étaient les antioxydants de Pulegone (8.10%), de pipériténone (7.68%), de dihydroactinidiolide (5.0%) et de carvone (4,18%). L'activité antioxydante du distillat a été obtenue à 75 ug / l en utilisant le test DPPH (2.2-diphényl-1-picryl-hydrazyl-hydrate). En général, les résultats de la présente étude ont démontré que le distillat de C. *intybus*, en tant que remède à base de plantes sans danger, peut atténuer les dommages oxydatifs induits par CCl4 en stimulant le système de défense antioxydant endogène.
Mots-clés: Chicorée, Catalase, Glutathion, Malondialdéhyde, Spectrométrie de Masse

Introduction

Reactive oxygen species (ROS) can be produced by the normal metabolism of cells or environmental factors (Mulla et al., 2018). Oxidative stress (OS) may result in multiple reproductive pathologies, such as polycystic ovarian syndrome, endometriosis, spontaneous abortion, infertility, and prenatal disorders (Darché et al., 2017).

The natural antioxidants in the body consist of both non-enzymatic and enzymatic systems, including catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione reductase (GR), (Gupta et al., 2006; Showell et al., 2017). Nowadays, high costs of treatment and safety problems have stirred up growing interest in the administration of alternative medicine for reproductive disorders (Ahangarpour et al., 2014).

Traditional medicine is considered a safe and natural therapeutic alternative among the public (Rashidi et al., 2017). Plant antioxidants have gained much attention for their ability to protect the cells from oxidative stress and are widely used for reproductive purposes (H Sekhon et al., 2010; Darché et al., 2017). There are various methods of traditional medicine preparation, including extraction, purification, fractionation, fermentation, concentration, and distillation.

Distillation products are among the most favorite drinks widely used in Iran, especially in Shiraz (Gohari et al., 2017). *Cichorium intybus L.* (Chicory or Kasni) belongs to the genus *Cichorium L.* and the *Asteraceae* family. The polyphenols-rich fraction of the plant exhibits antioxidant activity and inhibits hydrogen peroxide (Das et al., 2016). Chicory plants or extract are extensively used for the treatment of various diseases and improvement of reproductive organ status (Saric-Kundalic et al., 2011). *C. intybus* distillate (CI) has also been used for reproductive aspects of health in different regions of Iran. Nonetheless, the possible effects of CI on antioxidant status, as well as distillate composition, have not yet been investigated scientifically.

Therefore, to evaluate the effect of CI on antioxidant status in females, the present study aimed to assess the activity level of antioxidant enzymes, including malondialdehyde (MDA) and glutathione (GSH), in the serum of carbon tetrachloride (CCl4)-treated female rats and the chemical composition of *C. intybus* hydro-distillate.

Material and Methods

Preparation of *Cichorium intybus* Distillate. *Cichorium intybus* was collected from farms around Kashan, Isfahan province, Iran, and the genus and species were approved at the Herbarium of the Department of Botany, University of Isfahan, Iran. To obtain 1 liter of CI, 87.5 g of the dried plant was placed in a boiler with 1.75 liters of water as previously

described (Seghatoleslam et al., 2014). The outgoing steam was cooled, collected, and kept light-protected at 4ºC until use.

Extraction of Essential Oil. The essential oil was obtained from dried powder (100 g) of the aerial parts of *C. intybus* that was subjected to steam distillation using a Clevenger-type apparatus for 3h. Subsequently, the volatile fraction was isolated by hexane, dried by anhydrous sodium sulfate, and stored at 4°C in a closed vial until use for gas chromatography-mass spectrometry (GC/MS) analysis

Gas Chromatography-Mass Spectrometry Analysis. The oil was analyzed by GC/MS using Agilent technologies model 7890 B connected to a 5977A MSD. The separation was carried out by HP-5MS capillary column (5% phenyl methyl polysiloxane, 30 m×0.25 mm, and film thickness 0.25µm). The carrier gas was helium at a flow rate of 1ml/min (split; 10:1). The mass spectrometer was acquired in EI mode with ionization energy of 70 eV in a mass range of 50-550 m/z. The column temperature was maintained at 60°C for 4 min and then increased to 280°C at a rate of 5ºC/min and held at 280°C for 2 min. A sample volume of 50µl was diluted with 1000µl of hexane. Furthermore, 1µl was injected with a running time of 50 min. The components were identified based on a comparison with NIST (05a.L) and Willey (nl7) libraries spectra and the literature.

DPPH scavenging assay

The ability of the essential oil to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was assessed according to Moon et al. (Moon and Shibamoto, 2009). The oil IC_{50} value was calculated using an inhibition curve.

Animals. Adult female Sprague Dawley rats (180–200g) were obtained from Animal Breeding Center, Shiraz University of Medical Science, Shiraz, Iran. They were kept under standard conditions (12:12h light⁄dark, 25-35% humidity, and 20-22°C). All procedures were approved by the Institutional Animal Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC). To determine whether they had regular cycles, vaginal smears were obtained from all the rats before any drug administration.

Experimental Protocol. In this experimental study, 40 female rats were assigned to four groups: Control (receiving oral saline for four weeks), CI group (receiving 12.5 ml/kg/day CI orally for four weeks), CCl_4 group (receiving 1 ml/kg body weight CCl_4 via intraperitoneal (IP) injection twice a week for two weeks), and CCl_4+CI group (receiving both CI and 1 ml/kg of CCl_4 for two weeks). At the end of the experiment, all rats were anesthetized, blood samples were collected by cardiac puncture, and the sera were kept at -80°C for biochemical analysis.

Liver Function Tests. Liver injury induced by CCl_4 was evaluated by the measurement of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) enzymes activities using Biorex kit (Shiraz, Iran).

Measurement of Malondialdehyde Concentration. The MDA concentration was evaluated by a colorimetric method as previously described (Mashhoody et al., 2014). It was calculated in µmol/mg protein using 1,1,3,3-Tetraethoxypropane as a standard.

Measurement of Glutathione Concentration. GSH assay with DTNB [5, 5′-dithiobis-(2-nitrobenzoate)] dye was performed, followed by a standard Ellman's method with some modifications to evaluate GSH in µmol/mg protein (Mashhoody et al., 2014). The absorbance of the products was observed at 412 nm after 5 min.

Determination of Glutathione Peroxidase Activity. The GPx activity was measured using the method of Fecondo and Augusteyn with minor changes (Zal et al., 2014). The enzyme activity was expressed as mU/mg of the protein using the molar extinction coefficient of 6.22×10^6 M^{-1} cm^{-1} for NADPH.

Determination of Catalase Activity. CAT activity was estimated by monitoring H_2O_2 decomposition using the procedure of Aebi with minor modifications (Yarahmadi et al., 2017). It was expressed as mmol of H_2O_2 consumed per min/mg of protein using the molar extinction coefficient of 43.6/ M/cm for H_2O_2.

Determination of Glutathione Reductase Activity. GR activity was examined using the method of Carlberg and Mannervik with some modifications (Zal et al., 2018). GR catalyzes the reduction of GSSG to GSH using NADPH for the reduction of the GSSG molecule. The results were based on the molar extinction coefficient of $6.22\times10^6\ M^{-1}cm^{-1}$ for NADPH.

Statistical Analysis. Statistical analysis was performed in SPSS software (version 19), and the graphs were obtained using GraphPadPrism5 software (San Diego, CA, USA). Curve expert 1.3 was used for IC_{50} values. The data were depicted as mean±SEM. One-way ANOVA and Tukey's post-hoc tests were used for between-groups comparisons (n=10). A p-value less than 0.05 was considered statistically significant.

Results

Chemical Composition of *C. intybus*. The chemical composition of CI is presented in Table 1. A total of 68 compounds were identified using the GC-MS analytical method and literature comparison. They were mainly antioxidants, such as terpene and terpenoid, as well as flavonoid and phenolic compounds.

Antioxidant Activity. The IC_{50} value of chicory hydrodistillate was found to be 75µg /l using the DPPH method.

Liver Function Tests. As suggested by the results, the activities of ALT and AST were significantly increased in the serum of CCl_4-treated rats, compared to those in the control group. The administration of CI decreased the levels of these liver enzymes ($P<0.05$; Figure1 A and B).

Antioxidant Enzyme Activity. As displayed in Figure 2A, the GPx activity significantly decreased in the CCl_4 group, compared to that in the control group ($P<0.05$). Nevertheless, CI significantly increased the activity of GPx (approximately 33%), in comparison with that in the CCl_4 group ($p<0.05$).

A significant reduction was observed in GR activity ($P<0.05$) by CCl_4 administration, and CI administration ameliorated ($P<0.05$) its activity (Figure 2B). Moreover, the CAT level significantly reduced in the CCl_4 group, and it was restored ($P<0.05$) by CI treatment (Figure 2C). Nonetheless, the CI did not change the activities of enzymes in normal rats.

Glutathione and Malondialdehyde Levels. As illustrated in Figure 3, the administration of CCl_4 decreased GSH levels, while no significant change was observed in the CI-treated group. The rats treated with CCl_4 showed significantly increased levels of MDA (48%), compared to the control group (Figure 4). Moreover, the administration of CI in the CCl_4-treated group ameliorated the adverse effects of CCl_4 ($P<0.05$).

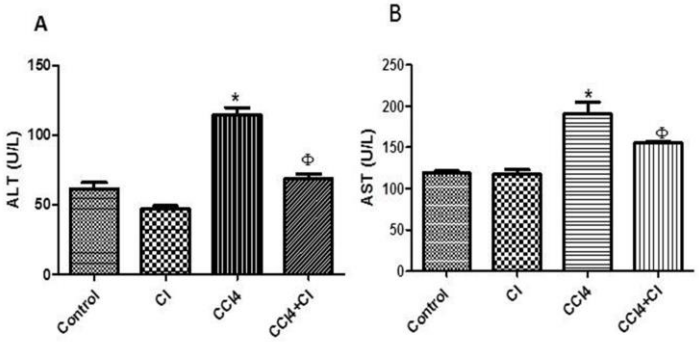

Figure 1. Liver function enzyme activities in female rats (n=10): ALT (A) and AST (B). The control group received saline, the CI group received *Cichorium intybus* distillate orally for four weeks, the CCl_4 group received 1 ml/kg BW CCl_4 via IP injection twice a week for two weeks, and the CCl_4+CI group received CI and 1 ml/kg BW of CCl_4 for two weeks. * stands for $P<0.05$ compared to the control group and Φ for $P<0.05$, in comparison with the CCl_4 group.

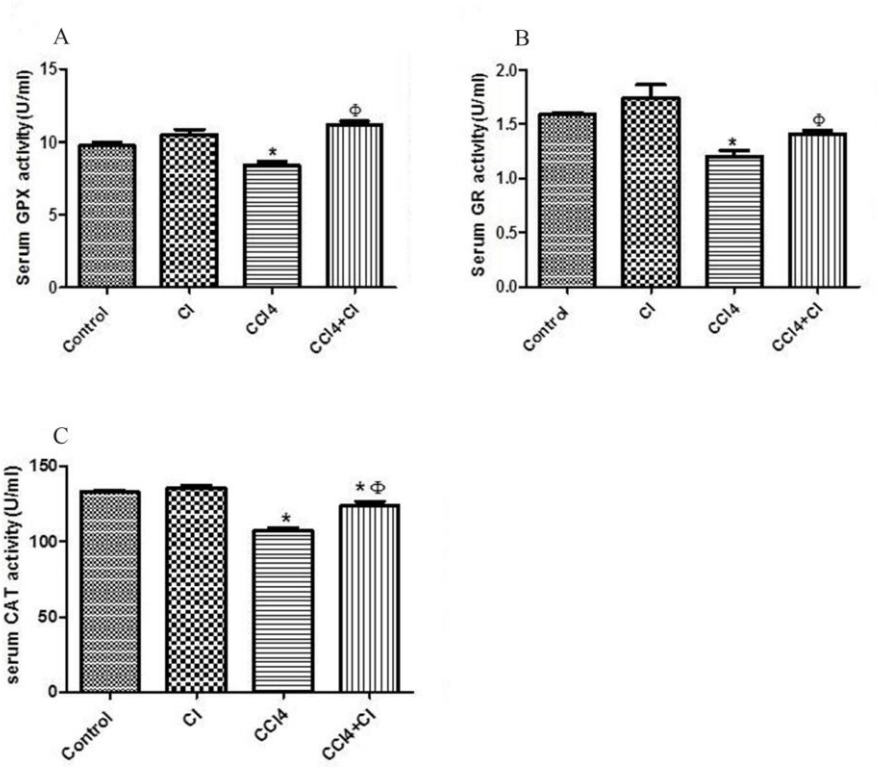

Figure 2. Serum enzyme activity in female rats (n=10): Glutathione peroxidase (GPx) (A), glutathione reductase (B), and catalase (C). The control group received saline, the CI group received *Cichorium intybus* distillate orally for four weeks, the CCl_4 group received 1 ml/kg BW CCl_4 via IP injection twice a week for two weeks, and the CCl_4+CI group received CI and 1 ml/kg BW of CCl_4 for two weeks. * stands for P<0.05, compared to the control group and Φ for P<0.05, in comparison with the CCl_4 group.

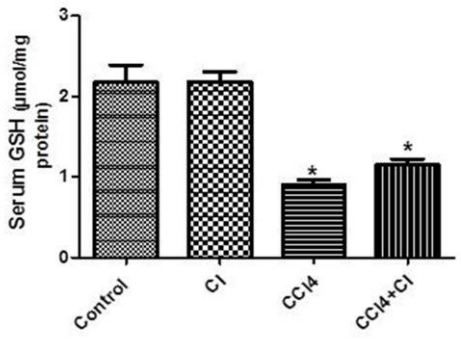

Figure 3. Level of serum reduced glutathione in female rats (n=10). The control group received saline, and the CI group received Cichorium intybus distillate orally for four weeks. The CCl4 group received 1 ml/kg BW CCl4 via IP injection twice a week for two weeks. The CCl4+CI group received both CI and 1 ml/kg BW of CCl4 for two weeks. * stands for P<0.05, compared to the control group and Φ for P<0.05, in comparison with the CCl4 group.

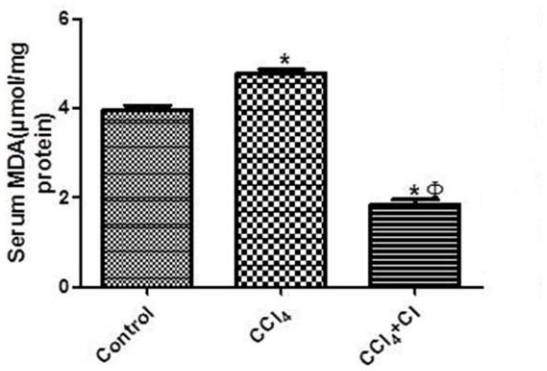

Figure 4. Level of serum malondialdehyde (MDA) in female rats (n=10). The control group received oral saline for four weeks. The CCl_4 group received 1 ml/kg BW CCl_4 via IP injection twice a week for two weeks. The CCl_4+CI group received both CI and 1 ml/kg BW of CCl_4 for two weeks. * stands for P<0.05, compared to the control group and Φ for P<0.05, in comparison with the CCl_4 group.

Table 1. Chemical composition analysis of essential oil from aerial parts of *Cichorium intybus* by gas chromatography-mass spectrometry

	RT	%Area	Compound	Mw	Activity action
1	3.654	0.12	1,2-Cyclopentanediol, 3-methyl	116	
2	6.307	0.32	2.-Beta.-Pinene	136.238	Antimicrobial (terpene)
3	6.392	0.48	2,3-Octanedione	142.19	
4	6.412	2.84	(E) 5-pentyloxy-2-Pentene	156.269	
5	7.906	3.38	l-Limonene	136.23	Anti-inflammatory Antioxidant
6	8.029	0.70	dl-Limonene	136.24	Antiaflatoxigenic Antioxidant (cyclic monoterpene)
7	8.318	3.74	Benzeneacetaldehyde	120.15	
8	8.863	1.14	Gamma.-Terpinene	136.238	Antioxidant
9	9.238	0.65	Formic acid	46.02	Antibacterial
10	9.275	0.46	1-Octanol	130.23	
11	9.896	1.28	3,4)-Methylenedioxy)toluene	136.15	
12	10.30	0.85	Linalool	154.25	Anti-inflammatory Antioxidant
13	10.479	1.24	Nonanal	142.22	Metabolite observed in cancer metabolism
14	10.591	2.77	Cyclohexanol	98.145	$C_6H_{10}O$
15	12.003	0.30	1-Cyclohexene-1-carboxaldehyde	110.156	$C_7H_{10}O$
16	12.393	0.88	7-Octenal	126.199	$C_8H_{14}O$
17	12.843	2.23	Borneol	154.25	Antioxidant ($C_{10}H_{18}O$) Sedative and antispasmodic

	RT	%Area	Compound	Mw	Activity action
18	13.319	1.29	3-Cyclohexen-1-ol	98.145	$C_6H_{10}O$
19	14.153	1.16	Cyclohexanone	98.15	$C_6H_{10}O$
20	14.966	0.73	1-Dodecene	168.319	$C_{12}H_{24}$
21	15.105	1.05	3,3-dimethyl-2-(1-methylethyliden cyclopentanone(Pulegone)	152.24 152.24	$C_{10}H_{16}O$
22	15.165	8.10	Pulegone	152.24	Insecticidal Terpene responsible for tissue necrosis ($C_{10}H_{16}O$)
23	16.047	4.18	Levo-carvone	150.22	Antioxidant ($C_{10}H_{14}O$)
24	16.314	5.25	p-Benzoquinone, 2,3,5,6-tetramethyl (Duroquinone)	164.204	Antioxidant $C_{10}H_{12}O_2$
25	16.469	1.13	3 – Carvomenthenone (Piperitone)	152.23	Antibacterial $C_{10}H_{16}O$
26	16.619	0.47	1-Cyclohexene-1-acetaldehyde	110.156	$C_7H_{10}O$
27	17.106	0.78	cis-Cinnamaldehyde (3-Phenyl-2-propenal)	132.162	C_9H_8O
28	17.983	1.34	Benzenemethanol	108.14	C_7H_8O
29	18.469	0.45	Quinoline	129.16	Antioxidant C_9H_7N
30	20.058	7.68	Piperitenone	152.23	Natural antioxidant and food preservative $C_{10}H_{16}O$
31	20.470	1.95	Camphene	136.24	Strong antioxidant capacity $C_{10}H_{16}$
32	20.967	0.36	4-Hydroxy-2,5-dimethyl-3(2H) - furanone (Furaneo)l (3H)-Furanone2	101.105	Antioxidant $C_4H_7NO_2$
33	21.138	0.81	2,,6-Xylohydroquinone	138.166	$C_8H_{10}O_2$
34	22.583	0.63	Tetradecane	198.39	$C_{14}H_{30}$
35	22.770	0.84	Methyl Eugenol	178.23	Anesthetic Antioxidant $C_{11}H_{14}O_2$
36	23.117	0.30	Beta-damascone	192.30	Strong antioxidant $C_{13}H_{20}O$
37	23.497	0.30	3-Fluoro-4-methoxyphenylacetonitrile	154.14	$FC_6H_3(OCH_3)$ CHO
38	25.139	0.77	Cinnamic acid ethyl ester	176.215	Potential protection in oxidative damage diseases: coronary heart disease, stroke, and cancers $C_{11}H_{12}O_2$
39	25.984	6.55	beta.-Ionon-5,6-epoxide	208.301	Antiproliferative and antioxidant potential of beta-ionone $C_{13}H_{20}O_2$
40	26.594	0.47	Pentadecane	212.42	$C_{15}H_{32}$
41	27.386	0.85	1- allyl-3,4-met hylen-dioxy-5-methoxy-benzene	162.188	$C_{10}H_{10}O_2$
42	27.557	5.00	Dihydroactinidiolide	180.24	Antioxidant $C_{11}H_{16}O_2$
43	30.215	0.56	Diethyl Phthalate	222.24	$C_{12}H_{14}O_4$
44	32.248	0.29	8,9-Epoxy-6,6-dimethyl-3,4-undecadien-2,10-dione (Geranylacetone)	194.318	$C_{13}H_{22}O$
45	32.814	0.33	Beta. Turmerone	218.34	Bioactive compound of Curcuma longa. $C_{15}H_{22}O$ Candidate for regeneration in neurologic disorders Anti-cancer

	RT	%Area	Compound	Mw	Activity action
46	35.168	1.02	cis-3,5-Dimethoxy-b-methyl-b-nitro styrene	223.084	$C_{11}H_{13}NO_4$
47	36.099	5.16	Methyl 2,4,5-Trimethoxy-6-methyl benzoate	182.237	$C_9H_{10}O_{2S}$
48	36.874	1.39	Caffeic acid	180.16	Hydroxycinnamic acid derivative and polyphenol, Potential antioxidant, anti-inflammatory, and antineoplastic activities $C_9H_8O_4$
49	39.126	1.95	Hexahydrofarnesyl acetone	268.47	Antioxidant $C_{18}H_{36}O$
50	41.816	0.48	Pentadecanoic acid	242.403	$C_{15}H_{30}O_2$
51	47.561	0.36	trans-Phytol	296.539	Strong antioxidant (Diterpene Alcohol) $C_{20}H_{40}O$
52	51.786	0.45	cis-9,10-Ethoxystearic Acid	296.495	$C_{19}H_{36}O_2$
53	3.504	0.18	2-Hexenal	98.145	$C_6H_{10}O$
54	3.622	0.59	2-Hexenal, (E)	98.145	$C_6H_{10}O$
55	3.680	0.11	trans-2-Hexen-1-al	98.14	$C_6H_{10}O$
56	3.723	0.16	2-Hexen-1-al	98.14	$C_6H_{10}O$
57	3.777	0.02	2-Hexen-1-al	98.14	$C_6H_{10}O$
58	5.665	0.09	Hydroxylamine, O-decyl	173.3	$C_{10}H_{23}NO$
59	7.007	0.59	Octanal	128.212	$C_8H_{16}O$
60	12.618	0.55	2-Dodecen-1-al	196.286	$C_{12}H_{20}O_2$
61	13.013	1.23	11, 13-Tetradecadien-1-ol	210.361	$C_{14}H_{26}O$
62	15.431	0.5	1-Thienylcyclohexene	164.266	$C_{10}H_{12}S$
63	17.186	0.55	Aza-4-methyl-6-1 hydroxybicyclo[3.3.0]octane	175.184	$C_7H_{13}NO_4$
64	18.384	0.27	6--Nitro-o-cresol	153.135	Antioxidant $C_7H_7NO_3$
65	18.683	0.34	Cinerolone	166.22	Antioxidant $C_{10}H_{14}O_2$
66	24.257	0.49	12-Oxatetracyclo [5,2,1,1(2,6).1(4,10)]dodecan-11-one	204.313	$C_{14}H_{20}O$
67	25.139	0.77	Ethyl cinnamate	176.21	Antioxidant $C_{11}H_{12}O_2$
68	27.279	5.58	5-methyl- 4-Hexen-3-one	112.172	$C_7H_{12}O$

Discussion

As evidenced by the results of the present study, the administration of CCl_4 increased ALT and AST levels, while CI administration improved the enzyme levels. Carbon tetrachloride is extensively used to induce liver toxicity via lipid peroxidation (Sharma and Agrawal, 2017). In line with the current study, several experiments reported that CCl_4 administration increased ALT and AST levels. They also indicated that treatment with antioxidant herbal plants, such as *Echium Amoenum*, *Terminalia bellirica* fruits, *Ficus religiosa*, and *Syzygium samarangense*, improved liver function enzymes (Kuriakose et al., 2017; Sobeh et al., 2018).

The results of CI GC-MS identified various classes, such as terpenes, polyphenols, and flavonoids. In the present study, the pattern and amount of certain substances in GC-MS analysis of hydrodistillate of *C. intybus* aerial parts differed from those obtained in

other studies (Gol, 2014). It was reported that (Būdienė, 2008) the predominant compositions of *C. intybus* in Lithuania were aliphatic hydrocarbons and their derivatives, while the quantities of terpenoids were minor.

Another study (Gol, 2014) revealed that the major and minor compositions were γ -terpenes and aliphatic hydrocarbons, respectively. In a similar vein, the present study detected the same pattern, except for the slight differences in values. Some data suggested that (Būdienė, 2008; Zahid Khorshid Abbas a and Nahla Zidan d, 2015) the hydro-alcoholic extract of chicory leaves possess higher values of flavonoids and phenolic acids. In agreement with previous reports (Haghi et al., 2012; Muhammad et al., 2014), the findings of the current study confirmed the presence of some terpenes, flavonoids, and polyphenols in hydrodistillates.

Nevertheless, the results of the present demonstrated comparatively lower amounts of flavonoid and phenolic contents. This discrepancy can be ascribed to differences in seasonal, climatic, and geographical conditions, species, and the use of distillation as the method of plant preparation. The results of the current study indicated that chicory distillate was rich in terpene and terpenoid, as well as flavonoid and phenolic compounds, which might be responsible for the observed antioxidant activity of the distillate.

Therefore, the present study for the first time demonstrated that the essential oil of chicory hydrodistillate as the widely used herbal remedy in traditional medicine exhibited antioxidant activity and introduced it as a new potential source of natural antioxidants. Anti-oxidant and anti-inflammatory effects had been reported for sesquiterpenes in previously conducted studies (Chadwick et al., 2013). The anti-oxidant and radical scavenging effects of CI flavonoids and sesquiterpene might be responsible for the ameliorative effects of *C. intybus* distillate on the CCl_4-induced oxidative stress.

In the present study, the observed decreases in the levels of the antioxidant enzymes in the CCl_4 groups might be due to the induced oxidative stress. Furthermore, the level of GSH was reduced by CCl_4 administration. In agreement with the results of the present study on female rats, it was reported that CCl_4 administration also decreased CAT, GPx, and GR activity in male rats (Al-Rasheed et al., 2016). In 2013, consistent with the present study regarding distillate, it was reported that *C. intybus* leaf powder also increased CAT activity in rats (Street et al., 2013).

The elevated MDA level after hepatic injury and its decrease after CI administration were in line with previous studies conducted on other plants (Gupta et al., 2011; Asirvatham and Usha, 2017; and Sobeh et al., 2018).

Conclusion

Based on the obtained results, CI could be suggested as a safe medicine supplement to improve antioxidant status in females via the attenuation of oxidative stress due to its beneficial effects and lack of hepatotoxicity. Nonetheless, the safety, dosage, stability, and efficacy of distillates, as well as their exact constituents need further investigations.

Authors' Contribution

Study concept and design: A. S. and F. Z.
Acquisition of data: Z. Kh., R. Gh. and M. M.
Analysis and interpretation of data: A. S., F. Z. and M. M.
Drafting of the manuscript: All the authors
Critical revision of the manuscript for important intellectual content: : A. S., F. Z., M. N., Sh. F. and R. Gh.
Statistical analysis: A. S., F. Z. and Z. Kh.
Administrative, technical, and material support: A. S., F. Z.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of the current study.

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