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In vitro and *in vivo* assessment of free radical scavenging and antioxidant activities of *Veronica persica* Poir

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Abstract: With the appearance of new disorders along with inability of some conventional therapies for the treatment of diseases without any side effects, the discovery of safe and efficient therapeutic agents is of utmost importance in the medical area. In this context, medicinal plants as promising therapeutic candidates can provide a reliable and efficient profile. Since free radicals are at the center of various disorder pathways, reducing their production or complete removal of these chemical species could be advantageous for prevention and treatment of many diseases. In this experiment, free radical scavenging and antioxidant activities of *Veronica persica* Poir., a known medicinal plant, were evaluated using *in vitro* and *in vivo* assays. Chemical characterization results showed a high phenolic content in the *V. persica* methanol extract. In addition, *in vitro* assays including DPPH radical-scavenging assay, nitric oxide-scavenging activity assay, hydrogen peroxide scavenging test and bleomycin-dependent DNA damage test revealed significant antioxidant power and radical scavenging capacity of this plant. In accordance, *in vivo* experiments showed inhibitory effects of the methanol extract on lipid peroxidation, a main cause of cell damage. Our findings revealed the promising potential of this plant in reducing free radicals through different pathways. Moreover, our data suggested a correlation between the high phenolic content of the *V. persica* extract and its free radical scavenging and antioxidant activities.

Key words: Plantaginaceae; Methanol extract; Lipid peroxidation; Oxidative stress; Polyphenols; ROS.

Introduction

Free radicals are chemical species that contain one or more unpaired electrons, which can be either neutral or positively or negatively charged. The important ones in the human body are superoxide anion ($O_2^{\cdot}$-), hydroxyl radical ($OH^{\cdot}$) and nitric oxide ($NO^{\cdot}$), with the ability to produce many other free radicals mostly derived from unsaturated fatty acids (1). These molecules with unstable nature have the affinity to reach stability by donating their unpaired electron to other molecules or taking another electron from other cell molecules (1). In low to moderate concentrations, these highly reactive fragmented atoms can play essential roles in hormonal regulations, signal transduction pathways, acting as transcription factors, upregulating defense genes and adaptive responses. However, they can be harmful in higher concentrations (2). They also play a critical role in the pathogenesis of various chronic degenerative disorders, including atherosclerosis, cancer, neurodegenerative diseases, diabetes mellitus, aging, rheumatoid arthritis and inflammation (3-5). Therefore, reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a dual role. ROS and RNS overproduction causes oxidative and nitrosative stress which leads to impairment of cell macromolecules, including lipids, DNA and proteins. However, moderate concentrations of ROS and RNS are beneficial during infections (the respiratory burst of macrophages), in cell signaling and mitogenic stimulation (2, 6). This delicate balance is accomplished by a mechanism called “redox regulation” (2). Synthetic antioxidants in foods might be a suitable choice; however, using such additives can result in toxicity since they contain phenolic compounds with various degrees of alkyl substitution. On the other hand, natural antioxidants include phenolic compounds, nitrogen-containing compounds, carotenoids, tocopherols, ascorbic acid and their derivatives (7, 8). These natural antioxidants possess redox properties giving them the ability to act as reducing agents or hydrogen-atom donors (9). Therefore, they can function as free-radical scavengers and chain breakers, chelators of pro-oxidant metal ions and quenchers of singlet-oxygen formation (10).

Plant-based foods can reduce ROS activities through

different mechanisms including *i*) avoiding ROS generation; *ii*) scavenging ROS and their byproducts; *iii*) exchanging ROS with less reactive molecules, or *iv*) improving the resistance of sensitive biological targets to ROS damages (11).

Until now, various investigations have been conducted to find any natural agent able to reduce free radical damages (12, 13). In the search for naturally occurring antioxidants from plant sources, *Veronica* species were found to have significant radical scavenging activity (11). The genus *Veronica* (Plantaginaceae) includes annual and perennial plants located in central and southern Europe and Turkey, and is represented by 79 species, 26 of which are endemic in the Turkish flora (12). Based on some investigations, these species have been traditionally used in the treatment of various diseases such as cancer, influenza, hemoptysis, laryngopharyngitis, hernia and respiratory disorders (14, 15). The genus *Veronica* is believed to contain mostly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, as well as some phenylethanoid and flavonoid glycosides (4). These flavonoids were found to be flavone glycosides often with additional hydroxyl substitution at C-6 or C-8 of the A-ring (12, 16). Iridoid glucosides, phenylethanoid glycosides, and alkaloids have also been reported from *Veronica persica* Poir. in previous studies (4). Therefore, our study aims to check free radical scavenging and antioxidant activities of *V. persica* using different *in vitro* and *in vivo* assays.

Materials and Methods

Plant material and preparation of the extract

Aerial parts (stems, leaves, and flowers) of *Veronica persica* Poir. were collected at flowering stage in April 2016 from wild plants in the mountains of Meymand, Firuzabad County, Fars Province, Iran (Coordinates: 28°52′04″N 52°45′12″E). The plant was taxonomically identified by a botanist. All parts of the plants were dried in the shade, pulverized into a fine powder using a grinder sieved through a No. 22 mesh sieve and stored in an air-tight container until analysis. A volume of 200 mL of 70% methanol was added to 20 g of powder and kept on a mechanical shaker for 72 h. The content was filtered and concentrated under reduced pressure and controlled temperature to obtain a dark gummy residue. The concentrated extract was stored dry in an amber-colored flask at 4°C for future analysis.

Animals

In this study, all animal manipulations were carried out according to the Helsinki Convention. The protocol study was approved by the local Ethical Committee (IR.SBMU.RETECH.REC.1396.586). Male Wistar rats (190 ± 4.5 g) and used for in vivo assays. The animals were kept in clean metabolic stainless-steel cages placed in a well-ventilated room and allowed to adapt to their controlled environmental conditions (24 ± 5 ˚C and 12: 12 hours light-dark cycle) before and during the tests. The animals were having free access to food and water. All animal tests were done during the light period.

Polyphenol contents

Total phenolics

The content of total phenolics in the *V. persica* methanol extract was determined spectrophotometrically by the Folin Ciocalteau reagent with the slightly modified method of Wolfe et al.(17). An aliquot of the extract (0.5 ml) was mixed with 3 ml of 10% Folin-Ciocalteu reagent and 2.5 ml of 75% w/v sodium carbonate (Sigma-Aldrich, St. Louis, Missouri, United States) solution. The resulting mixture was vortexed for 20 seconds and incubated at 40°C for 25 min for color development. The absorbance of the samples was measured at 765 nm against a blank in a Hitachi U-2001 spectrophotometer (Tokyo, Japan). All measurements were carried in triplicate and the total phenolic content was expressed as mg/g tannic acid equivalent.

Total flavonoids

The determination of total flavonoid content of the plant extract was performed according to Ordon et al. (2006) method (18). Briefly, a volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of the extract. After one hour of incubation at room temperature (25 ± 3 ºC), the absorbance was measured at 420 nm against a blank in a Hitachi U-2001 spectrophotometer (Tokyo, Japan). All measurements were carried in triplicate and the total flavonoid content was expressed as mg/g quercetin equivalent.

Total flavonols

The determination of total flavonol contents of the plant extract was performed according to the method described by Kumaran and Karunakaran (2007) (19). The reacting mixture consisted of 3 mL of the sample, 3 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/L) sodium acetate solution. The absorption at 440 nm was measured against a blank after 3 h at 20°C by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). All measurements were carried in triplicate and the total flavonol content was calculated as quercetin equivalent (mg/g).

Total proanthocyanidins

The determination of total proanthocyanidins was performed according to Sun et al. (1998) (20) with slight modifications. Briefly, 0.5 ml of 1 mg/mL extract solution was added 3 ml of vanillin-methanol (4 % v/v) and 1.5 ml of hydrochloric acid and, then, the mixture was vortexed shortly. The absorbance of the mixture was measured at 500 nm against a blank after 15 min at room temperature (25 ± 5 ºC). All measurements were carried in triplicate and the total proanthocyanidin content was expressed as catechin equivalents (mg/g).

In vitro antioxidant activities

DPPH radical-scavenging assay

The scavenging capacity of the plant extract against the stable free radical 2,2-diphenyl-1-pycrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA) was measured according to the method of Liyana-Pathiranan and Shahidi (2005) (21). Briefly, a solution of 0.135 mM DPPH in methanol was prepared, and 1.0 ml of this solution was mixed with 1.0 mL of the plant extract prepa-

red in methanol ranging from 0.025 to 0.5 mg. Butylated hydroxytoluene (BHT) and rutin were used as standard drugs separately. The reaction mixture was vortexed thoroughly and left in the dark at room temperature (25 ± 5 ºC) for 30 min. The absorbance of the mixture was measured at 517 nm against a blank by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). The ability of the plant extract to scavenge DPPH radical was calculated by the equation:

DPPH radical scavenging activity = [(Absorbance $_{control}$ – Absorbance $_{sample}$)/(Absorbance $_{control}$)] × 100

Where: absorbance control is the absorbance of DPPH radical plus methanol; absorbance sample is the absorbance of DPPH radical plus sample extract or standard.

Nitric oxide-scavenging activity

For the determination of nitric oxide (NO) scavenging activity of the plant extract the method of Garrat (1964) was used (22). Briefly, 2 mL of 10 mM sodium nitroprusside prepared in 0.5 mL phosphate buffer saline (pH 7.4) were mixed with 0.5 mL of the plant extract. Butylated hydroxytoluene (BHT) and rutin were used at different concentrations (0.025 - 0.5 mg/mL). The mixture was incubated at 25 °C. After 3 h, 0.5 mL of incubation solution was withdrawn and mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33 % prepared in 20 % glacial acetic acid) at room temperature (25 ± 5 ºC) for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature (25 ± 5 ºC) for 30 min, followed by the measurement of absorbance at 540 nm against a blank using Hitachi U-2001 spectrophotometer (Tokyo, Japan). The amount of nitric oxide radical inhibited by the extract was calculated using the following formula:

NO radical scavenging activity = [(Absorbance $_{control}$ – Absorbance $_{sample}$) / (Absorbance $_{control}$)] × 100

Where: absorbance $_{control}$ is the absorbance of NO radical plus methanol; absorbance $_{sample}$ is the absorbance of NO radical plus sample extract or standard.

Hydrogen peroxide scavenging test

The method of Ruch et al. (1989) was used to estimate the hydrogen peroxide scavenging activity of the plant extract (23). Briefly, 4 mL of the plant extract prepared in distilled water at different concentrations were mixed with 0.6 mL of 4 mM hydrogen peroxide (H_2O_2) solution prepared in phosphate buffer (0.1 M, pH 7.4) and incubated for 15 min. The absorbance of the solution was read at 230 nm against blank solution (plant extract without H_2O_2) by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

H_2O_2 radical scavenging activity = [(Absorbance $_{control}$ – Absorbance $_{sample}$)/ (Absorbance $_{control}$)] × 100

Where: absorbance $_{control}$ is the absorbance of H_2O_2 radical plus methanol; absorbance $_{sample}$ is the absorbance of H_2O_2 radical plus sample extract or standard.

Bleomycin-dependent DNA damage

In this test, the pro-oxidant activity of the plant extract and the standard (ascorbic acid) were evaluated by the Ng et al., (2003) method (24). Briefly, the reaction mixture contained 50 µg of 1 mL bleomycin sulfate, 1 mL of 5 mM magnesium chloride, 1 mL of 50 µM ferric chloride, 0.5 mL calf thymus DNA (10 µg/mL), and 1 mL of various concentrations of the plant extract. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL ethylenediaminetetraacetic acid (EDTA) (0.1 M). The color was developed by adding 0.5 mL thiobarbituric acid (TBA) (1% w/v) and 0.5 mL hydrochloric acid (25% v/v) followed by incubation at 37°C for 20 min. After centrifugation, the extent of DNA damage was measured at 532 nm by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). The ascorbic acid was the positive control.

Calculation of 50% inhibitory concentration (IC_{50})

The concentration (mg/mL) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at various different concentrations of the extract. The IC_{50} was calculated using the formula (25):

EXP (LN (conc >50%) - ((signal >50%-50)/ (signal >50%-signal <50%)*LN (conc >50%/conc <50%))).

In vivo study

Animal grouping and extract administration

Thirty-five male Wistar rats were randomized into five groups consisting of 7 each. The first group served as control and was given 0.5 mL distilled water alone per day for one week with the aid of oropharyngeal cannula. In the second group, animals served as hepatotoxic control, treated with carbon tetrachloride (CCl_4) in a single dose of 0.5 mL administered orally for one week. Groups 3 - 5 were treated like the hepatotoxic control except that they received 0.5 ml of the extract corresponding to 250, 500 and 1000 mg/kg body weight respectively. All the Wistar rats from each group were sacrificed by ether anesthesia. The liver from each animal was excised, rinsed in ice cold 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 × g for 60 min at 4 °C. The supernatant obtained was used for the estimation of superoxide dismutase and catalase activities, lipid peroxidation and reduced glutathione.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was evaluated with the method described by Misra and Fridovich (1972) (26). Briefly, a mixture was prepared contained 0.5 mL of hepatic post-mitochondrial supernatant (PMS), 0.4 mL of 25 µM nitroblue tetrazolium, 1 mL of 50 mM sodium carbonate, and 0.2 mL of freshly prepared 0.1 mM hydroxylaminehydrochloride. The reaction mixture was mixed quickly by inversion followed by the addition of supernatant of 0.1 mL of liver homogenate (10% w/v). The change in absorbance was read at 560 nm by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). Percentage inhibition was calculated using this equation:

% Inhibition of superoxide dismutase = [(Normal activity – Inhibited activity)/(Normal activity)] × 100.

Lipid peroxidation assay

Lipid peroxidation in the liver was measured using thiobarbituric acid reactive substances (TBARS) accor-

ding to the slightly modified method of Niehius and Samuelsson (1968) (27) Briefly, 0.1 mL of liver homogenate (10% w/v) was treated with 2 mL of (1:1:1 ratio) thiobarbituric acid (TBA)-trichloroacetic acid (TCA)-HCl reagent (TBA 0.37%, TCA 15% and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and then cooled. The amount of malondialdehyde formed in each of the samples was evaluated by measuring the absorbance of the clear supernatant at 535 nm against blank by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). Percentage inhibition was calculated using the equation:

% Inhibition of lipid peroxidation = $(A_c - A_s)/A_c \times 100$

Where: A_c is the absorbance of the control and A_s is the absorbance of the sample extract.

Catalase activity assay

The activity of catalase (CAT) was assayed by a method described by Pari and Latha (2004) (28). In brief, the liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuge at 5000 rpm. The reaction mixture consisted of 1 mL of 0.01 M phosphate buffer (pH 7.0), 0.4 mL of hydrogen peroxide (0.2 M), and 0.1 mL of liver homogenate (10% w/v). The reaction of the mixture was stopped by adding 2 mL of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ prepared with glacial acetic acid). The changes in the absorbance were measured at 620 nm and recorded by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). Percentage of inhibition was calculated using the equation:

% Inhibition of catalase = [(Normal activity – Inhibited activity) / (Normal activity)] × 100

Reduced glutathione assay

To determine the reduced glutathione (GSH), the Ellman (1951) method (29) with slight modifications was used. Briefly, an aliquot of 1 mL of the supernatant of liver homogenate was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm by using Hitachi U-2001 spectrophotometer (Tokyo, Japan). The percentage inhibition of reduced glutathione was calculated using the following equation:

% Inhibition of reduced glutathione = $(A_c - A_s)/A_c \times 100$

Where: A_c is the absorbance of the control and A_s is the absorbance of the sample extract.

Statistical analysis

The experimental results were expressed as the mean ± standard deviation (SD) of three replicates and were subjected to paired student's t-test. Significant levels were tested at $P < 0.05$.

Results

After determination of polyphenol contents in the *V. persica* extract, a high total phenol content (88.5 mg/g tannic acid equivalent). The amounts of total flavonoids, proanthocyandins and flavonols in the plant extract were 8.3 mg/g quercetin equivalent, 7.1 mg/g catechin equivalent and 6.5 mg/g quercetin equivalent, respectively (Figure 1).

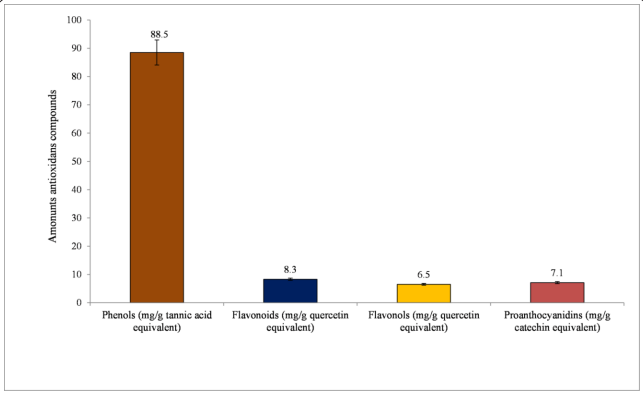

Figure 1. Polyphenol contents of *V. persica* extract.

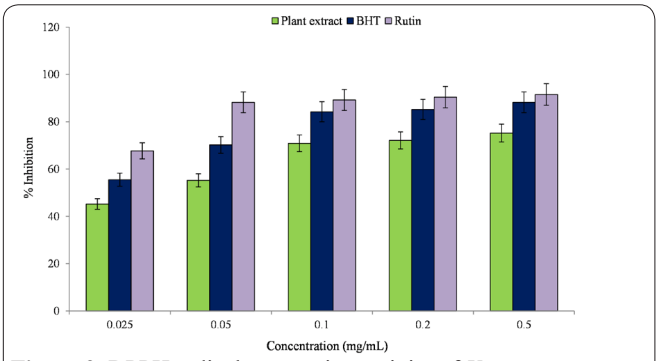

Figure 2. DPPH radical scavenging activity of *V. persica* extract

The *V. persica* extract exhibited a dose-dependent *in vitro* DPPH scavenging capacity (IC_{50} 0.03 mg/mL) (Figure 2). This antiradical activity was the weakest among the other reactive species evaluated.

The NO scavenging power test showed the ability of *V. persica* to inhibit NO in a moderate dose-dependent manner with an IC_{50} of 0.09 mg/mL, as shown in table 1.

The antioxidant activity of the plant extract was also assessed using hydrogen peroxide scavenging test. *V. persica* extract exhibited a dose-dependent scavenging capacity against H_2O_2 when compared with vitamin C and BHT used as controls (Table 2). The IC_{50} value for

Table 1. Nitric oxide radical scavenging activity of *V. persica* extract.

Sample	Concentration (mg/mL)	% Inhibition	IC_{50}
Plant extract	0.025	15.42 ± 0.29	0.09
	0.05	45.22 ± 0.01	
	0.1	51.11 ± 0.04	
	0.2	54.79 ± 0.65	
	0.5	59.80 ± 0.11	
BHT			< 0.025
Ascorbic acid			< 0.025

Values are expressed as mean ± SD.

Table 2. Hydrogen peroxide radical scavenging activity of *V. persica* extract.

Sample	Concentration (mg/mL)	% Inhibition	IC_{50}
Plant extract	0.025	35.29 ± 0.11	0.04
	0.05	54.17 ± 0.00	
	0.1	65.62 ± 0.41	
	0.2	72.44 ± 0.1	
	0.5	80.80 ± 0.09	
BHT			< 0.025
Ascorbic acid			< 0.025

Values are expressed as mean ± SD.

Table 3. Pro-oxidant activity of *V. persica* extract.

Sample	Concentration (µg/mL)				
	25	50	100	200	400
Plant extract	0.19 ± 0.04	0.16 ± 0.01	0.61 ± 0.00	0.08 ± 0.02	0.04 ± 0.01
Ascorbic acid	0.97 ± 0.04	0.75 ± 0.05	0.21 ± 0.09	0.03 ± 0.01	0.02 ± 0.01

Values are expressed as mean ± SD.

the plant extract was 0.04 mg/ml, whereas vitamin C and BHT showed IC_{50} values less than 0.025 mg/ml.

The pro-oxidant activity of the plant extract and the standard (ascorbic acid) was evaluated by their damaging effects to DNA in the presence of the bleomycin-Fe^{2+} complex. It was observed that the plant extract is devoid of pro-oxidant activity since the absorbance values decreased with the increase in the extract concentrations Table 3. This result was compared to ascorbic acid effect ($P < 0.05$).

The *in vivo* experiment after administration of different *V. persica* extract amounts (250, 500 and 1000 mg/kg body weight) revealed that the ability of the plant extract in the promotion of CAT and SOD activities, and GSH levels is in a dose-dependent manner ($P < 0.05$) (Table 4). Lipid peroxidation assay in rats treated with the plant extract demonstrated a remarkable increase in thiobarbituric acid reactive substances (TBARS) when compared to normal control group ($P < 0.05$).

Discussion

Medicinal plants have been applied for the treatment of various disorders since ancient times (30). From a simple cold to a complex disease, such plants can be used as suitable curing agents. Therefore, the discovery of new (phyto) therapeutic agents is of outmost importance in the medical area (31-37). Medicinal plants can be suitable candidates in this context (9, 38-49). *V. persica,* as a well-known medicinal plant, was selected for this study primarily because of its anti-inflammatory potential. Indeed, *V. persica*, also known as birdeye speedwell, a very common annual herb, has a long record in folk medicine (50, 51).

Until now, different investigations have been conducted to find biological activities of *V. persica*. In a study performed by Harput et al., (2002), the activities of five *Veronica* species including *V. cymbalaria*, *V. hederifolia*, *V. pectinata* var. *glandulosa*, *V. persica* and *V. polita* were evaluated (14). The results of their study showed that methanol extracts of all species exhibited inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated macrophages. The extracts also showed cytotoxic activity against KB epidermoid carcinoma and B16 melanoma cells. Such results indicated their radical scavenging activity which was compared to well-known antioxidants such as BHA and dl-α-tocopherol (14). In another recent experiment, the hydroalcoholic extract of *V. persica* was evaluated with various assays to study the antioxidant, anti-fungal and anti-inflammatory potentials of this plant (51). The chemical composition of the plant extract was characterized by high performance liquid chromatography (HPLC) which quantified 13 compounds. The extract showed a tremendous antioxidant activity using DPPH (79.91% inhibition) and phosphomolybdate (324.4 mg/g ascorbic acid equivalents) assays. The extract also exhibited an antifungal activity against *Aspergillus niger* and *Penicillium hirsutum*. Moreover, the anti-inflammatory activity of *V. persica* extract was confirmed using inflammation experimental models. Anti-inflammatory (52), antioxidant, antibacterial (53, 54), antimutagenic and antitumor (55) activities of different *Veronica* species were also shown by various investigations. These reports are evidences of the enormous potential of *Veronica* species in biomedical sector.

In this experiment, we evaluated the *in vitro* and *in vivo* antioxidant and radical scavenging activities of *V. persica* methanol extract using different assays. The analysis of the polyphenol content showed a low amount of total flavonoids, proanthocyandins and flavonols in the extract. On the other hand, a high amount of total phenolic was found in the extract. Results of all *in vitro* antioxidant assays including DPPH radical-scavenging, nitric oxide-scavenging and hydrogen peroxide-scavenging tests showed a remarkable antioxidant activity of the *V. persica* extract. DPPH assay results indicated a dose-dependent scavenging ability of the plant extract. However, this ability was not as high as the ascorbic acid or BHA one. The activity of the extract in scavenging NO also showed a dose-dependent trend, though with a higher IC_{50}. A similar pattern was observed in H_2O_2 scavenging test, where the extract exhibited a high capacity against hydrogen peroxide.

In addition, the plant extract did not display pro-oxidant activity. Since the extract contained phenolics, flavonoids, proanthocyandins and flavonols, the observed antioxidant effects of *V. persica* extract could be

Table 4. *V. persica* extract effects on lipid peroxidation, antioxidant enzymes and glutathione in CCl_4 induced liver damage in male Wistar rats.

Groups	Catalase	Superoxide dismutase	Glutathione	Lipid peroxidation
Control	105.42 ± 0.01 [a]	98.95 ± 0.11 [a]	90.44 ± 0.05 [a]	114.85 ± 0.29 [a]
Carbon tetrachloride	65.58 ± 0.12 [b]	39.33 ± 0.01 [b]	29.45 ± 0.12 [b]	39.34 ± 0.06 [b]
Carbon tetrachloride + *V. persica* (C1)	75.33 ± 0.19 [c]	58.21 ± 0.09 [c]	55.39 ± 0.02 [c]	65.92 ± 0.03 [c]
Carbon tetrachloride + *V. persica* (C2)	83.47 ± 0.03 [c]	75.32 ± 0.14 [d]	62.75 ± 0.23 [d]	73.29 ± 0.01 [d]
Carbon tetrachloride + *V. persica* (C3)	99.89 ± 0.51 [d]	83.33 ± 0.09 [e]	69.72 ± 0.29 [e]	83.72 ± 0.42 [e]

Values are mean of ± SD (n = 7 rats). C1 = 250 mg/kg body wt; C2 = 500 mg/kg body wt; C3 = 1000 mg/kg body wt. Results are expressed as percentage inhibition of the control. Mean values with different letters within a column are significantly different ($P < 0.05$; LSD).

mainly attributed to its polyphenol content. These findings are in agreement with other reports showing that the antioxidant power and radical scavenging capacity of plant extracts were related to their high amount of polyphenols (56-61). Another study reported that the *Veronica officinalis* L. extract exhibited the most potent radical scavenging activity among other species. After characterization, it was found that the *V. officinalis* extract had a high amounts of phenolic when compared to other extracts (12). Moreover, in another experiment, anti-inflammatory and antioxidant activities of the *Veronica* spp. extract was supposed to be correlated with its polyphenols and iridoid compounds (62). Such direct relationship is a proof for the importance of these phytochemicals and their antioxidant activities.

Our *in vivo* results demonstrated the capability of the plant extract to improve the production of antioxidant enzymes in animal. SOD and CAT are antioxidant enzyme that provide a natural defense against oxidative stress of the cell (63). Treatment of animal models with carbon tetrachloride along with the plant extract showed an increase in the amounts of these enzymes in animal cells. Similarly, the extract increased the production of non-enzymatic antioxidants such as GSH. The thiobarbituric acid reactive substances (TBARS) assay was used for measuring the effects of the plant extract on lipid peroxidation. According to our results, groups treated with the plant extract showed a dose-dependent increase in the TBARS, thus indicating the ability of the extract in lipid peroxidation inhibition. These results are in agreement with recent experiments demonstrating the lipid peroxidation inhibitory potential of different plant extracts (64, 65). As known, the anti-lipid peroxidation activity by plant extracts can also be attributed to their polyphenol content. For example, the phenolic-rich extract of *Tetrapleura tetraptera* fruits showed effectively inhibited xanthine oxidase as well as F^{2+}-induced lipid peroxidation in animal models (66). Lipid peroxidation can generate a high amount of ROS, which, in turn, can be involved in cardiovascular diseases, diabetes mellitus, carcinogenesis, etc.(67). Therefore, the use of plants with high phenolic content and anti-lipid peroxidation activity might be beneficial for the prevention of many disorders.

Since oxidative stress is a deep-rooted cause of various disorders, the prevention of ROS and RNS production in the cell metabolism is of utmost importance. Vast usage of medicinal plants in different therapeutic contexts led us to study *V. persica* in order to evaluate its antioxidant and radical scavenging activities. Our results indicated the tremendous potential of this plant in reducing free radicals through different pathways, possibly due to its high polyphenol content. However, more investigations should be carried out to clarify the specific correlations between the plant bioactives and the observed biological activities.

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Conflict of Interest
The authors declare no conflict of interest.

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