

## The Protective Role of Silymarine on Selenite-Induced Cataract in Rabbit Model

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Makale Kodu (Article Code): KVFD-2013-9658

### Summary

The study was designed to investigate silymarine as preventive agent in selenite-induced cataract in rabbits. Eighteen rabbits were concluded and divided equally into negative control (I), positive control (II) and test (III) groups, each 6. After clinical and ophthalmoscope, bio-microscope slit lamp equipped with a digital camera and ultrasonographical examinations of rabbits' eyes, selenite sodium was administered subcutaneously on the neck in all rabbits at day 0 of the study. After induction of anesthesia, lateral recumbency and making a pore in globe 0.1 ml of saline solution 0.9% in group II and 0.1 ml of silymarine were injected into vitreous in group III. Any agent did not administrate in group I. Selenite sodium was injected in days third and sixth in all rabbits subcutaneously. The bio-microscopic slit lamp study showed grade one and two cataract in day 4 and 8, and grade three in day 11 in all rabbits in group I and II. In group III, three rabbits on the eighth day and four until the end of the eleventh day presented grade one cataract. Anterior lens capsule thickness was less in group III in comparison to group I and II, and posterior capsule thickness in group III was significant statistically by ultrasonography on day 20 and the anterior-posterior lens diagonal length in the experimental group comparing to the control groups was longer significantly. Selenite sodium showed cataractogenic character and silymarine as probable protective role on selenite-induced cataract in rabbit model.

**Keywords:** *Cataract, Selenite sodium, Silymarine, Rabbit*

## Tavşan Modelinde Silymarinin Selenit ile Oluşturulmuş Katarakt Üzerine Koruyucu Rolü

### Özet

Bu çalışma tavşanlarda selenit ile oluşturulmuş katarakt üzerine önleyici ajan olarak silymarinin araştırılması için tasarlanmıştır. Her biri altılı gruplardan oluşan 18 tavşan negatif kontrol (I), pozitif kontrol (II) ve test (III) grupları olmak üzere eşit şekilde ayrıldı. Tavşan gözlerinin klinik ve oftalmoskop, dijital kameralı biyo-mikroskop yarı (slit) lamba ve ultrasonografik muayene sonrası sodyum selenit çalışmanın 0. gününde tüm tavşanlara boyuna subkutan olarak uygulandı. Anestezinin başlatılmasından sonra, yan yatırılarak ve küre üzerine gözenek oluşturularak 0.1ml %0.9'luk tuz solusyonu grup II'deki ve 0.1ml silymarin grup III'deki hayvanların vitreouslarına enjekte edildi. Grup I'deki hayvanlara ise herhangi bir madde enjeksiyonu yapılmadı. Selenit sodyum subkutan olarak tüm tavşanlara üçüncü ve altıncı günde enjekte edildi. Biyo-mikroskop yarı (slit) lamba çalışması birinci ve ikinci derece kataraktın 4. ve 8. günlerde ve üçüncü derece kataraktın ise 11.günde grup I ve II'deki tüm tavşanlarda gözlemlendiğini ortaya koydu. Grup III'de, üç tavşanda sekizinci günde ve dördünde ise onbirinci günün sonunda birinci derece katarakt gözlemlendi. Yirminci gündeki ultrasonografiye göre anterior lens kapsül kalınlığı, Grup I ve II'ye göre grup III daha az ve posterior lens kapsül kalınlığı grup III'de istatistiksel olarak anlamlı bulundu. Anterior-posterior lens diagonal uzunluğu deney grubunda kontrol grubuna göre önemli düzeyde daha uzun olarak bulundu. Çalışma sonuçları selenit sodyumun, tavşan modelinde selenit ile oluşturulmuş katarakt üzerinde kataraktojenik karakter ve muhtemel koruyucu rol oynadığını gösterdi.

**Anahtar sözcükler:** *Katarakt, Selenit sodyum, Silymarin, Tavşan*



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## INTRODUCTION

The lens is a transparent biconvex structure that is stabilized in place by zonular fibers [1,2]. A cataract disease is the gradual clouding that develops in the eye's lens that over time leads to impair vision and complete cloudiness (advanced mature cataract) which may progress to blindness [2]. Cataract is a congenital or an acquired condition; the recent type is associated with aging and is influenced by some structural and biochemical changes in the lens [3].

Today, mature cataract is treated by various surgical methods [2]. However, the less severe form and the non-developed form of cataract in diabetic patients and elderly make with some complications [4,5]. Therefore, some agents such as vitamins and antioxidants are used to reduce the progression of the disease [6,7]. Vitamin E and ascorbic acid are examples of such antioxidants utilized [6]. Considering not having the reliable response, the effort of the authors for introducing the appropriate agent in this regard is continued. Since the category of silymarine possesses antioxidant compounds, the present study has investigated the use of silymarine compounds in reduction of selenite-induced cataract in rabbit model.

## MATERIAL and METHODS

Eighteen rabbits of both genders (9 male and 9 female) weighing  $1100 \pm 430$  g were conducted in the experimental study. After clinical examinations and administration of anti-parasitic drugs at the research center, the rabbits' eyes were carefully examined, using ophthalmoscope, biomicroscope slit lamp equipped with a digital camera and ultrasonography upon arrival of the rabbits (day one) (Fig. 1). The rabbits were kept under constant environmental conditions and nutritional care, another careful eye examination was performed in the day 14.



**Fig 1.** Ultrasonography of normal rabbit eye  
**Şekil 1.** Normal tavşan göz ultrasonografisi

The structure of the eyes and normal lens diagonal size were performed to determine transcorneal ultrasonography (Sonosite Titan, USA) under physical restraint with minimum pressure applied on the head and the neck, while transducer horizontally placed on the surface of the cornea, in a position that the cornea, anterior and posterior capsules and the optic discs were at the same direction.

After ultrasonography examination, the eyes were rinsed using 0.9% normal saline solution. The rabbits were then divided randomly into three groups of six rabbits in each negative (I) and positive (II) control, and test (III) groups. The study was approved by the Animal Ethics Committee of the Iranian laboratory animal ethic frameworks under the referencecode IAEC 1-12/2.

### Stage 1

A mixture of 99.3% pure selenite sodium (Merk, Germany) and 0.9% sterile normal saline solution having a density of 0.1% was prepared and 1 mg/kg of body weight (1 ml) was administered subcutaneously on the neck in all three groups of rabbit at day zero of the study.

### Stage 2

Anesthesia was induced using combination of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg) intramuscularly. Then anesthesia rabbits in groups II and III were placed on their sides, and the eyes and the eyelids were irrigated with dilute solution of povidone-iodine. Prior to the administration of normal saline and silymarine (Madaus, Germany), a pore in the cornea using the gauge 27 needle from a distance of 2 mm of limbus was created to allow the release of pressure and neutralization after injection of silymarine into vitreous chamber.

In group I, no normal saline solution or silymarine was applied. In group II, 0.1ml of 0.9% normal saline solution and 0.1 ml of silymarine in group (test) III was injected into vitreous chamber under general anesthesia, respectively (Fig. 2). Then topical antibiotic therapy using



**Fig 2.** Injection in the vitreous chamber  
**Şekil 2.** Vitreus boşluğa enjeksiyon

chloramphenicol eye drop was administered post-injection every 6 h for 3 days.

### Stage 3

Days third and sixth of the study, the same dosage of selenite sodium was administered subcutaneously to all 3 groups of rabbits.

### Stage 4

For prevention infection, no ultrasonography of eyes was performed during three days after the injection. Ultrasonographic study was performed on day 20 after injection of silymarine to measure the diagonal of anterior and posterior of the lens capsules and diagonal of the antero-posterior of the lens. Bio-microscopic slit lamp study of the eyes was performed with 3 day intervals for 20 days. The degree of opacification was assessed according to a study that described as follows: grade 0, absence of opacification (gridlines clearly visible); grade 1, slight degree of opacification (minimal clouding of gridlines and gridlines still visible); grade 2, diffuse opacification involving almost the entire lens (moderate clouding of gridlines and gridlines faintly visible); grade 3, extensive thick opacification involving the entire lens (total clouding of gridlines and gridlines not seen at all [7].

### Stage 5

The blind method was applied to study the rabbits' eyes, and the results analyzed by the ANOVA.

## RESULTS

The results suggested that the injection of selenite sodium with 3 days intervals and three times, did not cause any death in the rabbits of 3 groups. All rabbit tolerated the experimental studies. The study of the eyes with bio-microscopic slit lamp showed that cataract of grade one on day 4, grade two on day 8 and grade 3 until on day 11 in the eyes of all rabbits in group I and II post-injection of selenite sodium. It is noteworthy that the degree and severity of cataract from day 11 until the end of the study remained without changes. Cataract grade one was seen in three rabbits on the eighth day in the group (test) III, and four rabbits on day 11. The cataract was not observed in two rabbits of group III (Fig. 3). Ultrasonographically, anterior lens capsule thickness was less in group III in comparison to group I and II, and posterior capsule thickness in group III was significant statistically ( $P < 0.05$ ). The anterior - posterior lens diagonal length in the group III comparing to the control groups was longer and showed a significant difference ( $P < 0.05$ ).

Results of ultrasonographic study of the eyes showed an increase in thickness and anterior and posterior capsule echogenicity in the 11 days which remained constant until the end of day 20 (Fig. 4, 5, 6, 7, 8).



**Fig 3.** Grade one of cataract in rabbit eye

**Şekil 3.** Tavşan gözünde birinci derece katarakt



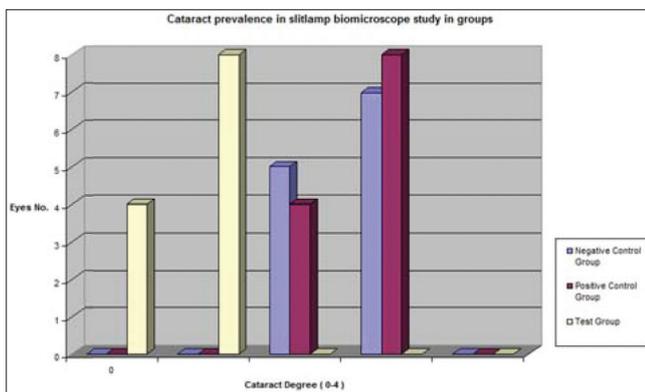
**Fig 4.** Increase of posterior lens capsule thickness following intraocular selenite sodium injection

**Şekil 4.** Göz içi selenit sodyum enjeksiyonu takiben posterior lens kapsül kalınlığı artışı

## DISCUSSION

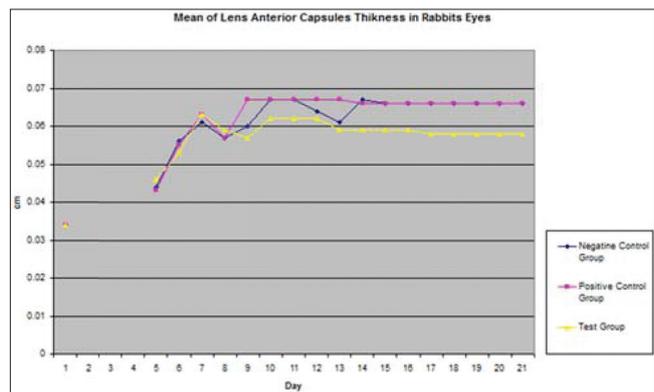
From past till now, different studies for induction the experimental cataract in various animal groups have been conducted [3,8,9]. There is a troublesome question that how could make the experimental cataract [8,10]. Many research implicated in finding out which medication and diet could induce cataract in animal model [10]. Medication or method for reduction or prevention of cataract in patients has not been known clearly [2].

There have been a lot of reports related to induction of acquired cataract due to the hyperglycemia, but laboratory trails for induction of cataract using hyperglycemia has been failed [1,2,8,11,12]. Investigations have induce temporary cataract in younger dogs and cats (under 6 months of age) using administering goat milk without argenine, but the cataracts was observed to be resolved after diet modifications [13]. In another study, it was shown that a diet



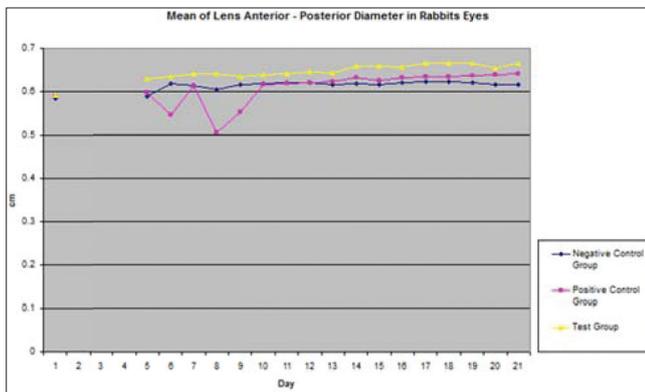
**Fig 5.** Cataract prevalence in slitlamp microscope study in groups

**Şekil 5.** Gruplardaki yarı (slit) lamba mikroskop çalışmasındaki katarakt yaygınlığı



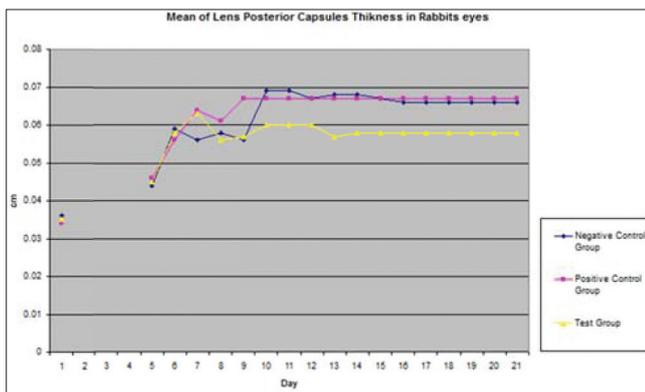
**Fig 8.** Mean of lens anterior thickness in rabbits' eyes

**Şekil 8.** Tavşanların gözlerinde lens anterior kalınlığı ortalaması



**Fig 6.** Mean of lens anterior-posterior diameter in rabbits' eyes

**Şekil 6.** Tavşanların gözlerinde lens anterior-posterior çap ortalaması



**Fig 7.** Mean of lens posterior thickness in rabbits' eyes

**Şekil 7.** Tavşanların gözlerinde lens posterior kalınlığı ortalaması

without methionine, resulted in irreversible cataract [12].

Reports revealed that the toxic agents such as paromycin (aminoglycosides drug) result in acute renal failure and cataract in cats [2]. They believed that cats suffering from enteritis has impaired intestinal epithelial layer and absorb toxic substance. Absorption of the toxin of healthy colon epithelium is not possible [3]. Other studies have showed that corticosteroid drugs (dexamethasone),

hydroxymethylglutaryl co-enzyme drugs, D-methylsulfoxide and diazoxid could also result in cataract in experimental dog models [14-16].

Investigators believe that cataract effects of the diazoxid drug and dexamethasone could be following hyperglycemia [4]. In other study, investigators showed that nephthalene in rabbits could cause induced-cataract in female rat model [4]. Many researches showed selenite sodium administration to infant rats induced cataract experimentally [4].

We have showed that injection of selenite sodium three times for three days with a dose of 1 mg/kg in rabbits during 11 days induced in different degrees of one to three. Therefore, the cataract effect of selenite sodium was statistically confirmed in our study. Pathological changes in the retina under the bio-microscopic slit lamp in any of three groups I, II, and III was not seen. Any signs of pathological changes in the liver, kidney, brain and spleen were not observed too. However, in our study, no gender predilection was found. Several studies have suggested that antioxidants retard the process of cataractogenesis by scavenging free oxygen radicals. The researchers have dedicated a lot of efforts using different agent to reduction cataract or the development of age-related cataract and or some of the acquired cataract conditions [17]. One research showed intraperitoneal injection of garlic aqueous extract in rat model appeared to effectively prevent selenite-induced cataract [18]. Doganay and his colleagues demonstrated that apricots reduced selenite induced cataract in Sprague-Dawley rat on the experimental cataract model [19]. The antioxidant effects and vitamin content play preventive role [20]. Various reports have shown that the use of enzyme inhibitors an anhydrous release carbon dioxide, ardistine antioxidants, N-stealcartozin and vitamin E, have reduced progression of cataract [4,20]. Geraldine and et al. [21] suggested that acetyl-L-carnitine (ALCAR) is able to significantly retard experimental selenite-induced cataractogenesis in Wister rat model.

We showed that increase of the anterior - posterior

diameter of the lens which is due to the antioxidant properties of silymarine increases elasticity of the lens and explains the increase of the anterior-posterior diameter in group III. The results of bio-microscopic slit lamp study confirmed that levels of cataract which were seen in groups I and II was more severe and significant than the group III, these findings also were confirmed by the ultrasonographical (increased capsule thickness).

In conclusion, our results demonstrated that selenite-sodium has cataractogenic effect on the experimental cataract model in rabbits and moreover silymarine has protective role on selenite-induced cataract in rabbit without gender predilection. We recommended that for achieving to reasonable results, histopathological study of globe and particularly lens and retina need to further studies.

#### ACKNOWLEDGEMENT

The authors would like to thank Dr. Nima Vazir.

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