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ORIGINAL ARTICLE

Investigation of the protective effects of horse mushroom (*Agaricus arvensis* Schaeff.) against carbon tetrachloride-induced oxidative stress in rats

Abdulahad Dogan[1] · Abdullah Dalar[2] · Cemil Sadullahoglu[3] · Abdulhamit Battal[4] · Yusuf Uzun[2] · Ismail Celik[5] · Kenan Demirel[6]

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Abstract

Wild and cultured mushrooms have been extensively used for food and medicinal purposes all around the world. However, there is limited information on chemical composition, health enhancing effects and contributions on diet of some mushrooms (e.g., *Agaricus arvensis*) widely distributed in many countries including United Kingdom, Australia, Turkey etc. Therefore, the present study was aimed to analyse the bioactive composition and ameliorative effects of *A. arvensis* via evaluating in vitro and in vivo antioxidant properties in CCl_4 induced rat model. The extract exhibited higher antioxidant capacities in vitro than that of the positive control (Reishi-Shiitake-Maitake standardized extract). Administration of the extract had significant regulative effects in the levels of AST, ALT, LDH, Urea and TRIG levels according to CCl_4 group. Additionally, lipid peroxidation and GSH in the brain, kidney and liver tissues was regulated by extract treated groups compared to the CCl_4 group. The supplementation of the extract at the dose of 100 mg/kg regulated the levels of GST, GR, CAT and GPx enzyme activities in brain and liver, but not in kidney tissue. There was approximately three fold increase in CAT enzyme activity in kidney tissue of extract treated groups compared to Control and CCl_4 groups. The extract contained a rich composition of bioactive compounds including phenolics (protocatechuic acid and *p*-hydroxybenzoic acid), volatile compounds (benzaldehyde, palmitic acid and linoleic acid) and mineral compounds (K, Si, Mg and Na). Data obtained within this study suggests that *A. arvensis* might be used for food industries in order to obtain nutritional products.

Keywords *Agaricus arvensis* · CCl_4 · Oxidative stress · Antioxidants · Bioactive compound

Abbreviations

Ag	Silver
ALT	Alanine aminotransferase
As	Arsenic

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✉ Abdulahad Dogan
ahadobi@hotmail.com; abdulahaddogan@yyu.edu.tr

Abdullah Dalar
dalar.abdullah@yyu.edu.tr

Cemil Sadullahoglu
csadullahoglu@smail.yyu.edu.tr

Abdulhamit Battal
abdulhamitbattal@yyu.edu.tr

Yusuf Uzun
y.uzun@yyu.edu.tr

Ismail Celik
icelik@yyu.edu.tr

Kenan Demirel
kenandemirel@odu.edu.tr

[1] Department of Biochemistry, Faculty of Pharmacy, Van Yuzuncu Yil University, 65080 Van, Turkey

[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, Van Yuzuncu Yil University, 65080 Van, Turkey

[3] Department of Biology, Faculty of Science, Van Yuzuncu Yil University, 65080 Van, Turkey

[4] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Van Yuzuncu Yil University, 65080 Van, Turkey

[5] Department of Molecular Biology and Genetics, Faculty of Science, Van Yuzuncu Yil University, 65080 Van, Turkey

[6] Department of Secondary Science and Mathematics Education, Faculty of Education, Ordu University, 52200 Ordu, Turkey

AST	Aspartate aminotransferase
B	Boron
Ca	Calcium
CAT	Catalase
CCl_4	Carbon tetrachloride
Cd	Cadmium
CHOL	Cholesterol
Cl	Chlorine
Co	Cobalt
Cr	Chromium
CREA	Creatinine
Cu	Copper
FCR	Folin–Ciocalteu reagent
Fe	Iron
FRAP	Ferric reducing antioxidant power
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione *S*-transferase
HDL-C	High-density lipoprotein cholesterol
K	Potassium
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
ORAC	Oxygen radical absorbance capacity
Pb	Lead
Se	Selenium
Si	Silicon
SOD	Superoxide dismutase
Na	Sodium
TG	Triglycerides
TP	Total protein
UA	Uric acid
Zn	Zinc

Introduction

Many environmental pollutants, alcohol, drugs and toxic materials engender oxidative stress such as hepatotoxicity, neurotoxicity, nephrotoxicity and lipid peroxidation. Carbon tetrachloride (CCl_4) is an extremely toxic chemical compound which has been commonly used to induce hepatic injury in experimental animal models [1]. CCl_4 induced oxidative stress might cause lipid peroxidation, protein and DNA damage in various rat tissues such as liver, kidneys and lungs [2]. A large amount of mushrooms have been investigated to eliminate the hepatic damage stimulated by toxic chemical substances [3, 4].

Oxidative stress generates free radicals such as peroxyl radicals, superoxide radicals, hydroxyl radicals etc. These free radicals suppress in vivo and in vitro antioxidant defence systems in living systems. Organisms avoid from harmful effects of free radicals by their in vivo protective enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH) and glutathione *S*-transferase (GST) [2–5].

Bioactive compounds present in mushrooms have been reported to increase the activity of antioxidant enzymes and suppress the formation of possible free radical sources [5, 6]. Several macrofungus samples exhibited pronounced antioxidant activity which has been associated to the presence of biologically active compounds including phenolics, glycosides, flavonoids, tocopherols, polysaccharides, carotenoids, ergothioneine, vitamins, ascorbic acid and minerals [7–11]. It was propounded that protective effects of antioxidants compounds present in macrofungus samples functioned mainly by two mechanisms: (I) chain breaking and free radical scavengers, (II) deactivation of metals, inhibition or breakdown of lipid hydroperoxides, regeneration of primary antioxidants and singlet oxygen quenching [11, 12].

Agaricus arvensis Schaeff. (Agaricaceae) known as horse mushroom has a widespread distribution in Europe (including in the United Kingdom and Turkey), Asia, Australia, New Zealand, and northern America. It is consumed globally in summer and autumn seasons and is collected from wild, particularly from scattered and rotten meadow areas. The hat zone is 7–20 cm in diameter and young white or pale yellowish as color. It has a thick structure, white or whitish colour without changing when damaged. *A. arvensis* has been traditionally utilized in Turkey for nutritional purposes. It is generally consumed freshly in salads or cooked with spices and vegetables in summer as food.

There is no or limited data regarding in vitro and in vivo antioxidant capacities, hepatoprotective and nephroprotective properties and chemical composition of *A. arvensis* in the scientific literature. Therefore, we aimed to determine health attributing properties of *A. arvensis* extract by evaluating antioxidant capacity in vitro and in vivo, toxicity level, protective properties on liver, kidney and brain, and general antioxidant defence system. Additionally, chemical composition of the extract was analysed by evaluating phenolic, volatile and mineral compounds.

Methods

Mushroom material

Fruiting bodies of *Agaricus arvensis* were collected from Akalin village, Yüksekova district, Hakkari City, in the

Eastern Anatolia Region of Turkey, (GPS coordinates 37°34′022″N; 44°14′582″E) on June, 2016. The identity of samples was confirmed by Prof. Dr. Kenan Demirel at the Department of Biology, Science Faculty, Van Yuzuncu Yil University, Turkey and a voucher specimen was deposited at the university's fungarium (Fungarium code: YYU-VAN, FUNGARIUM-7487). The fruiting bodies of fresh mushroom samples were cleaned properly and dried at room temperature in the dark until dry. Subsequently, the samples were ground and stored at − 20 °C until analysed.

Reagents

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA) and were of analytical or HPLC grade. Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Standardized Reishi-Shiitake-Maitake extract was purchased from Solgar (Istanbul, Turkey). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories L.T.D. (Antrim, UK).

Preparation of lyophilized extract

The lyophilized extract was prepared according to method by Dalar and Konczak [13]. Briefly, the ground mushroom material was mixed with a 10-fold volume of aqueous ethanol (80% ethanol, 20% H_2O, v/v), shaken for 2 h at room temperature (25 °C) and centrifuged for 20 min (15,320×*g*) at 4 °C (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial No. 02U8152, USA) with the supernatant collected. The extraction procedure was repeated one more time. The supernatants from the consecutive extractions were combined. Solvent was evaporated under reduced pressure at 40 °C. The derived fraction was dissolved in purified water and freeze-dried under a vacuum at—51 °C to obtain a fine lyophilized powder used for analysis.

Antioxidant capacity in vitro assay

Folin–Ciocalteu reducing capacity (Total phenolic content), total reducing capacity (FRAP assay) and oxygen radical scavenging capacity of the extract were determined as described previously by Dalar and Konczak [13]. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

Analysis of phenolic compounds

Identification and quantification of phenolic compounds by high liquid chromatography–diode array–mass spectrometry (LC–DAD–MS/MS) analysis were conducted as described previously with minor modifications [13].

Analysis of volatile and fatty acid compounds

Volatile compounds and fatty acids present in extracts were analysed by gas chromatography mass spectrometry (GC/MS) using a head space solid phase micro extraction and identified by the fragment ions and relative retention indices of their peaks with those of the MS library standards as described previously [14]. The headspace volume, heating temperature and time of the extraction were optimized according to Verzera et al. [15].

Mineral compounds analysis

Mineral composition of the extract was evaluated by using AAS, ICP-MS and ICP-OS. The analysis solution was prepared by dissolving the extract in HNO_3. Subsequently, the solution was subjected to microwave assisted extraction procedure. The identity of mineral compounds was confirmed by comparison of authentic standards.

Animals

Twenty-four Wistar albino male rats 2–6 months of aged were provided by the Experimental Animal Research Centre, Van Yuzuncu Yil University (Van, Turkey). The animals were housed in four groups, each group containing six rats. The animals were housed at 25 ± 1 °C in a daily light/dark (~ 12/12) cycle. All animals were fed a diet based on wheat and soybean meal and water ad libitum in stainless cages, and received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Van Yuzuncu Yil University (Protocol Number: 27552122-604.01.02).

Acute toxicity test

Acute toxicity was determined according to the OECD guidelines, test 423: acute oral toxicity method; 2002. A range of the extract (20, 50, 100, 250, 500, 1000 and 2000 mg/kg) administrated to the three rats orally via gastric gavage for 0.5, 2, 4, 8, 24, 48 and 72 h respectively. The animals were fed on food and water ad libitum. There is no symptoms of toxicity and mortality were observed over a period of 72 h.

Experimental design

The rats were randomly distributed into four groups; Control, CCl_4, CCl_4 + *A. arvensis* 100 mg/kg extract and CCl_4 + *A. arvensis* 500 mg/kg extract. Food and drinking water were supplied for all animals as ad libitum during experiments.

Control group (n = 5): the rats received drinking water and fed with standard pellet diet as ad libitum.

CCl4 group (n = 5): the rats received 0.5 ml CCl_4/kg rat weight intraperitonally twice per week. Dose of CCl_4 was selected on the basis of a 0.5 ml CCl_4/kg rat weight diluted in olive oil (1:1 dilution) [16].

CCI_4 + *A. arvensis* 100 mg/kg extract group (n = 6) CCI_4 + *A. arvensis* 100 mg/kg extract group (n = 6): the rats received 0.5 ml CCl_4/kg rat weight intraperitonally injection twice per week and *A. arvensis* extract (100 mg/kg, per day) was applied to rats via orally during 28 days.

CCI4 + *A. arvensis* 500 mg/kg extract Group (n = 6): CCI_4 + *A. arvensis* 500 mg/kg extract group (n = 6): the rats received 0.5 ml CCl_4/kg rat weight intraperitonally injection twice per week and *A. arvensis* extract (500 mg/kg, per day) was applied to rats via orally during 28 days.

Preparation of serum and tissue supernatants

At the end of the experiments, the rats were anesthetized by injection of ketamine (5 mg/100 g body weight) intraperitonally. The blood samples were taken using a cardiac puncture and were put immediately into silicon disposable glass biochemical tubes. Subsequently, the samples were centrifuged at 4000×*g* for 15 min at 4 °C in order to obtaining serum samples for the measurement of biochemical parameters. The liver, brain and kidney tissues were dissected and put into Petri dishes. Subsequently, the samples were taken and kept at − 78 °C until analysis. The tissues were homogenized for 5 min in 50 mM ice-cold potassium dihydrogen phosphate (KH_2PO_4) solution (1:5 w/v) using stainless steel probe homogenizer (SONOPULS HD 2200, Bandelin, Berlin, Germany), and subsequently centrifuged at 7000×*g* for 15 min. All processes were carried out at 4 °C. Supernatants were used to determine antioxidant defence systems (ADS) constituents and Malondialdehyde (MDA) contents as described previously [17, 18].

Antioxidant defence system enzyme analysis

The liver, brain and kidney in tissue supernatants reduced glutathione (GSH) levels, malondialdehyde (MDA) content, glutathione reductase (GR) activity, glutathione-*S* transferase (G*S*T) activity, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, catalase (CAT) activity were determined as described previously [18].

Measurement of biochemical parameters

Serum biochemical parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, uric acid (UA), creatinine (CREA), total protein (TP), sodium (Na), potassium (K), chlorine (CI), cholesterol (CHOL), triglyceride (TRIG) and HDL-cholesterol (HDL-C)] were measured by an auto analyser (ARCHITECT 16200, Abbott Park, IL 60064, USA) using the Abbott biochemistry kits (USA).

Analysis of data

All data were expressed as mean ± standard deviation (SD). The statistical analyses were made using the Minitab 13 for Windows packet program. One-way analysis of variance (ANOVA) statistical test was used to determine the differences between means of the experimental groups accepting the significance level at ($p \leq 0.05$).

Results

Extraction yield, total phenolic content and antioxidant capacity in vitro

A high extraction yield of (23.5%) was obtained in consequence of the extraction procedure. The extract exhibited high antioxidant capacities. As shown in (Table 1), the

Table 1 Extraction yield and in vitro antioxidant capacity

Parameters	*A. arvensis* extract Mean ± SD	Positive control extract[4] Mean ± SD
Extraction yield (%)	23.5	–
Total phenolic content (Folin–Ciocalteu)[1]	18.5 ± 0.4[a]	6.6 ± 0.8[b]
Ferric reducing antioxidant power (FRAP)[2]	195 ± 18.2[a]	74.7 ± 3.4[b]
Oxygen radical absorbance capacity (ORAC)[3]	533.6 ± 2.0[a]	188.8 ± 7.6[b]

Means with different letters in the same raw were significantly different at the level ($p < 0.05$); n = 3

[1]mg Gallic acid/g extract, [2]µmol Fe^{2+}/g extract, [3]µmol Trolox Eq./g extract, [4]Reishi–Shiitake–Maitake extract

extract exhibited 2.6–2.8 fold higher antioxidant capacities (ORAC and FRAP) and had 2.8 fold higher amount of total phenolics (FCR) than that of the positive control (Reishi-Shiitake-Maitake standardized extract), which indicated high antioxidant potential of the extract.

Chemical composition

Chemical composition of the extract was presented in (Table 2) as evaluated by HPLC–MS/MS (phenolics), GC–MS (volatiles and fatty acids) and AAS, ICP–MS VE ICP–OS (minerals). Phenolic composition of the extract mainly comprised of phenolic acids which had the maximum absorbance at 280 nm. No any compound was detected at 520 nm in the extract. The dominated phenolic compound identified based on m/z transition data and spectral and absorbance characteristics of HPLC peaks of the extract was detected as protocatechuic acid, followed by *p*-hydroxybenzoic acid as shown in (Table 2) and (Fig. 1). Volatile composition of the extract was mainly formed of benzaldehyde, palmitic acid and linoleic acid which contributed 80% of volatile composition as shown in (Table 2) and (Fig. 2). Major volatile compounds of the extract were identified as linoleic acid (54.03% contribution), followed by benzaldehyde (14.37% contribution) and palmitic acid (11.57% contribution) based on mass data (Table 2; Fig. 2). With regards to mineral composition of the extract, K, Si, Mg and Na were found as the dominant compounds of the extract, followed by Ca and B (Table 2). The heavy metals such as Cd, Co, As and Pb were found as trace levels in the extract (Table 2).

Acute toxicity test

Optimum tolerance dose was detected in animals against the application of *Agaricus arvensis* lyophilized extract. Non-lethal dose was obtained until 2000 mg/kg lyophilized extract. No any noticeable signs of toxicity and mortality were observed after daily administration of the extract orally at the end of the 72 h. Two different doses (100 and 500 mg/kg) were selected for further studies.

Table 2 Bioactive composition of *A. arvensis*

Phenolic compound	Phenolic composition	
	Protocatechuic acid (mg/g extract)	*p*-Hydroxybenzoic acid (mg/g extract)
MS/MS ($[M+1]^+/[M-1]^-$)	–/153	–/137
Fragmant ions (m/z) (+/−)	–/–	–/–
Concentration	5.1 ± 0.1	2.4 ± 0.3

Compound	Volatile and fatty acids composition	
	Fragmant ions	Relative concentration (%)
Benzaldehyde	51, 63, 77, 106	14.37
Palmitic acid	55, 74, 87, 97, 129, 143, 185 ,213, 227, 239, 270	11.57
Linoleic acid	67, 81, 95, 150, 220, 263, 294	54.03

Mineral compound	Mineral composition		
	Level (µg/g extract)	Mineral compound	Level (µg/g extract)
Ag	4.6 ± 0.0	Mn	2.6 ± 0.0
As	11.9 ± 0.1	Mo	0.31 ± 0.0
B	193.5 ± 2.4	Pb	0.67 ± 0.0
Cd	0.28 ± 0.0	Se	6.85 ± 0.1
Co	0.19 ± 0.0	Si	528.78 ± 11.3
Cr	1.66 ± 0.0	Zn	33.74 ± 1.8
Cu	65.87 ± 1.8	Na	381.78 ± 8.4
K	10136.4 ± 34.7	Fe	29.62 ± 2.6
Mg	527.6 ± 13.5	Ca	284.46 ± 7.4

Ag silver, *As* Arsenic, *B* boron, *Cd* cadmium, *Co* cobalt, *Cr* chromium, *Cu* copper, *K* pottasium, *Mg* magnesium, *Mn* manganese, *Mo* molybdenum, *Pb* lead, *Se* selenium, *Si* silicon, *Zn* zinc, *Na* sodium, *Fe* iron, *Ca* calcium

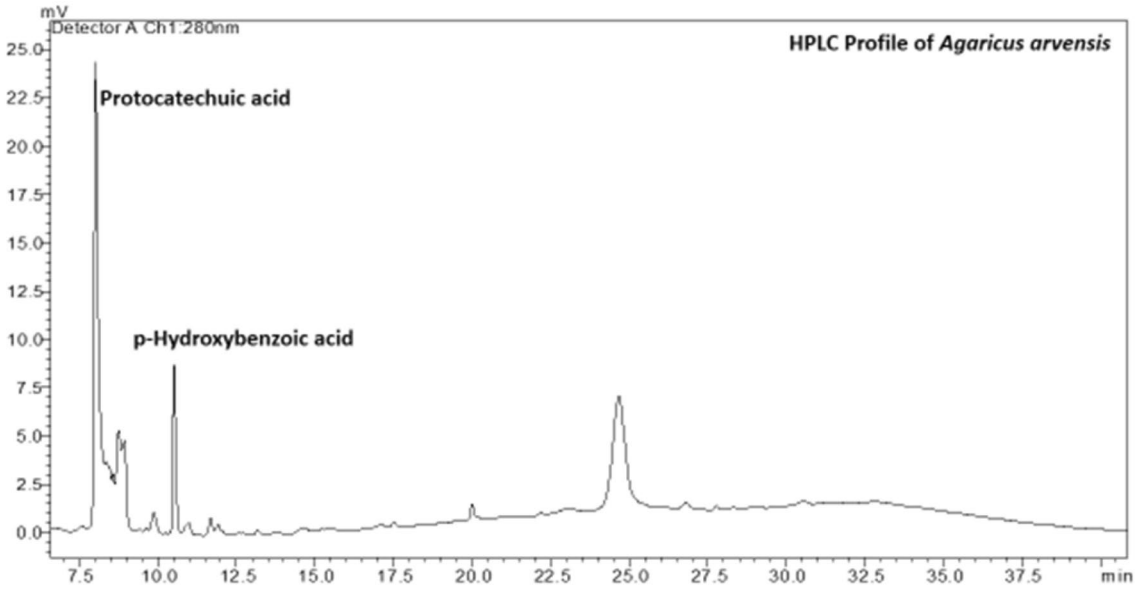

Fig. 1 HPLC profile of *A. arvensis* extract

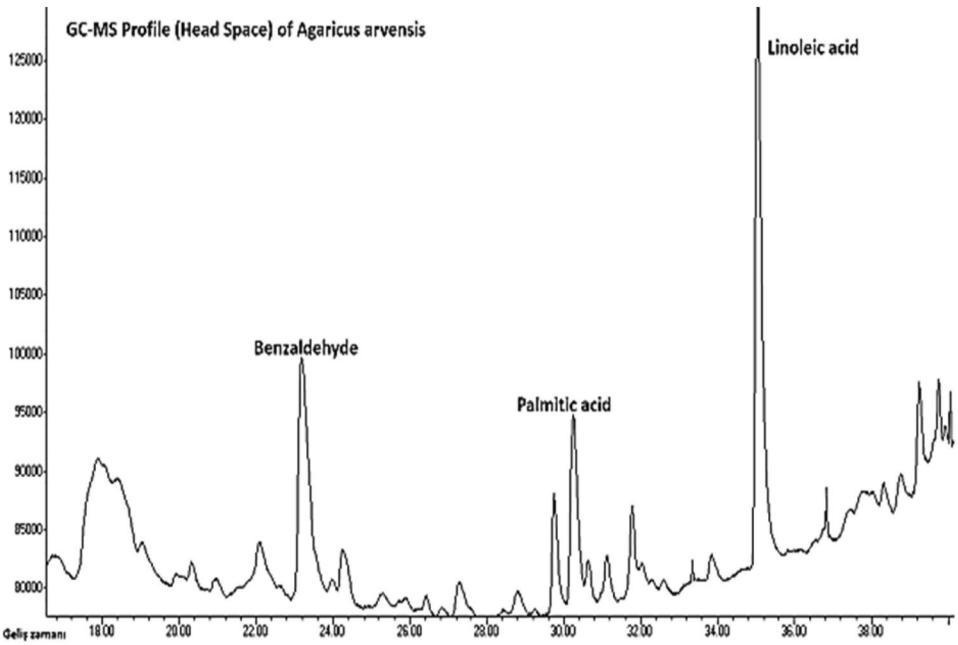

Fig. 2 GC–MS profile of *A. arvensis* extract

Effect of the extract on body weight

Changes in the initial and final body weight of the control and experimental groups were presented as supplementary material. Weight gain was significantly ($p \leq 0.05$) observed in final weight of all of the rat groups compared to initial weight.

Effect of the *A. arvensis* extract on biochemical parameters

As shown in (Table 3), the levels of AST, ALT and LDH significantly increased in CCl_4 group compared to control group. However, administration of the extract caused significant decreases in the levels of AST, ALT and LDH levels

Table 3 Effects of *Agaricus arvensis* extract on biochemical parameters in serum

Parameters	Control Mean ± SD	CCl_4 Mean ± SD	CCl_4 + *A. arvensis* (100 mg/kg extract) Mean ± SD	CCl_4 + *A. arvensis* (500 mg/kg extract) Mean ± SD
AST (U/L)	113.00 ± 8.77	139.01 ± 19.17[a]	127.83 ± 27.84	107.40 ± 18.68[b]
ALT (U/L)	36.40 ± 6.07	77.78 ± 13.11[a]	56.67 ± 12.52[ab]	57.80 ± 9.42[ab]
LDH (U/L)	961.40 ± 71.98	1722.01 ± 245.05[a]	1036.00 ± 148.04[b]	913.80 ± 148.23[b]
Urea (mg/dL)	55.20 ± 10.71	51.01 ± 7.39	46.67 ± 4.46	37.40 ± 1.14[abc]
UA (mg/dL)	1.68 ± 0.41	1.50 ± 0.24	2.07 ± 0.67	2.16 ± 0.62
CREA (mg/dL)	0.48 ± 0.02	0.47 ± 0.04	0.49 ± 0.05	0.50 ± 0.03
TP (g/dL)	5.66 ± 0.23	6.05 ± 0.31	5.77 ± 0.24	5.96 ± 0.30
Na (mmol/L)	141.60 ± 1.52	142.01 ± 2.31	141.17 ± 2.48	141.00 ± 0.71
K (mmol/L)	5.38 ± 0.41	6.03 ± 2.15	5.97 ± 1.78	5.78 ± 1.30
CI (mmol/L)	100.60 ± 3.21	101.75 ± 2.22	102.83 ± 1.60	103.40 ± 0.55
CHOL (mg/dL)	27.40 ± 6.69	28.75 ± 6.08	25.33 ± 8.66	23.01 ± 7.11
TRIG (mg/dL)	67.00 ± 11.68	42.75 ± 9.74[a]	39.17 ± 12.64[a]	35.00 ± 10.25[a]
HDL_C (mg/dL)	50.20 ± 4.99	47.88 ± 4.93	44.02 ± 6.94	43.94 ± 2.76[a]

Data were expressed as mean ± SD. One-way ANOVA followed by Tukey test, when appropriate (n = 5 or 6)

CCl_4 carbon tetrachloride; *AST* aspartate aminotransferase; *ALT* alanine aminotransferase; *LDH* lactate dehydrogenase; *UA* uric acid; *CREA* creatinine; *TP* total protein; *Na* sodium; *K* potassium; *CI* chlorine; *CHOL* cholesterol; *TRIG* triglyceride; *HDL_C* high density lipoprotein cholesterol

[a]Mean values were significantly different from control group ($p \leq 0{\cdot}05$)

[b]Mean values were significantly different from the CCl_4 group ($p \leq 0{\cdot}05$)

[c]Mean values were significantly different between the CCl_4 + *A. arvensis* extract group ($p \leq 0{\cdot}05$)

according to CCl_4 group. However, the effect of the extract didn't regulate the level of ALT compared to control group. Urea level was substantially lower in CCl_4 + *A. arvensis* 500 mg/kg group than all other groups. No significant correlation was found between UA, CREA, TP, Na, K, Cl and CHOL levels and extraction doses applied. TRIG level significantly increased in control group than other all groups. HDL level in the CCl_4 + *A. arvensis* 500 mg/kg group was significantly lower according to control group.

Effect of the *A. arvensis* extract on lipid peroxidation and antioxidant defence systems

Effects of *A. arvensis* extract and CCl_4 on MDA content, GSH level, enzyme activities of GST, GR, CAT, GPx and SOD in brain, kidney and liver tissues are presented in (Table 4). MDA content of CCl_4 group significantly increased in brain, kidney and liver tissues according to control group. On the other hand, lipid peroxidation in the brain, kidney and liver tissues was regulated by extract treated groups compared to the CCI_4 group (Table 4). GSH level of brain in CCl_4 + *A. arvensis* 100 mg/kg group markedly decreased compared to CCI_4 group. Additionally, GSH levels in liver markedly decreased in extract treated and CCI_4 groups compared to control group. Relatively decreases in GST, GR, CAT and GPx enzyme activities in tissues of CCl_4 group were observed according to control group. Generally, the supplementation of the extract at the dose of 100 mg/kg regulated the levels of GST, GR, CAT and GPx enzyme activities in brain and liver, but not in kidney tissue. There was approximately three fold increase in CAT enzyme activity in kidney tissue of extract treated groups compared to Control and CCl_4 groups (Table 4).

Discussion

Oxidative stress is a complicated consecution occurring as endogenous and exogenous sources, playing an important role in aging processes, increasing the risk of chronic disease and causing fatal consequences. It is known that oxidative stress plays an important role in various diseases such as cardiovascular disease, aging, mutations, cancer and neurodegenerative diseases [19]. Natural sources obtained from plant, fungi and lichens are rich in physiologically beneficial bioactive substances such as phenolics, glycosides, tocopherols, polysaccharides, carotenoids, alkaloids and ascorbic acid which possesses pronounced antioxidant capacities in vitro and in vivo [9, 11]. Therefore, investigation of bioactive rich edible mushroom samples is crucially important in order to find new and safe antioxidant agents which able to prevent and/or minimize the negative effects of oxidative stress.

Table 4 Effect of *A. arvensis* extract on lipid peroxidation and antioxidant defence systems CCl_4-induced in rats

Tissue	Parameters	Control Mean ± SD	CCl_4 Mean ± SD	CCl_4 + *A. arvensis* (100 mg/kg extract) Mean ± SD	CCl_4 + *A. arvensis* (500 mg/kg extract) Mean ± SD
Brain	MDA nmol/g	160.34 ± 20.35	207.24 ± 17.63[a]	84.71 ± 21.88[b]	45.08 ± 6.05[abc]
	GSH mg/g	35.79 ± 9.32	34.57 ± 2.30	29.72 ± 2.06[b]	30.62 ± 3.47
	GST U/g	8.04 ± 2.30	6.35 ± 0.93	7.06 ± 0.79	7.13 ± 0.96
	GR U/g	1.00 ± 0.30	0.93 ± 0.11	1.14 ± 0.08[b]	1.04 ± 0.11
	CAT U/g	41.01 ± 11.09	9.60 ± 1.14[a]	16.04 ± 3.64[ab]	23.09 ± 5.58[abc]
	GPx U/g	64.40 ± 11.31	44.32 ± 11.02[a]	72.12 ± 8.72[b]	59.88 ± 11.55
	SOD U/g	2173.62 ± 39.05	2195.46 ± 16.87	2208.61 ± 33.19	2193.10 ± 27.69
Kidney	MDA nmol/g	148.31 ± 39.30	279.29 ± 25.05[a]	179.40 ± 46.54[b]	128.23 ± 36.43[b]
	GSH mg/g	16.71 ± 2.54	16.92 ± 1.26	18.65 ± 1.28	17.03 ± 3.50
	GST U/g	7.72 ± 1.74	5.03 ± 1.77	10,27 ± 1.09[ab]	9,44 ± 2.29[b]
	GR U/g	0.35 ± 0.06	0.25 ± 0.08	0.13 ± 0.02[ab]	0.16 ± 0.05[a]
	CAT U/g	264.22 ± 32.94	212.35 ± 44.61	759.35 ± 163.99[ab]	745.95 ± 176.27[ab]
	GPx U/g	661.18 ± 165.29	518.28 ± 144.08	509.24 ± 95.39	837.15 ± 82.84[bc]
	SOD U/g	2202.74 ± 30.88	2204.98 ± 28.16	2073.10 ± 132.95	2198.91 ± 81.06
Liver	MDA nmol/g	136.39 ± 32.78	226.03 ± 64.99[a]	124.35 ± 26.51[b]	172.40 ± 37.04[abc]
	GSH mg/g	19.54 ± 2.87	11.95 ± 2.47[a]	12.45 ± 1.04[a]	13.10 ± 1.96[a]
	GST U/g	29.36 ± 5.24	16.36 ± 3.73[a]	22.87 ± 3.19[ab]	20.38 ± 2.74[a]
	GR U/g	0.26 ± 0.08	0.09 ± 0.03[a]	0.16 ± 0.03[ab]	0.08 ± 0.02[ac]
	CAT U/g	550.56 ± 116.86	485.95 ± 99.31	604.07 ± 77.09	580.71 ± 76.39
	GPx U/g	566.50 ± 124.41	513.52 ± 128,79	537.50 ± 133.89	533.39 ± 86.17
	SOD U/g	2105.98 ± 98.01	2152.48 ± 54.91	2150.03 ± 43.52	2157.80 ± 56.54

Data were expressed as mean ± SD. One-way ANOVA followed by Tukey test, when appropriate (n = 5 or 6)

CCl_4 carbon tetrachloride; *MDA* malondialdehyde; *GSH* reduced glutathione; *GST* glutathione *S*-transferase; *GR* glutathione reductase; *CAT* catalase; *GPx* glutathione peroxidase; *SOD* superoxide dismutase

[a]Mean values were significantly different from control group ($p \leq 0{\cdot}05$)

[b]Mean values were significantly different from the CCl_4 group ($p \leq 0{\cdot}05$)

[c]Mean values were significantly different between the CCl_4 + *A. arvensis* extract group ($p \leq 0{\cdot}05$)

Within this study, in vitro antioxidant capacity of the extract was evaluated by using three complementary antioxidant testing methods representing two basic antioxidant mechanisms. ORAC assay represented hydrogen atom transfer (HAT) mechanism and FCR and FRAP tests represented single electron transfer (SET) mechanism. ORAC values can be used as reference antioxidant effectiveness since ORAC assay is able to measure the radical chain breaking ability of antioxidants by monitoring the inhibition of peroxyl radical-induced oxidation. In order to distinguish dominant mechanisms for different antioxidants, ORAC, FRAP and FCR methods were suggested to reveal the complementary antioxidant potential of food sources [20]. As shown in (Table 1), the extract had more antioxidant activities than positive control (Reishi-Shitake-Maitake standardized extract) which was investigated by Islam et al. [21] and reported for their high antioxidant capacities. Pronounced ORAC, FRAP and FCR capacities of the extract might be due to the presence of rich chemical composition of the extract. Protocatechuic acid was found as the major phenolic compound of the *A. arvensis* extract in this study. Li et al. [22] investigated the antioxidant potential of protocatechuic acid using several antioxidant tests and detected more antioxidant activity of protocatechuic acid than trolox and associated its activity to both chelating metal transition ions as well as scavenging free radicals via donating hydrogen atom (H·) or electron (e) properties.

CCl_4 was used to create oxidative stress in different experimental animal models. CCl_4 activated by phase-II detoxifying enzymes to generate oxidative radicals in liver such as trichloromethyl (CCl_3) and peroxytrichloromethyl (CCl_3O_2). Peroxyl radicals induce lipid peroxidation causing damage on cell membrane in many tissues mainly liver, brain, kidney, lung and erythrocyte [23, 24]. In this study, in vivo antioxidant studies were conducted against CCl_4 induced oxidative stress. Our findings showed positive effect of CCl_4 on increasing the final body weights (Supplementary material) which was in accordance with previous studies [25, 26]. On the other hand, Connor et al. [27] suggested different doses (1.58 and 3.16 g/kg) of CCl_4 could cause an

increase for the first 5 days following by a decrease in live body weight. Hence it might be speculated that dose concentration and application period of CCl_4 might have different effects on live body weight.

As shown in (Table 2), within this study protocatechuic acid as major hydrophilic compound; linoleic acid as major lipophilic compound and K and Si as major mineral compounds were detected in *A. arvensis* extract. The levels of heavy metals were determined in the extract was substantially at low levels, which indicate the reliability of this mushroom in the context of nutrition. It was reported that economically important wild edible mushrooms contained significant amounts of valuable fatty acids [28]. Similar to our findings Barros et al. [29] detected linoleic acid as one of the major fatty acids in various mushroom samples including *A. arvensis, L.deliciosus, L. giganteus, S. imbricatus*, and *T. portentosum*. In another study conducted on pharma-nutritional properties of wild mushrooms, protocatechuic acid and *p*-hydroxybenzoic acid were detected as major phenolic compounds and linoleic and palmitic acids as major fatty acids of mushroom extracts [30]. Protocatechuic acid was reported for its potential hepatoprotective and nephroprotective activities in vivo [31] and *p*-hydroxybenzoic acid and its derivatives were detected as effective antioxidant compound for their abilities to scavenge free radicals and trigger endogenous antioxidant activity [11], which are in accordance with our findings.

As shown in (Table 3), although there was an increase in the levels of AST, ALT and LDH in the CCl_4 group compared to control group, supplementation of the extract regulated the levels of AST, ALT and LDH levels compared to CCl_4 group. Increases in serum AST, ALT, LDH, CRE and Urea levels generally indicate liver and kidney injuries. Özkol et al. [32] and Dogan and Celik [18] reported a positive correlation between serum liver enzymes and reactive oxygen species (ROS) which were associated with damage to the liver cells, leading to enzyme leakage into the plasma and cellular necrosis. Similar to our findings, some researchers reported increase in the levels of those enzymes in CCl_4 groups [16, 27]. Jayakumar et al. [5] evaluated *Pleurotus ostreatus* extract against CCl_4-induced hepatotoxicity and found regulative effects of the extract on serum AST, ALT and ALP levels. Furthermore, Jeong et al. [33] and Aniya et al. [34] demonstrated that the administration of *Artemisia campestris* extract decreased the toxicity induced by CCl_4 by healing of abovementioned enzymes. Treatment of *A. arvensis* extract might have improved biochemical parameters due to the presence of an antioxidative active compound—protocatechuic acid. We therefore suggest that pretreatment with the *A. arvensis* extract markedly reversed hepatotoxicity and nephrotoxicity caused by the CCl_4. The positive effect of extract on triglycerides (TRIG) and high density lipoprotein cholesterol (HDLC) was shown in Table 3. Goyal and Grewal [35] investigated the effects of *Agaricus bisporus* on serum total lipid and triglyceride levels and found a negative correlation between applied dose and levels of TRIG and HDLC. Additionally, ameliorative effects of various mushroom samples on TC, TRIG, LDL, HDL and VLDL levels were reported previously [36, 37].

As presented in (Table 4), the negative effect of CCl_4 which caused a significant increase in lipid peroxidation was regulated by extract applied in brain, kidney and liver tissues. Lipid peroxidation especially occurs on cell membrane as a result of unsaturated fatty acids oxidation. Malondyaldehyde (MDA) forms as a final product of lipid peroxidation in cells. MDA is one of the significant biomarker which have been used to observe lipid peroxidation and oxidative stress. Moreover, MDA might have important mutagenic, atherogenic, and cancerogenic actions, as it is capable of forming different, biologically relevant carbohydrates, nucleic acids and protein adducts [18]. It was reported that CCI_4 significantly increased MDA content in rat erythrocyte, liver, brain, kidney and spleen tissues compared to the control group [16, 24]. Similarly, Coballase-Urrutia et al. [26] reported significant increase in MDA levels and decrease in antioxidant enzyme activities in the liver and brain regions (cerebellum, cortex and cerebral hemispheres) of CCl_4 group. Treatment of rats with *A. arvensis* extract reversed the increase of lipid peroxidation compared to the control group. This could be explained by the presence of antioxidant compounds present in the extract including protocatechuic and linoleic acids. Our findings are in agreement with those of Lin et al. [38] and Jayakumar et al. [5] who reported the inhibitory potential of various mushroom samples on lipid peroxidation induced by CCl_4.

As shown in (Table 4), *A. arvensis* extract regulated the levels of GST, GR, CAT and GPx enzyme activities in tissues except kidney. Furthermore, the extract increased the CAT activity in kidney tissue threefold higher than those of Control and CCl_4 groups. Administration of CCl_4 causes generation of peroxyl radical and inactivation of antioxidant enzymes such as GPx, SOD, CAT and GST. Inactivation of these enzymes in organisms is associated with the accumulation of highly reactive molecules such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (OH·), hydroxyl ion (OH^-), nitric oxide (NO·) and hydrogen peroxide (H_2O_2) which contribute to the deleterious effects such as loss of coherence and function of cell membranes [5, 39–41]. When all the data are evaluated, this study suggests that *A. arvensis* has a protective capacity and antioxidant activity against CCl_4 toxicity in rtas, probably by promoting antioxidative defense systems.

In conclusions, *A. arvensis* extract which was rich in phenolics (protocatechuic acid and *p*-hydroxybenzoic acid), fatty acids (linoleic and palmitic acid) and various mineral compounds exhibited pronounced antioxidant capacities in vitro and in vivo. Data obtained within this study

suggest that *A. arvensis* extract might help gain protection against unwanted and harmful effects of oxidative stress such as hepatotoxicity, nephrotoxicity and lipid peroxidation induced by admistration of carbon tetrachloride and we suggest further clinical and industrial studies on *A. arvensis* extract in order to obtain a nutritional health enhancing food product both for food and pharmaceutical industries.

Compliance with Ethical Standards

Conflict of interest None of the authors has a conflict of commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices or with commercial providers of medical services. The authors are grateful to the University of Van Yuzuncu Yil Grant Commission for providing financial assistance of the research (Project Number: THD-2016-5377).

Research involving human and animal participants Animals used in this study (24 Wistar albino male rats 2–6 months of aged, weighting 200–350 g) were provided by the Experimental Animal Research Centre, Van Yuzuncu Yil University (Van, Turkey). All animals were fed and received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Van Yuzuncu Yil University (Protocol Number: 27552122-604.01.02). No human participants involved in the present research.

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