African Journal of Biotechnology Vol. 9(34), pp. 5542-5549, 23 August, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Biological and pharmacological effects of *Delphinium elbursense*

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Accepted 16 July, 2010

Antidepressant, antihypoxic and antioxidant activities of aerial parts of *Delphinium elbursense* were investigated employing nine various assay systems. Antidepessant activity was examined by using forced swimming test and tail suspension test in mice. The extracts at all tested doses show significant activity as compared to control group. Antihypoxic activity was investigated in two models, haemic and circulatory. The effects were pronounced and dose-dependent in both model of hypoxia. Extracts showed weak antioxidant activity in some models. IC_{50} for 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical-scavenging activity was 116.2 ± 5.6 µg ml^{-1}. Extracts showed nitric oxide-scavenging activity between 0.1 and 1.6 mg ml^{-1} (IC_{50} = 502.3 ± 18 µg ml^{-1}) and a very weak Fe^{2+} chelating ability (IC_{50} = 1.01 ± 0.03 mg ml^{-1}). It also exhibited low antioxidant activity in hemoglobin-induced peroxidation of linoleic acid but was capable of scavenging hydrogen peroxide in a concentration dependent manner. Extract show antihemolytic activity againts hydrogen peroxide (H_2O_2) induced hemolysis (558.7 ± 31 µg ml^{-1}). The total phenolic compounds in extract were determined as gallic acid equivalents (52.24 ± 1.7) and total flavonoid contents were calculated as quercetin equivalents (17.26 ± 0.6) from a calibration curve.

Key words: Antidepressant, antihypoxic, *Delphinium elbursense*, flavonoid contents, forced swimming test, medicinal plants, phenolic contents, tail suspension test.

INTRODUCTION

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury and death (McCord, 2000). Moreover, the oxidative stress caused from imbalance between the generation and the neutralization of free radicals by antioxidant mechanism is responsible for many human diseases, includeing aging, cancer and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's diseases (Nabavi et al., 2008b). Their deteriorative effects can be diminished by natural antioxidants available in foods. Also, oxidative reactions limit the shelf life of fresh and processed food stuffs and are a serious concern in food industry (Sökmen et al., 2004). Synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes. However, due to their unstable and highly volatile nature, questions about their safety and efficiency have been brought up ever since their first introduction to the food industry (Nabavi et al., 2008a). Consequently, the need to identify alternative natural and safe sources of food antioxidant arose and the search for natural antioxidants, especially of plant origin, has notably increased in recent years (López et al., 2007).

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Abbreviations: DPPH, Diphenyl-2-picryl hydrazyl; **BHA,** butylhydroxyanisole; **BHT,** butylhydroxytoluene; **EDTA,** ethylenediaminetetraacetic acid; **i.p.,** interperitoneal; **AD,** Alzheimer's disease.

Depression constitutes the second most common chronic condition in clinical practice (Whooley and Simon, 2000) and will become the second leading cause of premature death or disability worldwide by the year 2020 (WHO, 1999). Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing (Mora et al., 2006). Although there are many effective antidepressants available today, the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated. This necessitates the development of newer and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential has been assessed in a variety of animal models (Zhang, 2004).

Delphinium elbursense Rech. f. (*Ranunculaceae*) is native to Iran. Norditerpene alkaloids with antioxidant activity have been isolated from *Delphinium linearilobum* (Kolak et al., 2006). Some major flavonoids have been isolated from *Delphinium flexiosum* and *Delphinium elisabethae* (Arazashvili et al., 1975). Anticonvulsant activities of ethanolic extract and aqueous fraction from *Delphinium denudatum* was reported (Mohsin et al., 2001). To the best of our knowledge, there is no report on biological activity of *D. elbursense*. The aim of this study is to determine the antidepressant, antihypoxic, antioxidant and antihemolytic activities of *D. elbursense* aerial parts extract in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

MATERIALS AND METHODS

Chemicals

Imipramine was products of Darupakhsh Co (Tehran, Iran). Ferrozine, linoleic acid, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH) and potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Sodium nitrite, sodium fluoride, BHA, ascorbic acid, N-(1-naphthyl) ethylenediamine dihydrochloride, ethylenediaminetetraacetic acid (EDTA), gallic acid, sulfanilamide and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Plant materials and preparation of extract

D. elbursense were collected from central Elburz Mountains, north of Iran, in summer 2008. After identification of the plant by Dr. Bahman Eslami, a voucher (No. 441) was deposited in the Faculty of Pharmacy herbarium. Aerial parts of plant were dried at room temperature and coarsely ground before extraction. A known amount of aerial parts of plant was extracted at room temperature by percolation method using 70% ethanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Animals

Animals used in the experiments were male albino rats (200 – 250 g) and male NMRI mice (22 – 25 g), kept under standard conditions in animal house (water and food *ad libitum*, 12 h dark and light cycle). All experiments were conducted between 10:00 and 14:00 h and all experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory animals.

Antidepressant activity

Forced swimming test

The mouse was dropped into a glass cylinder (20 cm in height and 12 cm in diameter) containing 8-cm-deep water at 24 - 25°C and left there for 6 min. The duration of immobility during the final 4-min interval of the swimming test was measured (Ebrahimzadeh et al., 2009b; Hadizadeh et al., 2009). Two groups served as control: The first group was treated with Tween 80 plus 0.9% (w/v) saline solution and the second group was with olive oil; the other groups of mice received an interperitoneal (i.p.) injection of extracts (250, 500 and 1000 mg kg^{-1}) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (15 mg kg^{-1}), 1 h before the experiment. Imipramine was utilized as positive control of the test.

Tail suspension

Male mice weighing 20 – 25 g were used preferentially. They were housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 10 animals were treated with the extract (250, 500 and 1000 mg kg^{-1}) by i.p. injection 30 min prior to testing. For the test, the mice were suspended on the edge of a shelf, 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 min. Mice were considered immobile when they hang passively and completely motionless for at least 1 min. Imipramine (15 mg kg^{-1}) was used as positive control of the test (Mahmoudi et al., 2009).

Antihypoxic activity

Haemic hypoxia

Thirty minutes after i.p. administration of extract, 250, 500 and 1000 mg kg^{-1}, $NaNO_2$ (360 mg kg^{-1}) was applied i.p. to each mouse and antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes according to the method of Roshtina and Ostrovskaya (1981). Antihypoxic activity was expressed relative to the control.

Circulatory hypoxia

Thirty minutes after i.p. administration of extract, 250, 500 and 1000 mg kg^{-1}, NaF (150 mg kg^{-1}) was applied i.p. to each mouse and the antihypoxic activity was estimated in minutes as the latent time of evidence of hypoxia (Krasteva et al., 2004). Antihypoxic activity was expressed relative to the control

Maximum non-fatal dose

Different doses of extract were injected to separated groups of seven. After 48 h, the highest dose that did not induce any mortality was considered as the maximum non-fatal dose (Ebrahimzadeh et

al., 2010).

Determination of total phenolic compounds and flavonoid content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ghasemi et al., 2009; Dehpour et al., 2009). The extract sample (0.5 ml) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g l^{-1} sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoids were estimated according to the method of Ebrahimzadeh et al. (2009a). Briefly, 0.5 ml solution of plant extract were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

Antioxidant activity

DPPH radical-scavenging activity

The stable DPPH was used for the determination of free radical-scavenging activity of the extracts (Ebrahimzadeh et al., 2008a). Different concentrations of extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power determination

The reducing power of *D. elbursense* was determined according to the method of Ebrahimzadeh et al. (2009d). 2.5 ml of extract (25 - 800 µg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

Assay of nitric oxide-scavenging activity

For the experiment, sodium nitroprusside (10 mM) in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without extract but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Ebrahimzadeh et al., 2009e).

Metal chelating activity

The chelating of ferrous ions by *D. elbursense* was estimated using the method of Ebrahimzadeh et al. (2008b, c). Briefly, the extract (0.2 - 3.2 mg ml^{-1}) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left to stand at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- Fe^{2+} complex formation was calculated as

$$[(A_0 - A_s)/A_s] \times 100$$

Where, A_0 was the absorbance of the control and A_s the absorbance of the extract/ standard. Na_2EDTA was used as positive control.

Scavenging of hydrogen peroxide (H_2O_2)

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ebrahimzadeh et al. (2009e). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extract (0.1 - 1 mg ml^{-1}) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as

$$\% \text{ Scavenged } [H_2O_2] = [(A_o - A_1)/A_o] \times 100$$

Where, A_o was the absorbance of the control and A_1 the absorbance in the presence of the sample of extract and standard.

Antioxidant activity in a hemoglobin-induced peroxidation of linoleic acid

The antioxidant activity of extract was determined by a modified photometry assay (Kuo et al., 1999). Reaction mixtures (200 ml) containing 10 ml extract (10 – 400 mg), 1 mmol/l of linoleic acid emulsion, 40 mmol/l of phosphate buffer (pH 6.5) and 0.0016% hemoglobin, were incubated at 37°C for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the lipid peroxidation. The amount of peroxide value was measured in triplicate using the thiocyanate method by reading the absorbance at 480 nm after colouring with 100 ml, 0.02 mol/l of $FeCl_2$ and 50 ml of ammonium thiocyanate (30 g/100 ml). Vitamin C was used as positive control.

Preparation of rat erythrocytes

Male Wistar rats were sacrificed under anesthesia and blood was collected by heart puncture in heparinized tubes. Erythrocytes were isolated and stored according to the method described by Yuan et al. (2005). Briefly, blood samples collected were centrifuged (1500×g, 10 min) at 4°C; erythrocytes were separated from the plasma and buffy coat and were washed three times by centrifugation (1500×g, 5 min) in 10 volumes of 10 mM phosphate buffered saline (pH 7.4; PBS). The supernatant and buffy coats of white cells were carefully removed with each wash. Washed erythrocytes were stored at 4°C and used within 6 h for further studies.

Antihemolytic activity of extract against H_2O_2 induced hemolysis

Antihemolytic activity of the extract was assessed as described by

Table 1. Antidepressant activity of extract in forced swimming test (FST) and tail suspension test (TST).

Group	Dose (mg/kg)	Duration of immobility (s), FST	Duration of immobility (s), TST
Control	-	164.2 ± 1.3	157.8 ± 12
Extract	250	140.7 ± 8.8**	150.8 ± 8.3**
Extract	500	118.7 ± 6.7***	121.8 ± 4.1***
Extract	1000	104.1 ± 3.0***	88.1 ± 5.0***
Imipramine	15	88.2 ± 3.0***	82.0 ± 9.6***

[a] Data are expressed as mean ± SD (n = 10), (**P < 0.01, ***P < 0.001 , compared to control).

Table 2. Antihypoxic activity of *D. elbursense* aerial parts extract on two models of brain hypoxia.

Dose (mg kg^{-1})	Haemic hypoxia activity (min)	Circulatory hipoxia activity (min)
Control	10.55 ± 0.33	9.38 ± 0.03
250	13.34 ± 0.63**	11.05 ± 0.45**
500	14.89 ± 0.24***	13.26 ± 0.14***
1000	19.23 ± 1.94***	17.67 ± 1.36***

[a] Data are expressed as mean ± SD (n = 10), (**P < 0.01, ***P < 0.001 , compared to control).

Naim et al. (1976) with slight modifications. The erythrocytes from male Wistar rat blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. 1 g of extract ml^{-1} of saline buffer were added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 ml of H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to about 90% hemolysis of blood cells after 240 min. After incubation, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test. The IC_{50} values were calculated from linear regression analysis.

RESULTS

Antidepressant activity

Table 1 shows the result of effect of extract on duration of immobility during forced swimming test. The extract at all tested doses show significant activity as compared to the control group. The extract also showed very good activity in tail suspension test. All tested significant in doses and dose dependently decreased the immobility time as compared to control mice (Table 1). The extract at the dose of 1000 mg kg^{-1} showed the same activity as imipramine at 15 mg kg^{-1}, in decreasing immobility period ($p > 0.05$).

Antihypoxic activity

The extract showed good protective effect against hypoxia. It produced dose dependent effect on both haemic and circulatory hypoxia (Table 2).

Determination of total phenolic compounds and flavonoid content

The total phenolic content was 52.24 ± 1.7 mg gallic acid equivalent g^{-1} extract by reference to standard curve ($y = 0.0054x + 0.0628$, $r^2 = 0.987$). The total flavonoid content was 17.26 ± 0.6 mg quercetin equivalent g^{-1} extract, by reference to standard curve ($y = 0.0063x$, $r^2 = 0.999$).

Antioxidant activities

IC_{50} for DPPH radical-scavenging activity was 116.2 ± 5.6 µg ml^{-1}. The IC_{50} values for ascorbic acid, quercetin and BHA were 1.26 ± 0.04, 1.32 ± 0.07 and 13.49 ± 0.09 µg ml^{-1}, respectively. Figure 1 shows dose-response curves for the reducing powers of extract. It was found that the reducing powers of extract increased with increasing concentrations. The extract exhibited a fairy good reducing power at 25 and 800 µg ml^{-1}. The extract also showed good nitric oxide scavenging activity between 0.1 and 1.6 mg ml^{-1}. The % inhibition was increased with increasing concentration of extract. IC_{50} was 502.3 ± 18 for *D. elbursense* and 17.01 ± 0.4 µg ml^{-1} for quercetin. The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, that is, the activity was increased on increasing concentration from 0.2 to 1.6 mg ml^{-1}. Extract showed moderate Fe^{2+} chelating ability. IC_{50} was

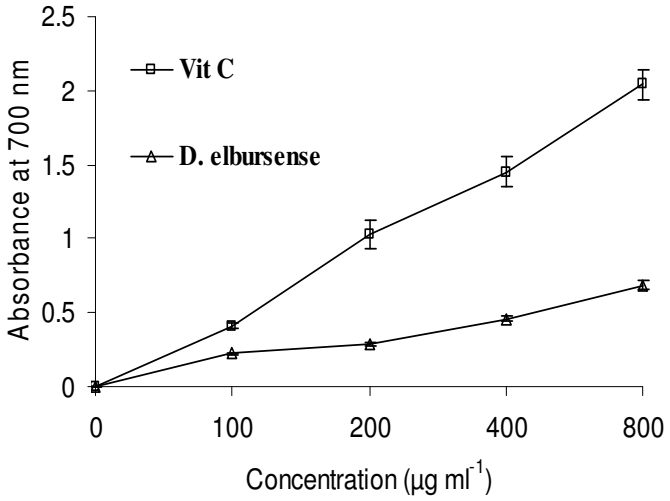

Figure 1. Reducing power of *D. elbursense*. Vitamin C was used as control.

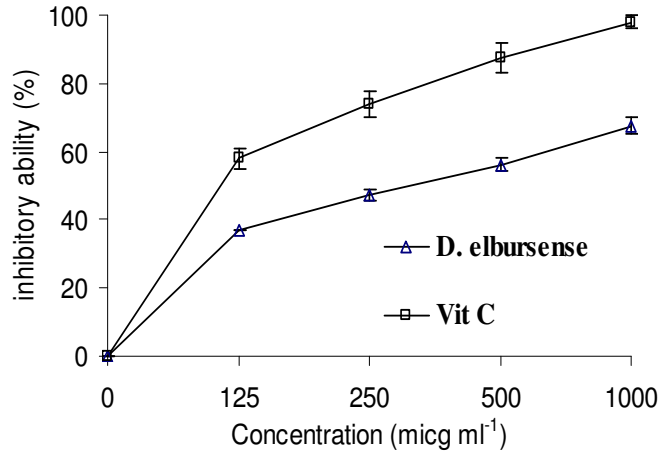

Figure 2. Effect of *D. elbursense* extract on hemoglobin-catalyzed peroxidation of linoleic acid. Vitamin C was used as control.

1012 ± 37 µg ml^{-1}. EDTA showed very strong activity (IC_{50} = 18 µg ml^{-1}). The extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. IC_{50} for H_2O_2 scavenging activity was 558.7 ± 31 µg ml^{-1}. The IC_{50} values for ascorbic acid and BHA were 21.4 ± 1.1 and 52.0 ± 2.3 µg ml^{-1}, respectively. Tested extract showed good activity in hemoglobin-induced linoleic acid system. There was significant difference between extract and control ($p < 0.05$) (Figure 2). In addition, extract showed good inhibition capacity on the hemoglobin-catalyzed peroxidation of linoleic acid with IC_{50} = 623 ± 27 µg ml^{-1}. Vitamin C exhibited IC_{50} value of 235 ± 9 µg ml^{-1}.The maximum non-fatal used dose of extract of *D. elbursense* aerial parts used in our experiment was 2 g kg^{-1}.

DISCUSSION

Behavioural despair was proposed as a model to test for antidepressant activity by Porsolt et al. (1991). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Table 1 showed effect of extract on the duration of immobility during forced swimming test. The extract at all tested doses, showed significant activity as compared to control group. The tail suspension test has been described by Steru et al. (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail. The extract at all tested doses, significantly and dose dependently decreased the immobility time as compared to control mice (Table 1). At the dose of 1000 mg kg^{-1}, extract showed the same activity as imipramine at 15 mg kg^{-1} in decreasing immobility period ($p > 0.05$).

There are literature data that shows that administration of sodium fluoride (substance that induces circulatory hypoxia) increases the blood histamine content and decreases the oxygen carrying capacity (Sumina et al., 1978). Our results may be supported strongly by some literature data that improved flavonoids increase in cerebral blood flow and possess antihypoxic activity. The mechanism of this protective action may be due in part to the antioxidant activity of quercetin (Meli et al., 1990; Karcher et al., 1984). The extract showed good protective effect against hypoxia. It produced dose dependent effect on both haemic and circulatory hypoxia (Table 2).

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (van Acker et al., 1996). Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (Hertog et al., 1993). The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee et al., 2003). DPPH is a stable nitrogen-centered free radical color which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore, radical scavengers (Dehpour et al., 2009). IC_{50} for DPPH radical-scavenging activity was 116.2 ± 5.6 µg ml^{-1}. Phenol and flavonoid contents of this plant may lead to its good DPPH-scavenging activity. Fe (III) reduction is often used as an indicator of electron donating activity, which is an

important mechanism of phenolic antioxidant action (Nabavi et al., 2009a; Nabavi et al., 2009b). In this assay, the presence of reductants (antioxidants) in the samples would result in reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows the dose-response curves for the reducing powers of *D. elbursense* extract. It was found that the reducing power of extract increased with the increase of its concentrations. The extract exhibited a fairy good reducing power at 25 and 800 µg ml^{-1}. The extract also showed good nitric oxide (NO) scavenging activity between 0.1 and 1.6 mg ml^{-1}. The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. The % inhibition was increased with increasing concentration of the extract. IC_{50} was 502.3 ± 18 and 17.01 ± 0.03 µg ml^{-1} for quercetin. In addition to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions (Moncada et al., 1991). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in human and thereby, improves quality of life and overall survival in some diseases such as thalassemia major (Hebbel et al., 1990). In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's disease (AD) pathology and so iron chelation could be considered a rational therapeutic strategy for AD (Ebrahimzadeh et al., 2009c).

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Ebrahimzadeh et al., 2009g). These processes can be delayed by iron chelation and deactivation. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (Ebrahimzadeh et al., 2009f). Because Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. The chelating of ferrous ions by the extract was estimated by our recently published paper (Ebrahimzadeh et al., 2008b). Ferrozine can quanti-tatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The absorbance of Fe^{2+} -ferrozine complex was decreased dose-dependently, that is, the activity was increased on increasing concentration from 0.2 to 1.6 mg ml^{-1}. *D. elbursense* extract showed moderate Fe^{2+} chelating ability. IC_{50} was 1012 ± 37 µg ml^{-1}. EDTA showed very strong activity (IC_{50} = 18 µgml^{-1}). Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh et al., 1999). It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). Scavenging of H_2O_2 by *D. elbursense* extracts may be attributed to their phenolics and other active components which can donate electrons to H_2O_2, thus neutralizing it to water (Halliwell and Gutteridge, 1990). The *D. elbursense* extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. IC_{50} for H_2O_2 scavenging activity was 558.7 ± 31 µg ml^{-1}. The IC_{50} values for ascorbic acid and BHA were 21.4 and 52.0 µg ml^{-1}, respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu, 2001). The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Nabavi et al., 2008a). Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. Tested extract show good activity in hemoglobin-induced linoleic acid system. There were significant differences between extract and controls (Figure 2).

Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lysis in rat blood. This assay is useful either for screening studies on various molecules and their metabolites, especially molecules having oxidizing or antioxidizing activity, or molecules having a long-term action (Djeridaneet al., 2006). Lipid oxidation of rat blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. Extract showed good inhibiting activity (IC_{50} =

623 ± 27).

Conclusions

Our studies indicate that the extract of *D. elbursense* aerial showed good antidepressant activity in forced swimming and tail suspension models and antihypoxic effect in both model of circulatory and haemic hypoxia. It is therefore very promissing for further pharmacological and biochemical experiments, which will focus on evaluating the mechanism of antihypoxic activity. It also exhibited good but different levels of antioxidant activity in some models studied. The extracts had good reducing power and nitric oxide scavenging activity. Further investigation of individual compounds, their *in vivo* antioxidant activities and different antioxidant mechanisms is needed.

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