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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (15): 743-747, 2010
ISSN 1028-8880

Prevention of Selenite-induced Cataractogenesis by *Origanum vulgare* Extract

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Abstract: The present study sought to assess antioxidant effect of *Origanum vulgare* extract in preventing selenite-induced cataractogenesis. This study was performed on Young white rats received sodium selenite (30 nmol g^{-1} birth weight) subcutaneously on day 13 post partum during two months in 2009. Cataract formation and intensity was detected and measured by slit-lamp. *Origanum vulgare* (Ov) extract (2 g kg^{-1}) was given (1-2 times) intraperitoneal at different times with respect to the selenite administration lens opacification was analyzed in selenite, selenite-Ov, Ov and control groups on day 7 after selenite administration. Ov extract have revealed a significant protective effect against selenite induced cataract when injected 1 and 2 day (2 times) before selenite injection. There is a protective effect of Ov against selenite induced cataract formation. It is supposed that the anticataract effect of Ov extract could be based on direct or indirect antioxidant mechanisms.

Key words: Selenite, cataractogenesis, *Origanum vulgare*, white rats, extract

INTRODUCTION

Cataract is a progressive opacification of the lens of the human eye that impairs vision and may cause blindness (Aeschbach *et al*., 1994). Cataractogenesis is a multifactorial pathological process in which many risk factors and different causes are involved (Bakkali *et al*., 2008; Devamanoharan *et al*., 1991). Age related cataract remains a major cause of blindness, affecting over 20 million of the nearly 45 million blind people worldwide with the highest incidence occurring in developing countries (Doganay *et al*., 2002; Geraldine *et al*., 2006; Gupta *et al*., 2003). Oxidative stress is a common initiator of many age-related conditions. The aging ocular lens is susceptible to oxidative insult and physiological damage through photocatalytic generation of various oxygen radicals (Gupta *et al*., 2005) and this is probably the most important mechanism in cataractogenesis. There are effective surgical procedures to combat this problem, but the requirement for highly trained personnel and the cost of surgery pose a significant economic problem. Thus, there is a need for chemical and pharmacological solutions for cataract prevention as well.

There are several endogenous defense mechanisms, which protect the lens against oxidative damage and these include the enzymes or components of the redox system. (Gupta *et al*., 2005; Hockwin, 1997). The model of scavenging of the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. The DPPH is a stable nitrogen-center free radical, the color of which changes from violet to yellow upon reduction, by either the process of hydrogen or electron donation substances able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Lee *et al*., 2003) (Table 1).

Selenite-induced cataract is a cataract model mainly dependent on oxidative stress, in which oxidation of the critical sulfhydryl group is essential for the initiation of cataractogenesis (Kokkini, 1997; Kulisic *et al*., 2004).

Table 1: Antioxidant activity of *Diospyros lotus* L. fruits extract and butylated hydroxyl toluene (BHT) against 1,1-diphenyl-2-picrylhydrazyl stable radical (DPPH)

Compound	Concentration (mg mL^{-1})	Inhibition % (Mean±SD)*
Diospyros lotus L.	0.5	32.47±3.08
	1	45.71±2.11
	2	59.12±1.18
	4	71.61±2.34
	8	86.43±1.27
BHT	0.01	34.11±2.48
	0.05	57.91±0.86
	0.1	75.16±1.91
	0.2	91.31±0.8
	0.4	95.57±0.51

*Each value in the table was obtained by calculating the average of 3 experiments±SD

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Selenium-induced oxidative stress mediated cataractogenesis has been shown to be prevented by antioxidative agents such as caffeic acid phenethyl ester (Lagouri *et al*., 1993) 2-ketoglutarate (McCay, 1985), lycopene (Muranov *et al*., 2004) and *Ocimum sanctum* (Nirmalan *et al*., 2003). However, the biochemical mechanisms for these activities have not been completely elucidated.

Recently, emphasis has been laid on exploring the possibility of using natural resources to delay the onset and progression of cataract. One of such natural products is *Origanum vulgare* extract. The genus *Origanum* belongs to the family of Labiatae (Orhan *et al*., 1999).

Recently, this plant has drawn more attention due to the antimicrobial, anti-fungal, insecticidal and antioxidative effects of this herb on human health (Shearer *et al*., 1987; Shearer *et al*., 1992; Skoula and Harborne, 2002).

Results of various studies indicated that the antioxidant effects of oregano might be related to the dominant components,carvacrol and thymol,of the essential oil (Thylefors, 1995, 1999; Varma *et al*., 1984).

In this study, an attempt has been made to determine whether *Origanum vulgare* extract can retard or prevent selenite-induced cataractogenesis in an experimental in vivo setting.

MATERIALS AND METHODS

Preparation of *Origanum vulgare* extract: After collection of *Origanum vulgare* from Northern Iran, upper crust of beans was separated and then the green beans grinded to the smaller segment, put into the glass container, then put into the oven and powdered in temperature of 45°C. After drying, the powder of beans was obtained from the dried beans by grinder. Aqueous ethanol (70%) was added to the powdered beans (250 g) and stirred for one hour. The mixture was kept at room temperature for 48 h. After filtration, methanol was evaporated under reduced pressure at 40°C. Finally, 26.5 g of extract powdered extract was obtained.

Animal care and cataract injection: White rat mothers and their litter were kept in separate 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 g^{-1} b.wt. 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 laboratory of sari medicinal college and observation of lenses were done in Booali hospital;Ophthalmology ward. b) Classification of cataract: Cataract was graded from 0 to 4 according to following explanation (Fig. 1-4). 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.

***Origanum vulgare* extract administration:** Rat pups received a single daily intraperitoneal injection (2 g kg^{-1})

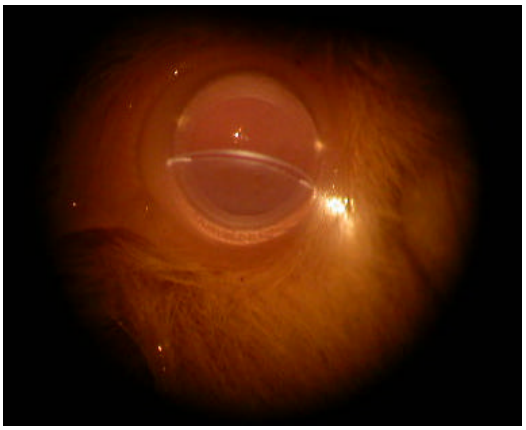

Fig. 1: Grade 0: Clear lens

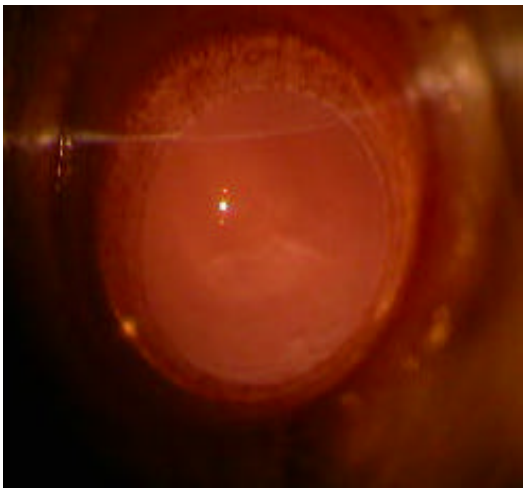

Fig. 2: Grade 1: Swollen fibers and subcapsular opacities observed

Table 2: Schemes of *Origanum vulgare* extract and sodium selenite application

	Post partum (days)							
Group	11	12	13	14	15	16	17	18
Control group 1 (G1)	-	-	-	-	-	-	-	-
Control group 2 (G2)	NS	NS	NS	NS	NS	NS	NS	NS
Group OV only (G3)	-	-	OV	-	-	-	-	-
Group Se only (30 umol g^{-1} b.wt.) (G4)	-	-	Se	-	-	-	-	-
Group 2 OV+ Se (G5)	OV	OV	Se	-	-	-	-	-
Group 1 OV+ Se (G6)	-	OV	Se	-	-	-	-	-

G1: No material injected, G2: Only N/S injection, G3: One injection of OV (2 g/kg/day) in 13 post partum day, G4: One injection of selenite (30 umol g^{-1} b.wt.) in 13 post partum day, G5: Two injection of selenite +2 injection of OV in 11 and 13 post partum day, G6: One injection of OV in 12 post partum day+one selenite injection at 13 post partum day

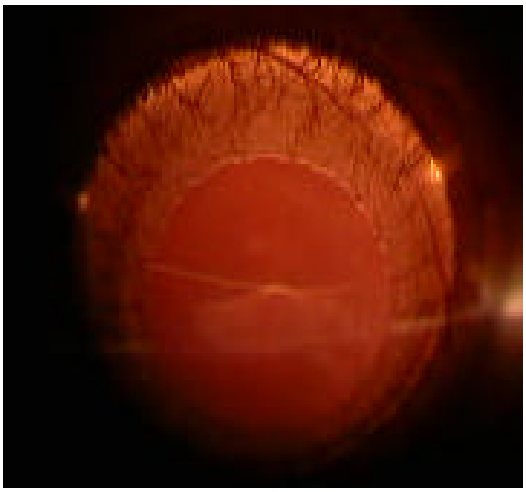

Fig. 3: Grade 2: Nuclear cataract in lens and swollen fibers in lens cortex

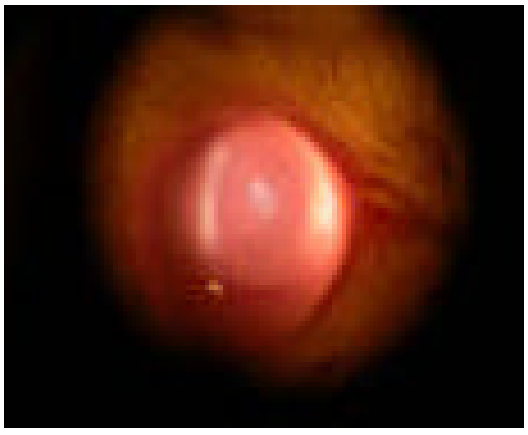

Fig. 4: Grade 3: Strong nuclear cataract with perinuclear area opacity in lens

of dry. *Origanum vulgare* extract according to the schemes shown in Table 2.

Measurement of free radical scavenging activity: Different concentrations of *Origanum vulgare* (0.5 to 8 mg mL^{-1}) were added, at an equal volume, to a methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. Butylated Hydroxy Toluene (BHT) was used as an antioxidant standard. Statistical analysis of data was carried out using descriptive statistics, the Mann-Whitney U-test and Kruskal Wallis nonparametric test.

Table 3: Influence of Ov extract on cataract formation

Groups	N	Grade (Mean±SD)
Control	5	0
Control (NS)	30	0.03±0.18
OV only	5	0
Se only	5	2.2±0.84
2 OV+Se	5	0.4±0.55
1 OV+Se	5	0.6±0.55

RESULTS

DPPH radical-scavenging activity: It was found that the radical-scavenging activities of both *Origanum vulgare* extract and BHT increased with increasing concentration. The maximum scavenging effects were obtained in 86.43% and 95.57% at 0.4-8 mg mL^{-1} for *Origanum vulgare* extract and BHT, respectively (Table 1).

Table 3 shows the cataract grades in rats that received Na_2SeO_3 and *Origanum vulgare* extract according to the schemes shown in Table 1. Single and double Ov extract injection decreased lens opacity significantly ($p<0.01$). Kruskal Wallis shows there is statistically significant difference among the Mean Ranks and $p<0.05$. The lenses of control group 1 and 2 rats did not show any opacity except a very slight haziness in one lens in control group 2 (n = 35) . The lenses of Ov-treated rats did not show any opacity (n = 5). Selenite injection (30 nmol g^{-1} body weight)caused formation of severe nuclear cataract as a rule,but cortical cataracts without nuclear opacity were found too (n = 5). As shown in Table 3 Ov extract significantly protects rat lenses from selenite-induced cataract when injected on 1 and 2 days before selenite administration.

DISCUSSION

Nowadays, cataract is one of the main ophthalmic problems in the world and yet there is no effective preventive drug for it. The data of the present study demonstrated that *Origanum vulgare* extract can protect against selenite-induced cataract formation. The pathogenesis of selenite-induced cataract is strongly related to oxidative damage: Selenite causes oxidation of protein and non-protein sulfhydryl groups; which leads to ion pump damage and electrolyteim balance. The intracellular calcium level increases, which activates the calcium-dependent protease calpain. Calpain partially hydrolyzes intracellular proteins, especially β-crystallin. Protein aggregates scatter light and lens opacity increases (Kokkini, 1997; Kulisic *et al*., 2004). Low oxidative stress induced by a low selenite dose (<15 nmol kg^{-1}) causes biochemical processes before calpain activation, such as disturbance of the lens membranes' ion permeability, water influx and swelling of the fiber cells. Cortical opacity is a marker of these events. Moderate oxidative stress (20-30 nmol kg^{-1} of selenite) causes nuclear opacity through that calpain proteolysis of lens proteins. High oxidative stress (>30 nmol kg^{-1}) causes intensified injury, i.e., damage of both nucleus and perinuclear area and eventually, of the whole lens (Bakkali *et al*., 2008). In this study, Ov extract protected the lens against the selenite-induced nuclear opacity (Table 3). We assume that the molecular mechanism of the Ov extract effect is connected with protection against mild or high oxidative stress induced by selenite.

We propose that the protective effect of Ov extract may be related to protection against oxidative stress induced by selenite.

Antioxidant activity of Ov extract demonstrated with DPPH test, but the molecular mechanism is unknown. Two mechanisms were suggested (Aeschbach *et al*., 1994) direct influence on free radical oxidation in the lens (Bakkali *et al*., 2008) an indirect effect through activation of a system that increases the antioxidant potential in the lens.

Therefore, augmentation of antioxidants is necessary to maintain a constant protective effect. For instance, several daily injections of water-soluble ascorbic acid after selenite exposure were effective against selenite-induced cataract (Varma and Hedge, 2004), as well as other antioxidants effectively prevent the selenite-induced cataract (Yanishlieva *et al*., 1999).

In conclusion, a protective influence of Ov extract against selenite cataract development can be assumed, based probably on direct or indirect antioxidant mechanisms.

REFERENCES

Aeschbach, R., J. Loliger, B.C. Scott, A. Murcia, J. Butler, B. Halliwell and O. I. Aruoma, 1994. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxicol., 32: 31-36.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils-a review. Food Chem. Toxicol., 46: 446-475.

Devamanoharan, P.S., M. Henein, S. Morris, S. Ramachandran, R.D. Richards and S.D. Varma, 1991. Preventation of selenite cataract by vitamin C. Exp. Eye Res., 52: 563-568.

Doganay, S., Y. Turkoz, C. Evereklioglu, H. Er, M. Bozaran and E. Ozerol, 2002. Use of caffeic acid phenethyl ester to prevent sodium selenite-induced cataract in rat eyes. J. Cataract Refract Surg., 28: 1457-1462.

Geraldine, P., B. Brijit Sneha, R. Elanchezhian, E. Ramesh, C.M. Kalavathy, J. Kaliamurthy and P.A. Thomas, 2006. Prevention of selenite-induced cataractogenesis by acetyl-L- carnitine: An experimental study. Exp. Eye Res., 83: 1340-1349.

Gupta, S.K., D. Trivedi, S. Srivastava, S. Joshi, N. Halder and S.D. Varma, 2003. Lycopeneattenuates oxidative stress induced experimental cataract development: An *in vitro* and *in vivo* study. Nutrition, 19: 794-799.

Gupta, S.K., S. Srivastava, D. Trivedi, S. Joshi and N. Halder, 2005. *Ocimum sanctum* modulates selenite-induced cataractogenic changes and prevents rat lens opacification. Curr. Eye Res., 30: 583-591.

Hockwin, O., 1997. Multifactorial pathogenesis of senile cataract. Nova Acta Leopoldina NF, 75: 37-44.

Kokkini, S., 1997. Taxonomy, diversity and distribution of *Origanum* species. Proceedings of the IPGRI International Workshop, (IIW'97), Italy, Rome, pp: 2-12.

Kulisic, T., A. Radonic, V. Katalinic and M. Milos, 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem., 85: 633-640.

Lagouri, V., G. Blekas, M. Tsimidou, S. Kokkini and D. Boskou, 1993. Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. Z. Lebensm. Unters. Forsch., 197: 20-23.

Lee, S.E., H.J. Hwang, J.S. Ha, H.S. Jeong and J.H. Kim, 2003. Screening of medical plant extracts for antioxidant activity. Life Sci., 73: 167-179.

McCay, P.B., 1985. Vitamin E: Interactions with free radicals and ascorbate. Ann. Rev. Nutr., 3: 35-57.

Muranov, K., N. Poliansky, R. Winkler, G. Rieger, O. Schmut and J.H. Winter, 2004. Protection by iodiade of lens from selenite-induced cataract. Graefes Arch. Clin. Exp. Ophtalmol., 242: 146-151.

Nirmalan, P.K., R. Krishnadas, R. Ramakrishnan, R.D. Thulasiraj, J. Katz, J.M. Tielsch and A.I. Robin, 2003. Lens opacities in a rural population of southern India. Invest. Ophthalmol. Vis. Sci., 44: 4639-4643.

Orhan, H., S. Marol, I.F. Hepsen and G. Sahin, 1999. Effect of some probable antioxidants on selenite-induced cataract formation and oxidative stress-related parameters in rats. Toxicology, 139: 219-232.

Shearer, T.R. L.L. David and R.S. Anderson, 1987. Selenite cataract: A review. Curr. Eye Res., 6: 289-300.

Shearer, TR., L.L. David, R.S. Anderson and M. Azuma, 1992. Review of selenite cataract. Curr. Eye Res., 11: 357-369.

Skoula, M. and J.B. Harborne, 2002. Taxonomy and Chemistry. In: Oregano: The Genera Origanum and Lippia. Medicinal and Aromatic Plants-Industrial Profiles 25, Kintzios, S.E. (Ed.). Taylor and Francis/CRC Press, USA., pp: 67–108.

Thylefors, B., 1995. Global data on blindness. Bull. World Health Organ., 73: 115-121.

Thylefors, B., 1999. Avoidable blindness. Bull. World Health Organ., 77: 453-453.

Varma, S.D., D. Chand, Y.R. Sharma, J.F. Kuck and R.D. Richards, 1984. Oxidative stress and cataract formation: Role of light and oxygen. Curr. Eye Res., 3: 35-57.

Varma, S.D. and K.R. Hedge, 2004. Effect of alpha-ketoglutarate against selenite cataract formation. Exp. Eye Res., 79: 913-918.

Yanishlieva, N.V., E.M. Marinova and M.H. Gordon, 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem., 64: 59-66.