Antioxidant Activity of Iranian *Echium amoenum* Fisch & C.A. Mey Flower Decoction in Humans: A cross-sectional Before/After Clinical Trial

Akram Ranjbar¹, Sara Khorami¹, Mehdi Safarabadi¹, Amene Shahmoradi¹, Ali Akbar Malekirad¹, Katyon Vakilian¹, Ali Mandegary²,³ and Mohammad Abdollahi³

¹Arak University of Medical Sciences, Arak, ²Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman and ³Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

Medicinal plants are recognized as sources of natural antioxidants that can protect from biological system oxidative stress. The present cross-sectional before/after clinical trial was carried out to investigate the antioxidant properties of the decoction of the flowers of *Echium amoenum* Fisch & C.A. Mey in humans. A group of 38 healthy subjects was invited to use the *E. amoenum* (7 mg kg⁻¹) twice daily for 14 days. Blood samples before and after entering the study were measured for lipid peroxidation level (LPO), total antioxidant capacity (TAC) and total thiol (SH) molecules. A significant reduction of blood LPO (24.65 ± 11.3 versus 19.05 ± 9.7, *P* = 0.029) was observed after 14 days of *E. amoenum* consumption. Blood TAC (1.46 ± 0.51 versus 1.70 ± 0.36, *P* = 0.018) and total thiol molecules (0.49 ± 0.11 versus 0.56 ± 0.12, *P* = 0.001) increased after 14 days of *E. amoenum* consumption. In conclusion, this antioxidative stress potential of *E. amoenum* may be due to its bioactive antioxidant components, especially rosmarinic acid and flavonoids. In recent years the importance of oxidative stress in the pathophysiology of many human disorders has been confirmed, thus use of this plant as a dietary supplement is highly recommended.

**Keywords:** antioxidant – decoction – *Echium amoenum* Fisch & C.A. Mey – human – oxidative stress

**Introduction**

Free radicals, like reactive oxygen species (ROS), nitrogen (RNS) and chlorine (RCS), are normal by-products of metabolism and they are introduced into the body from outside sources of harmful chemicals in the environment, unhealthy foods, stress, certain drugs, cigarette smoke, etc. Increasing the intake of antioxidants can neutralize free radicals and protect the body from cell damage. In the body, oxidative stress results from the imbalance between the extent of ROS formation and the antioxidant defense mechanisms. Links between oxidative stress and adverse health effects have been suggested for several groups of diseases, including cardiovascular, respiratory and neurological as well as for the general aging process. Such adverse effects are mediated by free radical damage to lipids, proteins and DNA. Protection from damage occurs through the action of multiple antioxidants, some endogenously produced and some provided through dietary intake (1–3). It is believed that medicinal plants are a potential source of antioxidants and ROS scavenger molecules (4). One of these plants is *Echium amoenum* Fisch & C.A. Mey that has been shown as a rich source of antioxidants, like rosmarinic acid (RA) and flavonoids. This plant belongs to the Boraginaceae family and is a biennial or perennial herb...
indigenous to the narrow zone of northern part of Iran and Caucasus, where it grows at highlands at the altitude ranging from 60 to 2200 m (5).

There has been an increased interest in Echium species, including E. amoenum (F.M.), because of their medicinal and nutritional properties. E. amoenum (F.M.) is one of the most important medicinal plants in Iranian traditional medicine (6). The flowers of this plant have been used as demulcent, anti-inflammatory and analgesic, anxiolytic, and sedative in folk medicine of Iran (5–7). Anxiolytic effect of the flower of this plant has been shown in two separate experimental studies in mice (8,9). In Western medicine, the flowers and the leaves of borage have been similarly used as antifebrile, antidepressant, anxiolytic, ameliorant of heart and pulmonary disturbances, poultice for inflammatory swellings, diuretic, laxative, emollient and demulcent, and recently as a possible protective factor against cancer (10–12). Extract of this plant has been shown to contain flavonoids, saponins, unsaturated terpenoids and sterols (8). Extract of Echium was effective at intraperitoneal doses of 80–125 mg kg$^{-1}$ in animals (10) and has not been toxic in doses as high as 6 g kg$^{-1}$ (13).

Phytochemical studies on E. amoenum revealed the presence of many chemicals such as RA, anthocyanidine, flavonoids, γ-linolenic acid and trace amount of alkaloids (6,14,15). The antioxidant properties of flavonoids (16,17) and RA (18–22) have been well established.

Regarding the above-mentioned information, we were interested in performing a cross-sectional before/after clinical trial study to explore antioxidant influences of E. amoenum (F.M.) in human by evaluation of blood total antioxidant capacity (TAC), lipid peroxidation (LPO) and total thiol (SH) molecules.

**Subjects and Methods**

**Study Design**

A clinical trial study with a total of 38 subjects was designed. Subjects were volunteer students of Arak University of Medical Sciences, located in the south-west of Iran, who all lived in the university dormitory. Subjects were selected on a simple random basis from volunteers. The study was conducted in complete accordance with the declaration of Helsinki. All participants were provided with specific written consents obtained prior to entrance into the study. Each individual was extensively interviewed by a specialized physician who filled in a structured questionnaire specifying gender, smoking, dietary habits, sports habits and history of special disease, before obtaining blood. Then the subjects were
administered *E. amoenum* (F.M.) flower decoction (7 mg kg\(^{-1}\)) twice daily (morning and evening) for 2 weeks. The dose was selected on the basis of a pilot study and traditional use information. A supervisor carefully checked to make sure that the volunteers were taking the decoction properly. Demographic characteristics of the subjects are presented in Table 1.

### Materials

5,5\(^0\)-Dithiobis-2-nitrobenzoic acid (DTNB), Tris base, 1,1,3,3\(^0\)-tetraethoxypropane (MDA) from Sigma, UK, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol from Merck, Germany, and 2,4,6-tripyrindyl-s-triazine (TPTZ) from Fluka, Italy, were used in this study.

### Plant Material

Flowers of *E. amoenum* (F.M.) were collected from a farm at 80 km north of Ghazvin (a city in western Iran) in June 2002. A total of 450 g air-dried flowers of *E. amoenum* was used to provide the decoction.

### Plasma Preparation

Five milliliters of heparinized blood was collected from each subject at the end of the 2 week treatment, centrifuged at 1200 \(g\) for 10 min at 4\(^\circ\)C and plasma frozeed at −80\(^\circ\)C until analysis. Blood samples were collected 12 h after the last dose of decoction was taken.

### Measurement of Plasma TAC

Antioxidant capacity of plasma was determined by measuring the ability of plasma to reduce Fe\(^{3+}\) to Fe\(^{2+}\). The complex between Fe\(^{2+}\) and TPTZ gives a blue color with absorbance at 593 nm (23).

### Measurement of Plasma Total Thiol Molecules

Total sulfhydryl content was determined in plasma by the method of Hu (24). A volume of plasma (0.20 ml) was mixed in a 10 ml test tube with 0.6 ml of Tris–EDTA buffer (Tris base 0.25 M, EDTA 20 mM, pH 8.2) followed by the addition of 40 \(\mu\)l of 10 mM of DTNB in methanol. The final volume of the reaction mixture was made up to 4.0 ml by adding 3.16 ml of methanol. The test tube was capped, and the color was developed for 15–20 min, followed by centrifugation at 3000 \(g\) for 10 min at ambient temperature. The absorbance of the supernatant was measured at 412 nm.

### Measurement of LPO

LPO of plasma was determined by the reaction of TBA with MDA and other lipid peroxides. Briefly, plasma samples were mixed with TCA (20\%) and the precipitate was dispersed in \(\text{H}_2\text{SO}_4\) (0.05 M). TBA (0.2\% in sodium sulfate) was added and heated for 30 min in a boiling water bath. LPO adducts were extracted by n-butanol and absorbance was measured at 532 nm (25).

### Statistics

Paired \(t\)-test was used to analyze the significance of differences observed between study groups. *F*-test was used to determine the normal distribution of variances between groups. *P*-values >0.05 were considered insignificant.

### Results and Discussion

A significant decrease (*P* < 0.05) in LPO was observed by use of *E. amoenum*. The mean ± SD before and after using were 24.65 ± 11.33 and 19.05 ± 9.7 nmol ml\(^{-1}\) (Fig. 1). After use of the decoction, the TAC level increased significantly (*P* < 0.05). The mean ± SD values before and after were 1.46 ± 0.51 and 1.70 ± 0.36 m\(\mu\)mol ml\(^{-1}\) (Fig. 2). A significant (*P* < 0.001) increase in total thiol molecules was observed after administration of the decoction (0.49 ± 0.11 m\(\mu\)mol ml\(^{-1}\) before versus 0.56 ± 0.12 m\(\mu\)mol ml\(^{-1}\) after (Fig. 3).

The Consumption of *E. amoenum* Markedly Decreases ROS Concentrations

In this study, the influence of usage of the decoction (7 mg kg\(^{-1}\)) twice daily (morning and evening) for 2 weeks on the oxidative stress status of healthy subjects was studied. Results indicate that TAC and thiol groups increased and LPO as a marker of ROS concentration markedly decreased after consumption of *E. amoenum*. In the body, antioxidants act as free radical scavengers and thus protect cells from being exposed to free radicals and further cellular damage. This is the mechanism by which they protect the human body from several diseases attributed to the reactions of radicals. Numerous substances have been suggested to act as antioxidants in this plant. Various phenolic antioxidants such as flavonoids, RA, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge radicals in a dose-dependent manner (26,27). In addition, flavonoids and RA have been introduced as the main constituents of *E. amoenum* (F.M.) in several phytochemistry studies (6,16,17,27,28).

### Table 1. Demographic characteristics of study subjects

| Using drug | Sport | Smoking | Age (year) | Sex | Number of subjects |
|------------|-------|---------|------------|-----|-------------------|
| No (86.8%)| 5 (13.2%) | 25 (65.8%) | 13 (34.2%) | 0 | 38 (100%) | 38 |
| Yes | No (65.8%) | Yes | 38 (100%) | 25 (65.8%) | 13 (34.2%) |
Antioxidant activity of Iranian Echium amoenum

The antioxidant potential of flavonoids has been well established (22,23). Flavonoids can highly scavenge most types of oxidizing molecules, including singlet oxygen and various free radicals, and thus act indirectly as an efficient antioxidant (29). They can also act directly by suppressing ROS formation (30).

RA Reduced Pro-inflammatory Molecule Expression and Enhanced Antioxidative Activity

RA, the other important constituent of this plant, is an ester of caffeic acid and 3,4-dihydroxyphenylacetic acid. It is commonly found in species of the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae. There are a number of reports on the antioxidative activities of RA which all confirm that RA has strong antioxidant activity even higher than vitamin E. In this regard, the reported positive effects of RA include enhancement of superoxide and hydroxyl scavenging (20), inhibition of both low-density lipoprotein (21) and oil oxidation (19), suppression of arachidonate metabolism formation (31), inhibition of hemolysis (32), and having hyaluronidase and h-hexosaminidase activities (33). In addition, RA inhibited lung injury in mice that is regularly induced by diesel exhaust particles. RA showed this by reduction of pro-inflammatory molecule expression and enhanced antioxidative activity (34).

In conclusion, the present findings well indicate that E. amoenum (F.M.) decoction has very good potential to improve human antioxidant status and prevent normal oxidative stress that happens daily due to normal exposure to many causal chemicals and conditions. This potential of E. amoenum (F.M.) seems to be due to its bioactive antioxidant components, especially RA and flavonoids. In recent years the importance of oxidative stress in the pathophysiology of many human disorders has been highlighted (35–44), thus use of this plant as a dietary supplement is highly recommended (45,46). Trials to establish efficacy and optimum dosage of the present herbal product for treating human chronic diseases with pathophysiology of oxidative stress are essential.

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References

1. Cochrane CG. Cellular injury by oxidant. Am J Med 1991;91:238–30S.
2. Abdollahi M, Ranbar A, Shadnia S, Niklar S, Rezaee A. Pesticides and oxidative stress: a review. Med Sci Monit 2004;10:RA141–7.
3. Halliwell B, Gutteridge JMC. The antioxidants of human extracellular fluids. Arch Biochem Biophys 1990;280:1–8.
4. Arora R, Gupta D, Chawla R, Sagar R, Sharma A, Kumar R, et al. Radioprotection by plant products: present status and future prospects. Phytother Res 2005;19:1–22.
5. Rechinger KH. Flora Iranica. No. 48. Graz: Akademische Druck-u.ver Lagentstalt. 1967, 215.
6. Hooper D. Useful Plants and Drugs of Iran and Iraq. Chicago, USA: Field Museum of Natural History, 1937, 115.
7. Wretensjo I, Svensson L, Christie WW. Gas chromatographic mass spectrometric identification of the fatty acids in borage oil using the picolyl ester derivatives. J Chromatogr A 1996:521:89–97.
8. Shafaghi B, Naderi N, Tahmash L, Kamelinaied J. Anxiolytic effect of Echium amoenum in mice. Iran J Pharm Res 2002;1:37–41.
9. Kast RE. Borage oil reduction of rheumatoid arthritis activity may be mediated by increased cAMP that suppresses tumor necrosis factor-alpha. Int Immunopharmacol 2001;1:2197–9.
10. Kapoor R, Klimaszewski A. Efficacy of borage oil in patients with atopic eczema. Br J Dermatol 2000;143:200–1.
11. Gonzalez CA, Sanz JM, Marcos G, Pita P, Bruillet E, Saigi E, et al. Borage consumption as a possible gastric cancer protective factor. Cancer Epidemiol Biomarkers Prev 1993;2:157–8.
12. Iranian Herbal Pharmacopoeia (IHP). Tehran: Ministry of Health Publication, 2002. 25–6, 2002. 667–71.
13. Savyah M, Savyah M, Kammalinejad M. A preliminary randomized double blind clinical trial on the efficacy of aqueous extract of Echium amoenum in the treatment of mild to moderate major depression. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:166–9.
14. Erdemoglua N, Kusmenoglua S, Vura M. Gamma-linolenic acid content and fatty acid composition of Boraginaceae seed oils. Eur J Lipid Sci Technol 2004;106:160–4.
15. Delorme P, Jay M, Ferry S. Iventaire phytochimique des borraginacees indigences. Planta Med 1977;11:5–11.
16. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radic Biol Med 1995;19:481–6.
17. Yao LH, Jiang YM, Shi J, Tomai-Barberan FA, Datta N, Singamons R, et al. Flavonoids in food and their health benefits. Plant Foods Hum Nutr 2004;59:113–22.
18. Nakamura Y, Htto Y, Murakami A, Ohigashi H. Superoxide scavenging ability of rosmarinic acid from Perilla frutescens Britton Var. Acuta, F. viridis. J Agric Food Chem 1998;46:4545–50.
19. Fuhrman B, Volkova N, Rosenblatt M, Aviram M. Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, rosmarinic acid, carnosic acid, or garlic. Antioxid Redox Signal 2000;2:491–506.
20. Frankel EN, Huang S, Aeschbach R, Prior E. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. J Agric Food Chem 1994;44:131–5.
21. Butterweck V, Hegger M, Winterhoff H. Flavonoids of St. John’s wort reduce HPA axis function in the rat. Planta Med 2004;70:1008–11.
22. Abdollahi M, Salehnia A, Mortazavi SHR, Ebrahimi M, Shafiee A, Fouladian F, et al. Antioxidant, antiadipic, antihyperlipidemic, reproduction stimulatory properties and safety of Satureja Khuzestanica essential oil as in rat in vivo; a toxicopharmacological study. Med Sci Monit 2003;9:BR331–5.
23. Benzi IF, Strain S. Ferric reducing antioxidant assay. Methods Enzymol 1999;292:15–27.
24. Hu ML, Dillared CJ. Plasma SH and GSH measurement. Methods Enzymol 1994;233:385–7.
25. Satoh K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978:90;37–43.
26. Czinner E, Hagymasi K, Blazovics A, Kery A, Szoke E, Lemberkovics E. Antioxid Redox Signal 2000;2:491–506.
27. D’Amelio FS. Botanics: A Phytoesthetic Desk Reference. London: CRC Press, 1999, 361.
28. Mehrabani M, Ghassemi N, Sajjadi E, Ghanamir A, Shams-Ardakani MR. Main phenolic compound of petals of Echium amoenum Fish. and C.A. Mey., a famous medicinal plant of Iran. DARU 2005;13:12; 65–9.
29. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Rev 1998;56:317–33.
30. van Acker SA, van den Berg DJ, Tromp MN, Griffin HO, van Bennekem WP, van der Vijgh WJ, et al. Structural aspects of antioxidant activity of flavonoids. Free Radic Biol Med 1996;20:331–42.
31. Kimura Y, Okuda H, Okuda T, Hatano T, Arichi S. Studies on the activities of tannins and related compounds. X. Effects of caffeic tannins and related compounds on arachidonate metabolism in human polymorphonuclear leukocytes. J Nat Prod 1987;50;392–9.
32. Englberger W, Hadding U, Etschenberg E, Graf E, Leyck S, Winkelmann J, et al. Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory activity. *Int J Immunopharmacol* 1988;10:729–37.

33. Ito H, Miyazaki T, Ono M, Sakurai H. Antiallergic activities of rabdosin and its related compounds: chemical and biochemical evaluations. *Bioorg Med Chem* 1998;6:1051–6.

34. Sanbongi C, Takano H, Osakabe N, Sasa N, Natsume M, Yanagisawa R, et al. Rosmarinic acid inhibits lung injury induced by diesel exhaust particles. *Free Radic Biol Med* 2003;34:1060–9.

35. Shadnia S, Azizi E, Hosseini R, Khoei S, Fouladlil S, Pazoumand A, et al. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. *Hum Exp Toxicol* 2005;24:439–45.

36. Mashayekhi F, Aghahoseini F, Rezaie A, Zamani MJ, Khorasani R, Abdollahi M. Alteration of cyclic nucleotides levels and oxidative stress in saliva of human subjects with periodontitis. *J Contemp Dent Pract* 2005;4:46–53.

37. Malekirad AA, Ranjbar A, Rahzani K, Kadkhodaei M, Rezaie A, Taghavi B, et al. Oxidative stress in operating room personnel: occupational exposure to anesthetic gases. *Hum Exp Toxicol* 2005;24:597–601.

38. Larjani B, Afsahi M, Astanehi-Axgari F, Mohajedi A, Rezaie A, Hosseininazhad A, et al. Effect of short-term carvedilol therapy on salivary and plasma oxidative stress parameters and plasma glucose level in type II diabetes. *Therapy* 2006;3:119–23.

39. Abdollahi M, Larjani B, Rahimi R, Salari P. Role of oxidative stress in osteoporosis. *Therapy* 2005;2:787–96.

40. Radfar M, Larjani B, Hadjibabaie M, Rajabi pou B, Mojtahedi A, Abdollahi M. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients; a randomized, double-blind placebo-controlled clinical trial. *Biomed Pharmacother* 2005;59:302–6.

41. Jahanshahi G, Motavaesel V, Rezaie A, Hashtroudi AA, Daryani NE, Abdollahi M. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig Dis Sci* 2004;49:1752–7.

42. Rahimi R, Nikfar S, Larjani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005;59:365–73.

43. Ranjbar A, Pasalar P, Sedighi A, Abdollahi M. Induction of oxidative stress in paraquat formulating workers. *Toxicol Lett* 2002;131:191–4.

44. Malekirad AA, Ranjbar A, Rahzani K, Filehvarian AA, Rezaie A, Zamani MJ, et al. Oxidative stress in radiology staff. *Environ Toxicol Pharmacol* 2005;20:215–8.

45. Azaizeh H, Ljubuncic P, Portnaya I, Said O, Cogan U, Bomzon A. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *Evid Based Complement Alternat Med* 2005;2:549–56.

46. Ahmed S, Anuntiyo J, Malemud CJ, Haqqi TM. Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis: a review. *Evid Based Complement Alternat Med* 2005;2:301–8.