

Research Article



Effect of *Foeniculum vulgare* Seed Aqueous Extract Eye Drops on Selenite induced Cataract in Rabbits

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ABSTRACT

Cataract is an opacity in the normally transparent focusing lens of the eye which leads to blindness. The aim of current study is to investigate the possible protective and therapeutic effects of aqueous extract of *Foeniculum vulgare* seed eye drops (0.5%) against selenite induced cataract in rabbits. Aqueous extract of *Foeniculum vulgare* seed prepared then formulated as eye drops. Sodium selenite used to induce cataract in rabbits right eye by single intravitreal injection. Thirty-six rabbits included in the study and divided into three equal groups (12 rabbits in each group): healthy control group, Cataract-Induced group and group treated by *Foeniculum vulgare* seed extract eye drops. Parameters include the score of lens opacity which was determined by using an ophthalmoscope, pupillary response to light, pupil diameter, Corneal sensation, Conjunctival redness, intraocular pressure (IOP) measurement, malondialdehyde (MDA) level in aqueous humor and scanning electron microscope (SEM) image of the eye lens. The results of this study revealed that eye drop of *Foeniculum vulgare* seed extract was highly significantly reduced the mean of lens opacity score in comparison with cataract-induced group after two weeks of cataract induction ($p < 0.01$). MDA level of the group treated with *Foeniculum vulgare* seed extract eye drops was highly significant less than the MDA level of cataract-induced group ($p < 0.01$). SEM image of rabbit's lens treated by *Foeniculum vulgare* seed extract eye drops shows normal appearances of the eye lens. In conclusion, eye drops prepared from aqueous extracts of *Foeniculum Vulgare* seed had protective and therapeutic effects against sodium selenite-induced cataract in the rabbits.

Keywords: Cataract, *Foeniculum vulgare* seed, lens opacity, oxidative stress.

INTRODUCTION

Cataract is defined as degenerative process produce opacity within the clear natural crystalline lens of the eye, which gradually results in vision deterioration. It is currently treated by surgery.¹ With aging, oxidative stress occurs which represent an imbalance between free radical production and antioxidant system. Oxidative stress is the primary factor in the genesis of senile cataract which is the commonest cataract type.² Cataract may occur due to metabolic disorders like galactosemia¹ and diabetes mellitus.³ Many drugs can contribute to cataracts formation including Corticosteroids, Thiazolidinedione, Amiodarone, Chlorpromazine.⁴⁻⁷

Foeniculum vulgare Mill (Fennel) is a medicinal plant belonging to the Umbelliferae (Apiaceae) family. It is an ancient seasonal herb, and it grew in the southern Mediterranean region for its edible fruit or seeds. Different Phytochemical studies have shown the presence of numerous valuable compounds, such as volatile compounds, flavonoids, and phenolic compounds. It has several pharmacological properties such as anti-inflammatory, oculohypotensive activity, and antioxidant activities.⁸ The purpose of this study was to investigate the protective and therapeutic effects of *Foeniculum vulgare* seed extract eye drops against selenite-induced cataract in rabbits.

MATERIALS AND METHODS

Preparation of *Foeniculum vulgare* seed extract

Seeds of *Foeniculum vulgare* bought from a local market in Baghdad-Iraq and identified by Dr. Ibrahim S. Abass, Assistant Professor, Department of Pharmacognosy and Medicinal Plants, Al-Mustansiriyah University, Baghdad, Iraq.

Firstly, the seeds cleaned and coarsely grinded, then 70 g of solid matter mixed with 1,4 L water. The experiment at 70 °C was carried out in a thermostatic shaker for 2 hours after that the extract was filtered using Whatman No.1 filter paper.⁹

lyophilization was used to obtain dry powder. The sample was frozen then under a vacuum for 10 hours the ice sublimating directly into water vapor, and dry powder remains.

Preliminary qualitative phytochemical analysis

This tests made on aqueous extract of *Foeniculum vulgare* seed using standard procedure to identify the active constituents present in this extracts. To identify flavonoids lead acetate test used. Wagners and molish tests were conducted to identify alkaloids. Liebermann-Bur chard test, forthin test, salkowski test and Ferric chloride test were conducted to identify steroids, saponins, terpenoids, and tannins respectively.¹⁰⁻¹²



Preparation of herbal extract eye drops

500 mg from extract dry powder dissolved in an appropriate volume of the isotonic buffer with mixing, then 1ml benzalkonium chloride (1% w/v) added and the volume completed to 100 ml by the isotonic buffer to made (0.5% w/v) eye drops.

Preparation of sodium selenite solution

10 mg of sodium selenite powder was dissolved in 100 ml of distilled water and mixed well to obtain (0.01% w/v) of selenite solution. A fresh solution prepared for each group.

Induction of cataract

The rabbits were anesthetized by used intramuscular injection of ketamine (50 mg/ml) at dose (50 mg/kg) and intramuscular injection of diazepam (10 mg/2 ml) at dose (2 mg/kg). Proparacaine (0.5%) eye drops applied to the eye to obtain additional anesthesia. A single injection of 0.1ml sodium selenite solution (0.01% w/v) used to induce cataract. It injected intravitreally (about 4 mm behind the limbus in sclera) using a needle of gauge (27 x 13 mm).¹³ After injection, rabbits' right eye monitored every day for cataractogenesis.

Experimental animals

Thirty-six rabbits (*Oryctolagus cuniculus*) included in the study. The aged about one year and their weight in the range of (1.5-2 kg). Animals kept on a fresh diet, water, suitable temperature and normal light. Rabbits divided into three equal groups: healthy control group (no cataract induction and distilled water instilled to both eyes), cataract-induced group and treatment (*Foeniculum Vulgare* seed extract eye drops) group. Distilled water (cataract-induced group) and tested plant extract eye drops (treatment group) instilled to rabbits' right eyes twice daily for 5 days before induction cataract and 21 days after cataract induction. The experiments were approved by Animal Ethical Committee, College of Medicine/Al- Nahrain University, Baghdad, Iraq.

Parameters

Lens opacity:

Ophthalmoscope was used to perform the red reflex test and to determine the score of cataract maturity according to Chylack and Kador classification.^{14,15}

Pupil diameter:

Pupil diameter measured by using the pupil gauge. Results expressed in millimeter unit.¹⁶

Pupillary response to light:

In a dark room, swinging flashlight used to detect an afferent pupillary defect. The response presented as either it was intact or absent.¹⁷

Corneal sensation

A wisp of cotton wool applied and moved from side to side to examine corneal sensation. The results expressed as either it was intact or absent.¹⁶

Conjunctival redness

Conjunctiva of both eyes checked in order to detect if the redness present or not.¹⁷

IOP measurement

Schiotz tonometer was used to measure IOP. A Proparacaine (0.5%) eye drop was instilled to both eyes in order to produce local anesthesia then tonometer footplate directly put on the cornea. The instrument held vertical to the eye to allow the plunger to move freely. Since the Schiotz tonometer does not measure pressure directly, conversion tables used to convert scale readings into estimates of intraocular pressure.¹⁸

MDA measurement

This test performed to measure MDA level in the aqueous humor of rabbits' right eyes. The principle of this procedure is the reaction between a Thiobarbituric acid (TBA) and MDA, which is resulting in a color compound measured spectrophotometrically at 532nm.¹⁹

Scanning electron microscope (SEM)

At the end of the study, one rabbit from each group sacrificed, and the lens removed and fixed by Glutaraldehyde (3%) solution then lenses examined by scanning electron microscopic (SEM).

Statistical analysis

The obtained quantitative data presented as mean \pm S.E.M (standard error of the mean). Student paired t-test used to determine the effectiveness of employed therapy for the right eyes of rabbits in the same group. Student unpaired t-test used to assess the significance of the difference between the results of right and left eyes of rabbits in the same group or between the results of the right eyes of rabbits of any two groups. If the P value > 0.05 the obtained difference was not significant, the obtained difference was considered significant when $0.01 < P \leq 0.05$ and highly significant when $P \leq 0.01$.²⁰

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical analysis showed the presence of different compounds in this extracts (Table 1).

The mean of lens opacity score of rabbits' right eyes of all study groups shown in (Table 2) and (figure 1). lens opacity score of rabbits' right eyes of the cataract-induced group after one week of sodium selenite injection was (2.58 ± 0.193) and in comparing to the mean of lens opacity score of the healthy control group (0 ± 0.0) the difference was highly significant ($p < 0.01$) and it remained at this level until the trial reached to end. The mean of lens opacity score of rabbits' right eyes of treatment



group after one week of cataract induction was (1.58 ± 0.378) which was significant ($P < 0.05$) less than the mean of lens opacity score in cataract-induced group. After two weeks of cataract induction, the mean of lens opacity score of rabbits' right eyes was reduced to (1.33 ± 0.376) which is show highly significant ($P < 0.01$) difference as compared to cataract-induced group. Right eyes of the cataract-induced group lost the pupillary response to light after cataract induction while the pupillary response to light remained intact after using eye

drops of *Foeniculum vulgare* seed extract .Mean of MDA concentration in aqueous humor of cataract-induced group was highly significant ($p < 0.01$) increase in comparison with healthy control group ($0.2552 \mu\text{Mol/l} \pm 0.03156$, $0.0327 \mu\text{Mol/l} \pm 0.00567$ respectively). MDA concentration of the rabbits group treated with eye drop of *Foeniculum vulgare* seed extract was ($0.1515 \mu\text{Mol/l} \pm 0.01622$) which was highly significant ($P < 0.01$) less than the MDA concentration of cataract-induced group,(Table 3) and (figure 2).

Table 1: Preliminary qualitative phytochemical analysis

Plant	Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Anthraquinone	Terpenoids
<i>Foeniculum vulgare</i>	+	+	+	+	+	+	+

Table 2: mean of lens opacity score of rabbits' right eyes

	First week	Second weeks	Third weeks
Healthy control group	0.0	0.0	0.0
Cataract-induced group	2.58	2.58	2.58
Treatment group	1.58	1.33	1.33

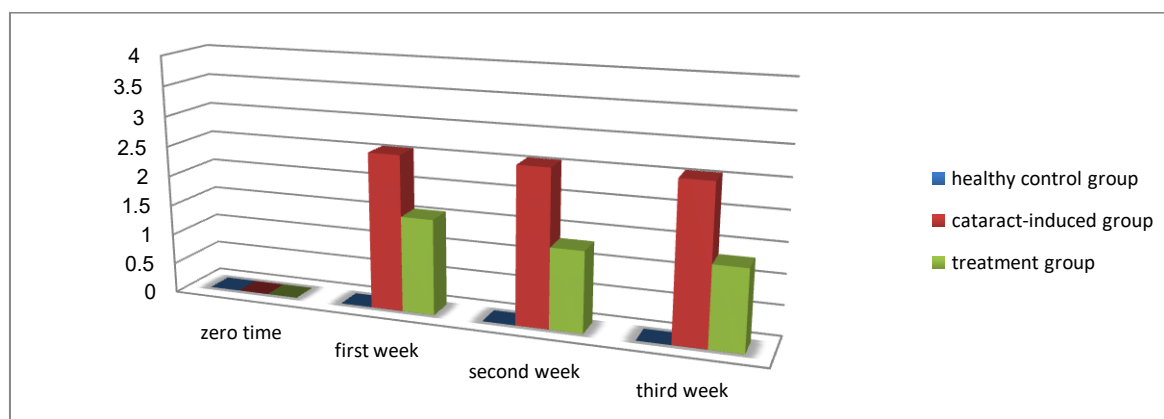


Figure 1: Mean of lens opacity score of healthy control, cataract-induced and treatment groups

There is no significant change in IOP, pupil diameter, and corneal sensation of rabbits' right eyes of treatment group when compared with the healthy control group. No conjunctival redness occurs in rabbits eyes after use *Foeniculum vulgare* seed extract (0.5%) eye drops.

Table 3: MDA ($\mu\text{Mol/l}$) level in aqueous humor

	Healthy control group	Cataract-induced group	Treatment group
MDA level ($\mu\text{Mol/L}$)	0.0327	0.2552	0.1515

SEM image shows the homogeneous structure of healthy control group lens (Figure 3A) while the image of cataract-induced group lens show it loses the homogeneous structure and large gap between the lens fibers occur (Figure 3B).

SEM image of rabbit's lens treated by *Foeniculum vulgare* seed extract eye drops shows the homogeneous structure of lens and there is no large gap between the lens fibers (Figure 3C).

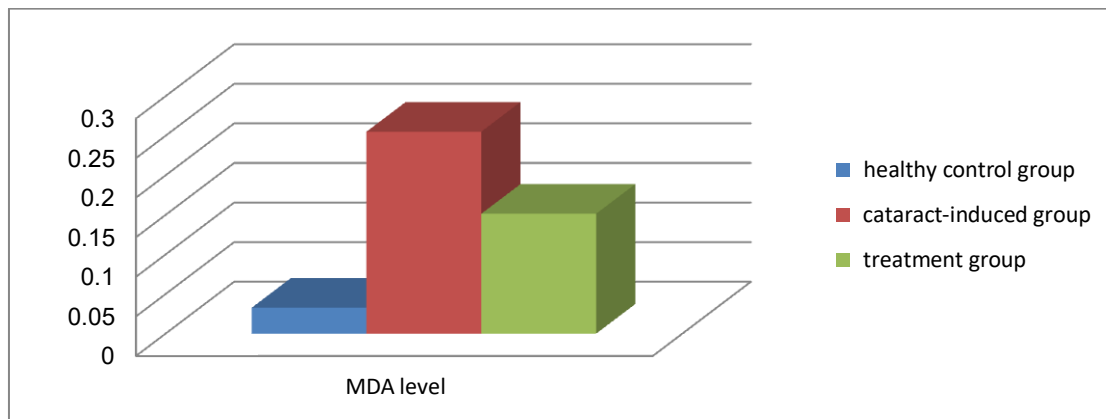


Figure 2: Mean of MDA level of rabbits' right eyes of healthy control, cataract-induced and treatment group.

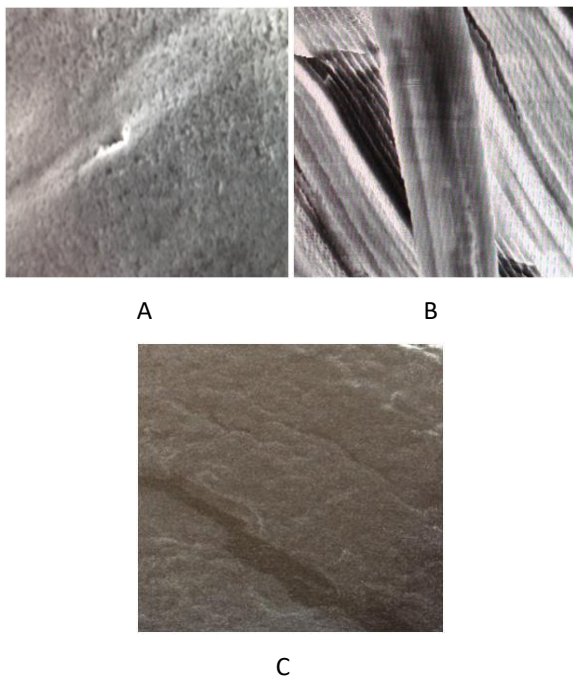


Figure 3: SEM image of rabbits' lens at 2000X. A: normal lens. B: cataractous lens. C: lens treated with *Foeniculum Vulgare* seed extract eye drops.

In this study sodium selenite (Na_2SeO_3) used to induce cataract in rabbits. Sodium selenite partially mimics senile nuclear cataract in humans and it acts by induced oxidative stress in lens tissue.²¹ the results show the ability of *Foeniculum vulgare* seed extract eye drops to reduce cataract maturity score and the concentration of MDA in aqueous humor. *Foeniculum vulgare* is a good source of natural antioxidants like polyphenols and flavonoids.²² The preliminary qualitative phytochemical analysis made on a sample of the aqueous extract of *Foeniculum Vulgare* seed show the presence of flavonoids in this extract. the antioxidant activity of 100 mg of water extracts was evaluated by Shahat and co-worker (2011) and the results showed that water extract had 77.5% of antioxidant activity which was greater than the antioxidant properties of alpha-tocopherol (36.1%) with the same dose.⁸

Glutathione Peroxidase is one of the enzymatic antioxidants of the eye lens.²³ Sadeghpour and co-worker

(2015) record (70%) Ethanolic extract of *Foeniculum vulgare* seed increases the blood level of glutathione peroxidase in female albino mice.²⁴

Hydrogen peroxide (H_2O_2) is the one of major oxidant involved in cataract formation, and NF-kappa B signal pathway has an important role in the development of H_2O_2 -induced cataract.²⁵ *Foeniculum vulgare* was significantly decreased NF-kappaB in lipopolysaccharide animal model of acute lung injury. it may act by increasing the expression of NF-kB inhibitor, I κ B- α .²⁶

Nitric oxide has an important role in cataract induction by selenite in rates.²⁷ The aqueous extract of *Foeniculum vulgare* showed a highest nitric oxide scavenging effect of 79.75% at 62.5 $\mu\text{g}/\text{mL}$ as compared to Ginkgo Biloba (the positive control) which has 36.22% scavenging effect at a similar concentration.²⁸ Methanolic extract of *Foeniculum vulgare* seed has the ability to decrease nitric oxide formation in an animal model of renal dysfunctions by tienilic acid.²⁹

CONCLUSION

Eye drops prepared from aqueous extracts of *Foeniculum Vulgare* seed has protective and therapeutic effects against sodium selenite-induced cataract in rabbits which was reflected in decrease lens opacity score and enhanced the image of scanning electron microscope. The anti-oxidant effect was the probable mechanism of protective and therapeutic effects of this extract.

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