

Prevention of selenite-induced cataractogenesis by hydroalcoholic extract of *Echium amoenum*: An experimental evaluation of the Iranian traditional eye medication**Kiumars Noroozpour Dailami¹, Mohammad Azadbakht^{2*}, Marjan Lashgari¹, Zahra Rashidi²**¹Department of Ophthalmology, Boali Sina Hospital, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran²Department of Pharmacognosy, Faculty of Pharmacy, Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran*Received: Jan 5, 2016, Revised: Jan 17, 2016, Accepted: Jan 26, 2016***Abstract**

Echium amoenum is an Iranian indigenous medicinal plant. In this research the effect of hydroalcoholic extract of this plant on sodium-selenite induced cataract formation was evaluated. Fifty five white rat pups were selected and divided into six groups. In first group (control 1) no elements injected. Second group (control 2) was received normal saline on 11-18 postpartum. Hydroalcoholic extract of *Echium amoenum* flower (400 mg/kg/day) intraperitoneally was injected into third group. Rats in group 4th received sodium selenite (30 nmol/kg) on day 13 postpartum. In group 5th, the extract was injected 2 days before selenite injection (once a day for two days). And in last group one day before selenite injection, the rats received a single dose of the extract. Cataract development was measured by slit-lamp. Lens opacification was analyzed in each group on day 7 after selenite administration. All lens in control and 3th groups were clear. However, it was found different type of cataract grades formation in selenite group (2.2 ± 0.83). Cataract grades were 0.4 ± 0.5 and 0.6 ± 0.5 in groups 5th and 6th respectively. *Echium amoenum* extract significantly was shown a protective effect on selenite-induced cataract in rat. This effect is probably associated with antioxidant activity of this medicinal plant.

Keywords: Experimental cataract, *Echium amoenum*, selenite-induced

Pharm Biomed Res 2015; 1(4): 40-47

DOI: 10.18869/acadpub.pbr.1.4.40

Introduction

Cataract is a major health problem and characterized by cloudiness or opacification of the lens of the human eye (1,2). It is the leading cause of blindness and contributes to physiologically 50% of blindness (3). Age related cataract, affecting over 20 million of the nearly 45 million blind people worldwide with the highest

incidence occurring in developing countries (1,4,5). Cataractogenesis is a multi-factorial disease associated with several risk factors such as ageing, diabetes, malnutrition, diarrhea, sunlight, smoking, hypertension, and renal failure (6,7). Free radical-induced oxidative stress is an important risk factor in the development of age-related

* E-mail: azadbakht110@gmail.com

cataract (8). This hypothesis is supported by the anticataractogenic effect of various antioxidant compounds such as: melatonin (9), resveratrol (10), acetyl-L-carnitine (11,12), ellagic acid (13), L-cysteine and vitamin C (14), N-acetylcysteine (15), rutin (16), vitamin C (17), Lycopene (18), vitamin-E (19, 20) and extracts such as: *Origanum vulgare* (5), *Cineraria maritima* (21), soya bean (22), *Pleurotus ostreatus* (23), Onion juice (24), *Embelica officinalis* (25) in experimental animals. Surgery is generally accepted as the only treatment for human cataract (10) but requirement for highly trained personnel and the cost of surgery pose a significant economic problem (1). Thus, development of a drug that prevents or delays the onset of cataract will reduce the number of blind patients. Selenite cataract is a useful rodent model for rapid screening of potential anticataract agents. The morphological and biochemical characteristics of this model have been extensively investigated (26). In this model, an overdose of sodium selenite induces cataracts in young rats by various mechanisms, including calpain-induced hydrolysis and precipitation of lenticular proteins (27). *Echium amoenum* Fisch and C.A. Mey. (Boraginaceae), is a biennial or perennial herb indigenous to the narrow zone of northern part of Iran (Neka and Tonekabon montaines) and Caucasus, where it grows at an altitude ranging from 60-2200 m (28,29). *Echium* genus has 4 species in Iran (30) and only *E. amoenum* has medicinal uses (28). The flowers are bright blue and star-shaped and the fruit consists of four brownish-black nutlets (31). The petals of *E. amoenum* have flavonoid, anthocyanidine

, volatile oil, mucilage, potassium nitrate, phenolic compounds like rosmarinic acid, cyanidine and delphinidin (28), and trace of pyrrolizidine alkaloids (32). The seeds of *E. amoenum* are potent source of γ -linolenic acid (GLA) (33). Petals of *E. amoenum*, is known in traditional medicine of Iran as Gol-e-Gavzaban, has long been used as a tonic, tranquillizer, diaphoretic, demulcent and as a remedy for common cold, cough, sore throat and pneumonia (28, 34,35). Also, this plant is analgesic (36), stimulating the immune system (37), anxiolytic (38), and antioxidant (39). The petals are used in circulatory heart diseases, as a poultice for inflammatory swellings, as a diuretic (due to potassium nitrate), laxative, emollient and demulcent (due to the mucilage), and recently as a possible cancer protective factor (40). In old Persian medical textbooks the plant has sedative effects, emollient, anti cough, decreasing heart palpitations and reducing anxiety (41-43).

We have previously shown that a hydroalcoholic extract of *Origanum vulgare* has an anticataractogenic effect in an animal model (5). In this study, because of high content of phenolic compounds such as flavonoid in *Echium amoenum* petals, prevention of selenite-induced cataractogenesis by a hydroalcoholic extract of this plant is evaluated.

Materials and methods

Plant material

Petals of *Echium amoenum* were collected from Neka mountains, 35-Km south of Neka, Mazandaran province, Iran, at an altitude 850 m. The plant was identified by plant systematic specialist and the voucher specimen (E-36-24)

was deposited at the herbarium of the faculty of pharmacy, Mazandaran university of medical sciences, Sari, Iran.

Extraction from petals of Echium amoenum

All materials and standards supplied from Merck company (Germany). The providing process for extraction was percolation method: using ethanol 70% (plant-solvent ratio was 1/10). The end of extraction was identified by the cyanidine test (44). The extract was then evaporated in a rotating evaporator and it was solidified by a freeze-drier machine to give a residue.

Standardization of the plant extract

Total flavonoid content was measured by the aluminum chloride colorimetric assay (44,45). About 5.0 g of the extractives were transferred to a flask and 10.0 mL acetone, 2.0 mL hydrochloric acid and 1.0 mL of hexamethylenetetramine 0.5% added. The mixture was refluxed on a water bath for 30 min. After cooling, the final volume was made up to 50.0 mL with acetone (S). 20.0 mL of S and 20.0 mL water were treated once with 15.0 mL and three times with 10.0 mL ethyl acetate. The ethyl acetate phases were washed twice with 50 mL water and made up to 50.0 mL (P). 10.0 mL of P plus 2.0 mL of $AlCl_3$ ethanolic solution was made up to 25.0 mL with methanol/ acetic acid to produce the test solution (T). A second 10.0 mL of P was diluted to 25.0 mL with acetic acid methanolic solution (C). After 30 min the absorbance of T was read at 420 nm against C. The same procedure was repeated for 30.0 and 40.0 mL of S. Total flavonoid content was calculated using equation (DAB10=German

pharmacopoeia). The total flavonoid assay was measured three times for each *Echium amoenum* extract.

Animal care and cataract induction

This study was approved by research committee of Mazandaran university of medical sciences. white rat mothers and their litter were kept in individual cages. They were fed a laboratory chow rodent diet and water. Temperature was maintained at 20 °C and light was turned on and off at 12 h intervals. To initiate cataract, the rat pups were injected subcutaneously on day 13 post partum with a solution of sodium selenite, Na_2SeO_3 , dissolved in 0.9% NaCl to give dose of 30 nmol/kg body weight of selenite. Following selenite injection, opacification progressed rapidly to maturity by day 4 or 5 post injection. Observations of lens opacification were made on day 7 after selenite administration under photo slit-lamp microscope and photographed. The pupils were dilated with a drop of 1% atropine. All of injections were done in an animal house (Faculty of Pharmacy) and observations of lenses were done in Bu-Alisina hospital, Sari, Iran.

Classification of cataract

Cataract was graded from 0 to 4 scales to following:

Grade 0: clear lens; grade 1: swollen fibers and subcapsular opacities observed; grade 2: nuclear cataract in lens and swollen fibers in lens cortex; grade 3: strong nuclear cataract with perinuclear area opacity in lens; grade 4: total opacity of lens (Fig. 1).

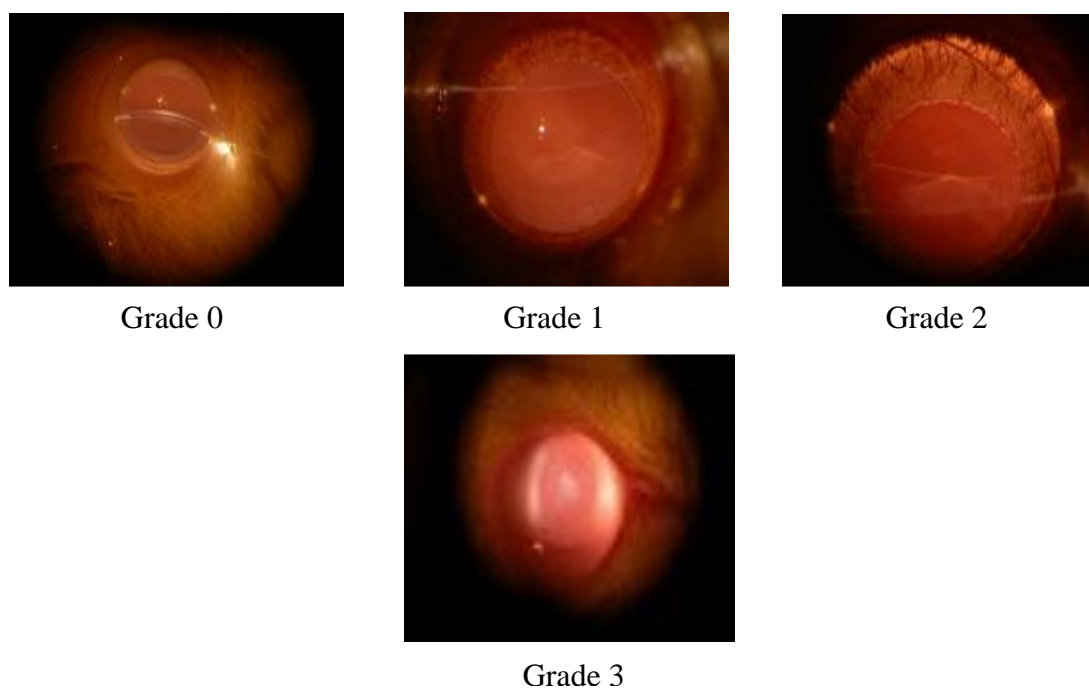


Figure 1 Grades from 0 to 3 scales of cataracts

Echium amoenum extract administration

Rat pups received daily a single intraperitoneal injection (400 mg/kg) of day *Echium amoenum* extract according to the schemes shown in table 1.

Statistical analysis

Kruskal-Wallis test was used for qualitative variables, and T-test was performed for quantitative variables. Values $p < 0.05$ were considered significant.

Results

Dried extract and total flavonoid contents were 20% and 0.371%, respectively.

Table 2 shows the cataract grades in rats that received Na_2SeO_3 and *Echium amoenum* extract according to the schemes shown in table 1.

Single and double *Echium amoenum*

extract injection decreased lens opacity significantly ($p < 0.01$). The lenses of both control group (1 and 2) rats did not show any opacity ($n = 35$). The lenses of rats that treated by *Echium amoenum* extract did not show any opacity ($n = 5$). Selenite injection (30 nmol/kg body weight) caused formation of strong nuclear cataract as a rule, but cortical cataracts without nuclear opacity were found too (grade 4) ($n = 5$).

As shown in table 2, *Echium amoenum* extract significantly protects rat lenses from selenite-induced cataract when injected on one and two days before selenite administration.

Discussion

Today, cataract is one of the main eye problems in the world and aging is the most common cause of cataract. The only current treatment for cataracts is

Table1 Schemes of *Echium amoenum* extract and sodium selenite application*

Group	Post partum (Days)							
	11	12	13	14	15	16	17	18
Control group 1	-	-	-	-	-	-	-	-
Control group 2	NS	NS	NS	NS	NS	NS	NS	NS
Group (3) EA	-	EA	-	-	-	-	-	-
Group (4) Se	-	-	Se	-	-	-	-	-
Group (5) EA 2day+ Se	EA	EA	Se	-	-	-	-	-
Group (6) EA 1day+ Se	-	EA	Se	-	-	-	-	-

*NS (normal saline), EA (*Echium amoenum*), Se (sodium selenite)

Table 2 Effect of *Echium amoenum* extract on cataract formation*

Application scheme	N	Grade (mean ± SD)
Control 1	5	0
Control 2 (NS)	30	0
EA	5	0
Se	5	2.2 ± 0.83
2 day EA + Se	5	0.6 ± 0.49
1 day EA + Se	5	0.8 ± 0.4

*NS (normal saline), EA (*Echium amoenum*), Se (sodium selenite)

limited to surgical removal of the clouded lens (46). Although evidence indicates that cataract is multifactorial but processes of oxidative stress were shown in the development of cataract (5,8). Oxidative damage plays an important role in the opacification of the lens. Free radicals disturb cellular homeostasis through modification of proteins and lipid peroxidation. Like other organs, the lens has a well-designed system of defense against oxidation. Primary defenses include non-enzymatic antioxidants, such as glutathione, vitamin E, vitamin C, and carotenoids (21,47). The using of sodium selenite is the easiest and most accessible method that has been used for cataract formation model in young rats. The method first described by Ostadalva and colleagues at 1987. By subcutaneous injection of 20-30 nmol / kg body weight of selenite, cataracts begins after 3-4 days in the lens of young rat eyes and is completed until a week (48). Various pharmacological agents possessing antioxidant properties to neutralize oxidative stress have been shown to protect against selenite-induced cataractogenesis in experimental animal models (11-13,15,21). The data of the present study demonstrate that *Echium amoenum* extract can protect against selenite-induced cataract. Selenite causes an oxidation of protein and non-protein sulfhydryl groups; this leads to ion pump damage and to disturbance of the electrolytic balance. The intracellular calcium level increases, which activates the calcium-dependent protease calpain. Calpain partly hydrolyzes intracellular proteins. Protein aggregates scatter light, and lens opacity increases (26). In our study, *Echium amoenum* extract protected the lens against the selenite-

induced nuclear opacity (table 2). We assume that the molecular mechanism of the *Echium amoenum* extract effect is connected with protection against mild or high oxidative stress induced by selenite. Various phenolic antioxidants such as flavonoids, rosmarinic acid, tannins, coumarins, xanthenes and, more recently, procyanidins have been shown to scavenge radicals in a dose dependent manner (28). In addition, flavonoids and rosmarinic acid have been introduced as the main constituents of *E. amoenum* (F.M.) in several phytochemistry studies (28,49). The antioxidant potential of flavonoids has been well established. Flavonoids can highly scavenge most types of oxidizing molecules, including singlet oxygen and various free radicals, and thus act indirectly as an efficient antioxidant. They can also act directly by suppressing ROS formation (49, 50). In researches by Vats, the effects of *Pterocarpus marsupium* (PM), *Ocimum sanctum* (OS) and *Trigonella foenum-graecum* (TF) extracts on sodium-selenite induced cataract formation were evaluated. The extracts of PM and TF were effective but OS extract was not valuable (51). Some medicinal extracts such as *Cineraria maritime*, *Onion* juice, *Embelica officinalis* were prevented cataractogenesis in the experimental animal models (21,24,25). We have previously reported that a hydroalcoholic extract of the *Origanum vulgare*, could prevent cataractogenesis in an animal model of selenite-induced cataracts (5). In this research, we emonstrated that the extract of *Echium amoenum* was a significant role in prevention selenite-induced cataract.

Conclusion

In conclusion, we have demonstrated, for the first time, the prevention of selenite-induced cataractogenesis in rats by administration of *Echium amoenum* extract. This effect is may be dependent to antioxidant activity of this medicinal plant.

References:

1. Nirmalan PK, Krishnadas R, Ramakrishnan R, Thulasiraj RD, Katz J, Tielsch JM, et al. Lens opacities in a rural population of southern India: the Aravind comprehensive eye study. *Invest Ophthalmol Vis Sci* 2003;44:4639-43.
2. West S. Epidemiology of cataract: accomplishments over 25 years and future directions. *Ophthalmic Epidemiol* 2007;14:173-8.
3. WHO. Prevention of avoidable blindness and visual impairment, provisional agenda item 2005;4.9.EB117/35, 117 Session 22.
4. Thylefors B. Avoidable blindness. *Bull. World Health Organ* 1999;77:453.
5. Noroozpour DK, Azadbakht M, Rashidi Z, Lashgari M. Prevention of selenite-induced cataractogenesis by *Origanum vulgare* extract. *Pak J Biol Sci* 2010;13:743-7.
6. Harding J. The epidemiology of cataract. In: Harding, J. (Ed.), *Cataractbiochemistry, Epidemiology and Pharmacology*. Chapman & Hall, Madras; 1991.p.83.
7. Sakthivel M, Elanchezhian R, Ramesh E, Isai M, Nelson JC, Thomas PA, et al. Prevention of selenite-induced cataractogenesis in Wistar rats by the polyphenol, ellagic acid. *Exp Eye Res* 2008;86:251-9.
8. Kassem SS, Aziz MA, El-Sheikh NM, Kholeif TE, Al-Balkini MS, Gomaa AM, et al. Modulation of Selenite-Induced Cataract by Dietary Supplement of Broccoli in Experimental Animals. *World Appl Sci J* 2013;26:1643-52.
9. Yağci R, Aydin B, Erdurmus M, Karadağ R, Gürel A, Durmuş M, et al. Use of melatonin to prevent selenite-induced cataract formation in rat eyes. *Curr Eye Res* 2006;31:845-50.
10. Doganay S, Borazan M, Iraz M, Cigremis Y. The effect of resveratrol in experimental cataract model formed by sodium selenite. *Curr Eye Res* 2006; 31:147-53.
11. Geraldine P, Sneha BB, Elanchezhian R, Ramesh E, Kalavathy CM, Kaliamurthy J, et al. Prevention of selenite-induced cataractogenesis by acetyl-L-carnitine: an experimental study. *Exp Eye Res* 2006; 83:1340-9.
12. Elanchezhian R, Ramesh E, Sakthivel M, Isai M, Geraldine P, Rajamohan M, et al. Acetyl-L carnitine prevents selenite-induced cataractogenesis in an experimental animal model. *Curr Eye Res* 2008; 32:961-71.
13. Sakthivel M, Elanchezhian R, Ramesh E, Isai M, Jesudasan CN, Thomas PA, et al. Prevention of selenite-induced cataractogenesis in Wistar rats by the polyphenol, ellagic acid. *Exp Eye Res* 2008; 86:251-9.
14. Jahadi Hosseini HR, Aminlari M, Khalili MR. Prevention of selenite-induced cataract by L-cysteine and vitamin C in rats. *Iran Red Cres Med J* 2008;10:281-7.
15. Aydin B, Yagci R, Yilmaz FM. Prevention of selenite-induced cataractogenesis by N-acetylcysteine in rats. *Curr Eye Res* 2009;34:196-201.
16. Isai M, Sakthivel M, Ramesh E, Thomas PA, Geraldine P. Prevention of selenite-induced cataractogenesis by rutin in Wistar rats. *Mol Vis* 2009;15:2570-7.
17. Devamanoharan PS, Henein M, Morris S, Ramachandran S, Richards RD, Varma SD. Prevention of selenite cataract by vitamin C. *Exp Eye Res* 1991;52:563-8.
18. Gupta SK, Trivedi D, Srivastava S, Joshi S, Halder N, Verma SD. Lycopene attenuates oxidative stress induced experimental cataract development: An *in vitro* and *in vivo* study. *Nutrition* 2003;19:794-9.
19. Mathew JP, Thomas VC, Thomas I. Selenite cataract and its attenuation by vitamin E in Wistar rats. *Indian J Ophthalmol* 2003;51:161-70.
20. Sneha BB, Geraldine P, Thomas PA. Selenite cataract and its attenuation by vitamin-E in Wistar rats. *Indian J Ophthalmol* 2003;51:365-6.
21. Anitha TS, Annadurai T, Thomas PA, Geraldine P. Prevention of Selenite-Induced Cataractogenesis by an Ethanolic Extract of *Cineraria maritima*: An Experimental Evaluation of the Traditional Eye Medication. *Biol Trace Elem Res* 2011;143:425-36.
22. Agarwal SS, Gupta SK, Kalaiselvan, Sushma Srivastava, Rohit Saxena. Potential of traditional food supplement, soya bean, as a novel anticataract agent. *I J Integr Biol* 2008;3:9-17.
23. Isai M, Elanchezhian R, Sakthivel M, Chinnakkaruppan A, Rajamohan M, Jesudasan CN, et al. Anticataractogenic effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, in an

Conflict of interest

The authors declared no potential conflict of interest with respect to the authorship, and/or publication of this study.

- experimental animal model. *Curr Eye Res* 2009;34:264-73.
24. Javadzadeh A, Ghorbanihaghjo A, Bonyadi S, Rashidi MR, Mesgari M, Rashtchizadeh N, et al. Preventive effect of onion juice on selenite-induced experimental cataract. *Indian J Ophthalmol* 2009;57:185-9.
 25. Nair Kavitha Nair, Kirti Patel, Tejal Gandhi. Effect of Aqueous Extract of *Embelica officinalis* on Selenite Induced Cataract in Rats. *Iran J Pharm Res* 2010;9:147-52.
 26. Shearer TR, Ma H, Fukiage C, Azuma, M. Selenite nuclear cataract: review of the model. *Mol Vis* 1997; 3:8.
 27. Dong Chang, Xuefei Zhang, Sheng zhong Rong, Qian Sha, Peipei Liu, Tao Han, and Hongzhi Pan. Serum Antioxidative Enzymes Levels and Oxidative Stress Products in Age-Related Cataract Patients. *Oxid Med Cell Longev* 2013;2013:587826.
 28. Mehrabani M, Ghassemi N, Sajadi E, Ghannadi AR, Shams-Ardakani MR. Main phenolic compound of petals of *Echium amoenum* Fisch. and C. A. Mey., a famous medicinal plant of Iran. *Daru* 2005;2:65-9.
 29. Mozaffarian V. A dictionary of Iranian plant names. Tehran: Farhang Moaser; 1996; p.198.
 30. Sadizadeh M, Abbassi F, Baghizadeh A, Yazdanpanah A. The effects of salicylic acid and ascorbic acid on some of resistance mechanisms to drought stress in *Echium amoenum*. *American-Eurasian J Agric Environ Sci* 2009;6:262-7.
 31. Iranian herbal pharmacopoeia (IHP), Vol. 2. Tehran: Ministry of Health Publication; 2002;p.667-71.
 32. Azadbakht M, Nematzadeh Gh A, Hosseinpour Azad N, Shokri E. Qualitative and quantitative analysis of pyrrolizidine alkaloids in root, leaf, petal and seeds of *Echium amoenum* Fisch & Mey. *J Mazand Univ Med Sci* 2011; 21(suppl 1):131-7.
 33. Hosseinpour Azad N, Nematzadeh Gh A, Azadbakht M, Kazemitabar SK, Shokri E. Investigation on fatty acids profile in two ecotypes of Iranian. *Echium amoenum* Fisch & Mey. *Iran J Med Arom Plants* 2012;27:587-95.
 34. Ghassemi N, Sajjadi SE, Ghannadi AR, Shams-Ardakani MR, mehrabani M. Volatile constituents of a medicinal plant of Iran, *Echium amoenum* Fisch. C.A. Mey. *Daru* 2003;11:32-3.
 35. Shafaghi B, Naderi N, Tahmasb L, Kamalinejad M. Anxiolytic effect of *Echium amoenum* L. in mice. *Iran J Pharm Res* 2002;1:37-41.
 36. Heidari MR, Moein-Azad E, Mehrabani M. Evaluation of the analgesic effect of *Echium amoenum* Fisch and C.A. Mey. Extract in mice: Possible mechanism involved. *J Ethnopharmacol* 2006;103:345-9.
 37. Amirghofran Z, Azadbakht M, Keshavarzi F. *Echium amoenum* stimulate of lymphocyte proliferation and inhibit of humoral antibody synthesis. *Iran J Med Sci* 2000;25:119-24.
 38. Rabbani M, Sajjadi SE, Vaseghi G, Jafarian A. Anxiolytic effects of *Echium amoenum* on the elevated plus-maze model of anxiety in mice. *Fitoterapia* 2004;75:457-64.
 39. Ranjbar A, Khorami S, Safarabadi M, Shahmoradi A, Malekirad AA, Vakilian K, et al. Antioxidant activity of Iranian *Echium amoenum* Fisch and C.A. Mey flower decoction in humans: A cross-sectional Before/After Clinical Trial. *Evid Based Complement Alternat Med* 2006;3:469-73.
 40. Mohsen Abolhassani. Antiviral activity of borage (*Echium amoenum*). *Arch Med Sci* 2010;6:366-9.
 41. Avicenna. The canon of medicine. Tehran: soroush press 1997:202-3.
 42. Moemen, M. Tohfat-Al-Hakim Moemen, 2nd edR Mahmoodi Press, Tehran. 1967; p. 763.
 43. Hooper, D. Useful Plants and Drugs of Iran and Iraq. Field Museum of Natural History, Chicago, USA.1937;p.115.
 44. Azadbakht M, Bahaeddini A, Shorideh SM. The effect of "Vitex agnus-castus" leaf and fruit flavonoidal extracts on serum prolactin concentration. *J Med Plants* 2005;4:56-61.
 45. Shams Ardekani MR, Hajimahmoodi M, Oveisi MR, Sadeghi N, Jannat B, Ranjbar AM, Gholam N and Moridi T. *Comparative Antioxidant Activity and Total Flavonoid Content of Persian Pomegranate (Punica granatum L.) Cultivars*. *Iran J Pharm Res* 2011;10:519-24.
 46. Roirdan P, Whitche J. Vaughan and Asbury s: General ophthalmology.16th edition. United state of America. The MC Graw-Hil companies; 2004: P.173-80.
 47. Augusteyn RC. Protein modification in cataract: possible oxidative mechanisms. In: Duncan G (Ed) Mechanisms of cataract formation in the human lens. Academic, New York 1981; pp72-115.
 48. Ostadvalva I, Babicky A, Obenberger J. Cataract induced by administration of a single dose of sodium selenite to sucking rats. *Experientia* 1987;34:222-3.
 49. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, et al. Flavonoids in food and their health benefits. *Plant Foods Hum Nutr* 2004;59:113-22.
 50. Ranjbar A, Khorami S, Safarabadi M, Shahmoradi A, Malekirad AA, Vakilian K, Mandegary A, Abdollahi M. Antioxidant Activity of Iranian *Echium amoenum* Fisch & C.A. Mey Flower Decoction in Humans: A cross-sectional Before/After Clinical Trial. *eCAM* 2006;3:469-73.
 51. Vats V, Yadav SP, Biswas NR, Grover JK. Anti activity of *Pterocarpus marsupium* bark and *Trigonella foenum-gracum* seeds extract in alloxan diabetic rats. *J Ethnopharmacol* 2004;93:289-94.