International Journal of Multiphysics
Volume 18, No. 4, 2024
ISSN: 1750-9548

Evaluating the Effects of Alpha-Lipoic Acid Treatment on Experimentally Induced Cataracts in Rabbit Model by Ultrasonography and Slit Lamp Examination

Sima Nikkhah[1], Farrokhreza Kabir[1*], Farnoosh Arfaee[1], Alireza Jahandideh[1], Hesameddin Akbarein[2]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.
*Corresponding author

Abstract

Background and Objective: Cataracts refers to an optical condition associated with turbidity and cloudiness of the lens. It is a slow-progressing disease and ultimately results in diurnal and/or nocturnal vision impairment. Alpha-lipoic Acid (ALA) is a potent anti-oxidant agent used to assist to impede the progress of hepatic conditions, such as hepatitis, and fatty liver disease. Given the great potency of this compound as an antioxidant, it is possible that ALA can be more efficient than vitamin E in halting the progress of cataractss. This study intends to evaluate the effects of ALA treatment on experimentally induced cataractss in rabbit models by methods of ultrasonography and slit lamp examination.

Design: A total number of 18 rabbits were assigned to three groups (n=6): a) ALA, b) positive control, and c) negative control. Each group was followed up for 20 days and the incidence of cataracts and its grade was recorded independently.

Findings: While the highest grade of cataracts recorded for ALA treated group was grade 1, grade 2 and grade 3 cataractss were present in positive control and negative control groups.

Conclusions: ALA is effective in preventing the progression of cataractss to later stages and protecting the lens from further damage.

Keywords: eye, cataracts, alpha-lipoic acid, ultrasonography, slit lamp examination

Introduction

Not long ago, no specific approach was available to control the progressive process of cataracts. In most cases, surgery was required to remove the affected lens and substitute it with a synthetic intraocular lens (IOL) after the condition had fully progressed; this would maintain a semi-satisfactory degree of vision for the patient. From 1977 onwards, researchers began to evaluate the effects of different compounds to prevent, delay, or if possible, halt the progress of senile cataracts and some acquired forms of this condition. Non-surgical treatments of cataracts using different compounds have been reported in experimental designs using mouse and rabbit models (Kabir 2009, Hogan et al. 1971, Nautrup and Tobias 2000, Maggs et al. 2008).

Until now, researchers have experimentally induced cataracts by oral administration of high doses of glucose and, consequently, inducing diabetic cataracts, exposure of animals to ionizing radiation, subcutaneous injection of sodium selenite salt (in immature mouse), and oral administration of naphthalene (in rabbit), to assess the potential of different compounds, such as carbonic anhydrase inhibitors, or a wide range of antioxidant agents including erdosteine, N-acetylcarnosine (NAC), silymarin, vitamin E, and ALA in preventing further progression of the

disease. Many reports claim to have achieved satisfactory results (Kabir 2009, Hogan et al. 1971, Nautrup and Tobias 2000).

Maitra et al. (1994), studied the effect of ALA as a potent antioxidant agent against cataracts in neonatal mice treated with L-buthionine-(S,R)-sulfoximine (BSO). They found that a dosage of 25 mg/kg (body weight) prevented cataracts in 60% of subjects. BSO is an inhibitory agent in the process of glutathione synthesis and causes cataracts if administered to neonates. Therefore, this study presented a potential model for studying the protective role of therapeutic antioxidants against cataracts in animals (Varma et al. 1995, 35). ALA induces major biochemical changes in the lens, such as increased levels of glutathione, ascorbate, and vitamin E, contributing to its protective effect against BSO. Moreover, ALA intensifies the catalytic action of glutathione peroxidase, catalase, and ascorbate free radical reductase enzymes in lenses of animals treated with BSO, but does not affect glutathione reductase or superoxide dismutase (Varma et al. 1995, 35; Vică, M. L., 2022).

Conclusively, ALA may take up some functions attributed to glutathione (i.e. maintaining a higher level of ascorbate, an indirect role in recycling vitamin E). Increased levels of glutathione in the lens also can be attributed to the direct protection of protein thiols mediated by ALA. Therefore, ALA can be a potential therapeutic agent for preventing cataracts and subsequent complications (Varma et al. 1995, 35). In another study, Kojima et al (2007) evaluated the efficacy of oral administration of dihydrolipoate-LA in treating streptozotocin (STZ) induced diabetic cataracts in mice; subjects were divided into three separate groups: a) control (C), b) diabetes mellitus (DM), c) diabetes mellitus treated by dihydrolipoate-LA (DM+LA). Diabetes was induced by injecting 50 mg/kg STZ. DM+LA mice were also fed 30 mg LA daily. Lens integrity was evaluated using the dynamic light scatter (DLS) test. The DLS increase in the DM+LA group was significantly lower than in the DM group five weeks after induction ($P < 0.05$). Highest and lowest blood glucose levels at every point until 111 days were recorded in the DM and C groups, respectively ($P < 0.05$). LA treatment delays the progress of diabetic cataracts induced by STZ in mice (Kılıç Et al. 2007, 4; Delcea, C., Bululoi, 2023).

Overdose injection of sodium selenite causes nuclear cataracts in juvenile mice. The first use of this method was reported in 1977 and consisted of a subcutaneous injection of 3.28 mg sodium selenite dissolved in normal saline to 10 to 14-day-old rabbits. Cataracts were induced 4 to 6 days later (Maggs et al. 2008, 258-276, Scott Luper 1988, 3).

Given the larger diameter of the eye in rabbits compared to mice, experimental induction of cataracts can contribute to research in this field. Furthermore, the development of a method for quick induction of cataracts (about 10 days) in rabbits is of great importance for future studies. Through the course of their investigations on the quick induction of cataracts in rabbits using sodium selenite, Kabir et al. found that subcutaneous injection of 3.28 mg sodium selenite can result in rabbit fatality in less than 6 hours. Afterward, lower doses of this compound were used for the same purpose with 1.5 mg/kg (live rabbit body weight) causing death in a maximum duration of 18 hours. Therefore, even lower doses were used and it was finally concluded that subcutaneous injection of 1 mg/kg of sodium selenite in a 0.1% solution on days 0, 3, and 6 can induce posterior subcapsular cataracts (PSCC) in rabbits without fatality (Kılıç et al. 2007, 4). Furthermore, administration of the same dose of this compound can cause cataracts in both mature and juvenile rabbits. Using this method, all subjects (100%) showed symptoms of cataracts from day four onwards, with the maximum degree of affliction on day 11. No fatality or side effects were observed in other organs.

The efficacy of intravitreal injection of silymarin in preventing experimentally induced cataracts was evaluated by Kabir and others, with comparable results to the administration of silymarin in the rabbit model (Kabir 2009). Based on a study by Eman and his colleagues, ALA is effective in preventing the incidence of cataracts or delaying its progress in rabbits (Eman et al. 2011, 21). Another study by Demir et al. (2005), showed similar effects for ALA in protecting the eyes against free radicals produced by ultraviolet radiation (Demir et al. 2005, 219). Williams (2017) also indicated that oral administration of ALA in diabetic dogs was effective in the prevention of cataracts (Williams 2017, 4).

In another study, Kojima et al (2007) evaluated the efficacy of oral administration of dihydrolipoate-LA in treating streptozotocin (STZ) induced diabetic cataracts in mice; subjects were divided into three separate groups: a)

control (C), b) diabetes mellitus (DM), c) diabetes mellitus treated by dihydrolipoate-LA (DM+LA). Diabetes was induced by injecting 50 mg/kg STZ. DM+LA mice were also fed 30 mg LA daily. Lens integrity was evaluated using the dynamic light scatter (DLS) test. The DLS increase in the DM+LA group was significantly lower than in the DM group five weeks after induction ($P < 0.05$). Highest and lowest blood glucose levels at every point until 111 days were recorded in the DM and C groups, respectively ($P < 0.05$). LA treatment delays the progress of diabetic cataracts induced by STZ in mice (Kılıç Et al. 2007, 4).

Overdose injection of sodium selenite causes nuclear cataracts in juvenile mice. The first use of this method was reported in 1977 and consisted of a subcutaneous injection of 3.28 mg sodium selenite dissolved in normal saline to 10 to 14-day-old rabbits. Cataracts were induced 4 to 6 days later (Maggs et al. 2008, 258-276, Scott Luper 1988, 3).

The present study evaluates the effect of ALA oral administration on experimentally induced cataracts in rabbit models using ultrasonography and slit lamp examination. One of the most important objectives of this study is to assess the efficacy of this approach with a minimum number of side effects possible. Since there is no report regarding the use of ALA in halting the progress of cataracts, our objective is to study the effects of the aforementioned compound on controlling the progress of experimentally induced cataracts in rabbits. This study aims to assess the preventive properties of ALA in halting the progress of cataracts in rabbits, using ultrasound and slit lamp methods, and to question whether this approach can be effective against cataracts.

Design (Methods):

Eighteen Iranian mixed breed rabbits weighing 1000 to 1500 g were subjected to this study, regardless of their sex. Following thorough clinical examination and health confirmation, ophthalmic examination was performed and healthy rabbits were included in this study. Subjects were relocated to their shelter, where each rabbit was examined and weighed separately. Individual record forms were filled and each subject received antiparasitic medication. Rabbits were kept in separate cages for two weeks and fed a diet consisting of carrots, apples, bread, and rabbit food produced by Pars Daneh (Savadkooh). After two weeks and following another round of clinical examinations, the ophthalmic examination was performed to ascertain the integrity of rabbit eyes, using an ophthalmoscope, slit lamp biomicroscope equipped with a digital camera, and ultrasound device in a veterinary clinical center.

After confirming the health status of the eyes, normal dimensions of the lens and other orbital components were measured using an ultrasound technique. Only physical restraint was used for ophthalmic ultrasonography, with no general or local anesthesia. Transcorneal ultrasound examination was performed in sterile conditions using sufficient volumes of coupling medium. After the application of ultrasound gel, the transducer was placed on the cornea and all measurements were taken when ultrasound impressions of the cornea, anterior capsule, exterior capsule, and optic disk were all in the same orientation. Eyes were washed using normal saline solution following each session. Subsequently, subjects were divided into three groups (n=6): a) negative control (NC), b) positive control (PC), and c) test (T). Subjects in each group were then weighed with a precision of 10 g. Sodium selenite 99.3% (Merck) was dissolved in normal saline to yield a concentration of 0.1% by a pharmacologist in a professional manner; all rabbits were then subcutaneously injected with the final solution at a dose of 1 mg/kg (body weight). The positive control group was administered 1 ml of normal saline per day, while the test group received daily administration of 150 mg ALA (oral solution 25 mg/ml).

Following another weight measurement on days 3 and 6, Sodium selenite was once again injected subcutaneously at a dose of 1 mg/kg (Kabir 2009). Using ultrasound technique, the diameters of anterior and posterior capsules, and the anterior-posterior diameter of lens were measured and recorded daily. Slit lamp examination was performed every three days and on day 20, final grading was performed for each subject and recorded accordingly in tables designed for each group.

Data analysis was performed using SPSS 26.0 software.

Findings:

As shown in Table (1), slit lamp examination on the negative control group revealed that by day 4, all subjects had developed grade 1 cataracts. On day 8, two subjects were diagnosed with grade 1 cataracts and four subjects with grade 2 cataracts. On day 11, 5 subjects had progressed to grade 2 cataracts while one subject was suffering from grade 3 cataracts. Similar results were observed on day 14. On day 17, 3 subjects were diagnosed with grade 2 cataracts and 3 subjects with grade 3, with no change on day 20.

Table (2) shows slit lamp exam results for the positive control group. On day 4, all 6 subjects were diagnosed with grade 1 cataracts. On day 8, 3 cases of grade 1 cataracts, 1 case of grade 2 cataracts, and 2 cases of grade 3 cataracts were observed. On day 11, 4 subjects suffered from grade 2 cataracts, and 2 subjects were diagnosed with grade 3 cataracts. Similar results were observed on day 14. On day 17, 2 cases of grade 2 cataracts, and 4 cases of grade 3 cataracts were reported, with no change on day 20.

Table (3) shows the results for the test group (ALA treatment). On day 4, no case of cataracts was diagnosed. On day 8, signs of cataracts had already become evident. On day 11, 4 subjects (66.7%) showed signs of cataracts with a maximum grade of 1, with no further progression until the end of the study; in 2 subjects, no sign of cataracts was present throughout the trial (33.3%).

As shown in Table (4), no significant difference ($p=0.074 > 0.05$) was observed when comparing the presence or absence of cataracts in all groups using the chi-square test.

As shown in Table (5), there is a significant difference ($p=0.007 < 0.05$) when comparing the presence or absence of mild cataracts in the test group with both control groups, indicating a higher portion of mild cataracts in the former. Conversely, based on results presented in Table (6), all subjects in control groups were affected by either grade 2 or grade 3 cataracts, while no greater progress of cataracts than grade 1 was present in the test group. Therefore, incidence of advanced stages of cataracts was significantly lower in the test group ($p=0.00 < 0.05$, based on the chi-square test).

Ultrasound images of the eye lenses of rabbits in positive control and negative control groups indicate an increase in thickness of anterior and posterior capsules from day 4 onwards, until coming to a halt on day 12. An increase in anterior-posterior diameter of lenses was also observed from day 4 onwards (table 7, table 8).

In the test group (rabbits that received ALA administrations), the thickening of capsules was less evident, with a mean increase of 0.34 mm to 0.58 mm in anterior capsule thickness. On the other hand, the mean increase in capsular thickness was 0.35 mm to 0.66 in the negative control group, and 0.34 mm to 0.66 mm in the positive control group (table 9). The mean value of the anterior-posterior diameter of the lens in the test group was 5.9 mm at the beginning, with an increase to 6.6 mm on the last day, while the same parameter changed from 5.8 mm to 6.1 mm in the negative control group, and from 5.8 mm to 6.4 mm in the positive control group.

Discussion:

This study evaluated the effects of ALA treatment on experimentally induced cataracts in rabbit models by methods of ultrasonography and slit lamp examination. The reasoning behind selecting this approach was therapeutic efficacy of oral solution associated with the minimum incidence of side effects and complications.

Ultrasound examination of rabbit eye lenses revealed that the thickening of anterior and posterior capsules of the lens started on day 4 and halted on day 12. Anterior-posterior diameter of the lens also increased from day 4 onwards. The test group showed lesser thickening of lens capsules compared to other groups, with an average increase of 0.34 mm to 0.58 mm in anterior capsule diameter. On the other hand, the negative control group showed an average change of 0.35 mm to 0.66 mm, while the positive control group showed an average increase of 0.34 mm to 0.66 mm. In the test group, the mean measurement for the anterior-posterior diameter of the lens was 5.9 mm on the first day and 6.6 mm at the end, while the same parameter changed from 5.8 mm to 6.1 mm in the negative control group, and from 5.8 mm to 6.4 mm in the positive control group. Moreover, slit lamp results indicated that in the test group, the lowest and highest grades of cataracts were grade 1 and grade 2, while the stage of cataracts was between grade 2 and grade 3 in the control groups. This means the progress of cataracts

was attenuated in the test group compared to others as evaluated using slit lamp examination, which is in agreement with ultrasound results in this study.

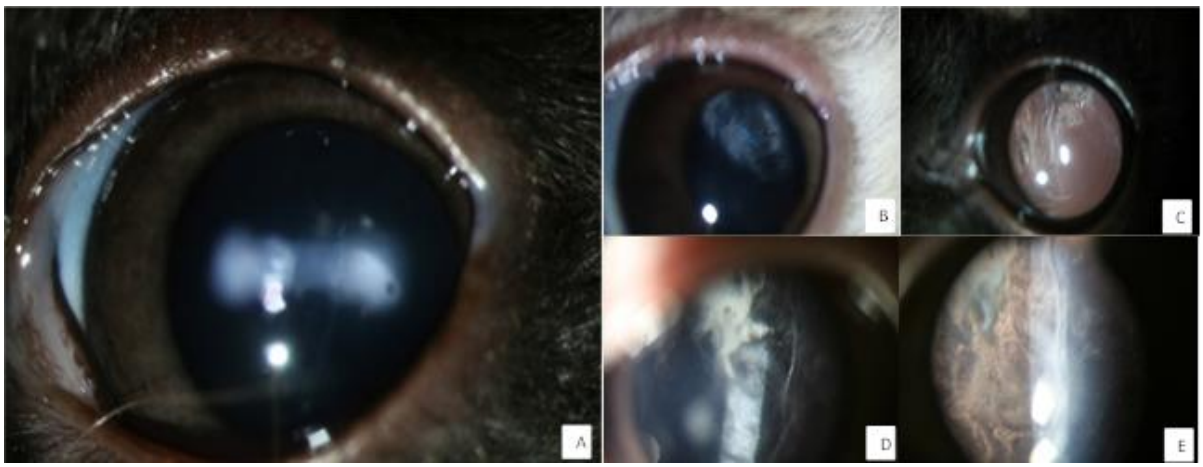
Currently, surgery is considered to be the only definitive treatment for cataracts. It is estimated that a 10-year delay in the onset of cataracts can result in a 50% decrease in the necessity of surgical intervention. Reports indicate that reactive oxygen species (ROS) have a profound effect on eye health, and may contribute to the incidence of cataracts (Vogel et al. 1975, 25).

Lower levels of blood plasma antioxidant agents, such as α-tocopherol, ascorbic acid, uric acid, and glutathione, are associated with lower antioxidant potential of blood plasma and higher incidence of cataracts. Many reports also indicate that oxidative stress is an important factor in the incidence of diabetic cataracts (63, 53). Consequently, recent investigations evaluating the effects of ALA (an antioxidant agent) on diabetic cataracts induced using STZ in rabbits, have gained attention (Kılıç et al. 2007, 4). In his work, Williams (2017) showed that oral administration of 2 mg/kg ALA to dogs suffering from diabetic cataracts, prevents further progress into later stages of the disease (Williams 2017, 4).

Eman et al. (2011) also proved that ALA is effective in preventing and slowing down the progress of cataracts in rabbits (Eman & Eman 2011, 21). Demir et al. (2005) also concluded that ALA could provide effective protection against free radicals produced as a result of ultraviolet radiation (Demir et al. 2005, 219).

Conclusion:

This study indicates that the administration of ALA is effective in protecting the eyes against cataracts in rabbits. It is therefore recommended that the effect of different doses of ALA in different animal models be evaluated in future studies. For ultrasound evaluation, comparing B mode results with A mode results is recommended.



E: grade 4, D: grade 3, C: grade 2, B: grade 1, A: grade 0 / Figure 3-8: Grading of lens turbidity

Figure 1: Different grades of lens turbidity. A) 0, B) 1, C) 2, D) 3, E) 4

Table 1: Incidence of varying grades of cataracts in the negative control group

	Day 4	Day 8	Day 11	Day 14	Day 17	Day 20
Grade 1 cataracts	6 cases (100%)	4 cases (33.3%)	0	0	0	0
Grade 2 cataracts	0	4 cases (33.3%)	5 cases (83.3%)	5 cases (83.3%)	3 cases (50%)	3 cases (50%)
Grade 3 cataracts	0	0	1 case (16.7%)	1 case (16.7%)	3 cases (50%)	3 cases (50%)

Table 2: Incidence of varying grades cataracts in positive control group

	Day 4	Day 8	Day 11	Day 14	Day 17	Day 20
Grade 1 cataracts	6 cases (100%)	3 cases (50%)	0	0	0	0
Grade 2 cataracts	0	1 case (16.7%)	4 cases (66.7%)	4 cases (66.7%)	2 cases (33.3%)	2 cases (33.3%)
Grade 3 cataracts	0	2 cases (33.3%)	2 cases (33.3%)	2 cases (33.3%)	4 cases (66.7%)	4 cases (66.7%)

Table 3: Incidence of varying grades of cataracts in the test group (ALA treatment)

	Day 4	Day 8	Day 11	Day 14	Day 17	Day 20
Grade 1 cataracts	0	3 cases (50%)	4 cases (66.7%)	4 cases (66.7%)	4 cases (66.7%)	4 cases (66.7%)
Grade 2 cataracts	0	0	0	0	0	0
Grade 3 cataracts	0	0	0	0	0	0

Table 4: Statistical comparison between groups regarding the presence or absence of cataracts

		Cataracts		Total number	P value Chi-square
		No	Yes		
Groups	Test	2	4	6	0.074
	Positive control	0	6	6	
	Negative control	0	6	6	
Total number		2	16	18	

Table 5: Statistical comparison between groups regarding the presence or absence of mild cataracts

		Mild cataracts		Total number	P value Chi-square
		No	Yes		
Groups	Test	2	4	6	0.007
	Positive control	6	0	6	
	Negative control	6	0	6	
Total number		14	4	18	

Table 6: Statistical comparison between groups regarding the presence or absence of grade 2 and grade 3 cataracts

		Grade 2 or grade 3 cataracts		Total number	P value Chi-square
		No	Yes		
Groups	Test	6	0	6	0.00
	Positive control	0	6	6	
	Negative control	0	6	6	
Total number			12	18	

Table 7: Mean changes in ultrasound measurements in the positive control group (in centimeters)

Day	Mean thickness of the anterior capsule	Mean thickness of the posterior capsule	Mean anterior-posterior diameter of the lens	Slit lamp results
0	0.034	0.034	0.589	Completely healthy
1				
2				
3				
4	0.043	0.046	0.597	All cases diagnosed with grade 1 cataracts
5	0.055	0.056	0.545	-
6	0.063	0.064	0.613	-
7	0.057	0.061	0.594	-
8	0.067	0.067	0.553	All cases diagnosed with grade 1, grade 2, and grade 3 cataracts
9	0.067	0.067	0.615	-
10	0.067	0.067	0.618	-
11	0.067	0.067	0.621	All cases diagnosed with grade 2 and grade 3 cataracts
12	0.067	0.067	0.623	-
13	0.066	0.067	0.632	-
14	0.066	0.067	0.625	All cases diagnosed with grade 2 and grade 3 cataracts
15	0.066	0.067	0.631	-
16	0.066	0.067	0.635	-
17	0.066	0.067	0.635	All cases diagnosed with grade 2 and grade 3 cataracts
18	0.066	0.067	0.637	-
19	0.066	0.067	0.639	-
20	0.066	0.067	0.641	All cases diagnosed with grade 2 and grade 3 cataracts

Table 8: Mean changes in ultrasound measurements in the negative control group (in centimeters)

Day	Mean thickness of the anterior capsule	Mean thickness of the posterior capsule	Mean anterior-posterior diameter of the lens	Slit lamp results
0	0.035	0.035	0.583	Completely healthy

1				
2				
3				
4	0.044	0.044	0.589	All cases diagnosed with grade 1 cataracts
5	0.056	0.059	0.619	-
6	0.061	0.056	0.614	-
7	0.057	0.058	0.605	-
8	0.060	0.056	0.615	All cases diagnosed with grade 1 and grade 2 cataracts
9	0.067	0.069	0.619	-
10	0.067	0.069	0.620	-
11	0.064	0.067	0.621	All cases diagnosed with grade 2 and grade 3 cataracts
12	0.061	0.068	0.617	-
13	0.067	0.068	0.619	-
14	0.066	0.067	0.617	All cases diagnosed with grade 2 and grade 3 cataracts
15	0.066	0.066	0.621	-
16	0.066	0.066	0.623	-
17	0.066	0.066	0.623	All cases diagnosed with grade 2 and grade 3 cataracts
18	0.066	0.066	0.621	-
19	0.066	0.066	0.615	-
20	0.066	0.066	0.615	All cases diagnosed with grade 2 and grade 3 cataracts

Table 9: Mean changes in ultrasound measurements in the test group (in centimeters)

Day	Mean thickness of the anterior capsule	Mean thickness of the posterior capsule	Mean anterior-posterior diameter of the lens	Slit lamp results
0	0.034	0.035	0.590	Completely healthy
1				
2				
3				
4	0.046	0.045	0.629	Completely healthy
5	0.053	0.058	0.635	-
6	0.063	0.063	0.640	-
7	0.059	0.056	0.642	-
8	0.57	0.57	0.634	Some cases diagnosed with grade 1 cataracts
9	0.062	0.60	0.639	-
10	0.062	0.60	0.642	-
11	0.062	0.60	0.646	Some cases diagnosed with grade 1 cataracts
12	0.059	0.057	0.643	-
13	0.059	0.058	0.660	-
14	0.059	0.058	0.659	Some cases diagnosed with grade 1 cataracts

15	0.059	0.058	0.657	-
16	0.058	0.058	0.665	-
17	0.058	0.058	0.665	Some cases diagnosed with grade 1 cataracts
18	0.058	0.058	0.665	-
19	0.058	0.058	0.665	-
20	0.058	0.058	0.665	Completely healthy to grade 1 cataracts

References:

[1] Delcea, C., Bululoi, A. S., Gyorgy, M., & Rad, D. (2023). Psychological distress prediction based on maladaptive cognitive schemas and anxiety with random forest regression algorithm. *Pharmacophore*, *14*(5-2023), 62-69.
[2] Delcea, C., Bululoi, A. S., Gyorgy, M., & Siserman, C. V. (2023). Medico-legal approach to incestuous sexual orientation in men. *Archives of Pharmacy Practice*, *14*(4-2023), 69-74.
[3] Demir, Ü., Demir, T., & Ilhan, N. (2005). The protective effect of alpha-lipoic acid against oxidative damage in rabbit conjunctiva and cornea exposed to ultraviolet radiation. Ophthalmologica, volume 219, issue 1.
[4] Eman, M. A., & Eman, S. M. (2011). The role of alpha-lipoic acid in streptozotocin-induced diabetic cataracts. Rom. J. Biophys, volume 21, issue 4.
[5] Hogan, M. J., Alvarado, J. A., Weddell, J. E. (1971). Histology of the Human Eye: An Atlas and Textbook. United Kingdom: Saunders.
[6] Kabir, Farrokh Reza. 2018. Study of ultrasound changes of intravitreal injection of silymarin in preventing the development of experimental cataracts in rabbits. PhD dissertation in veterinary radiology. Faculty of Veterinary Medicine. University of Tehran.
[7] Kılıç, A., Selek, Ş., & Erel, Ö. (2007). Effects Of Erdosteine On Oxidative-Antioxidative Equilibrium And On Cataracts Formation In Rat Pups With Selenite-Induced Cataracts. European Journal of General Medicine, volume 4, issue 4.
[8] Lettéron, P., Labbe, G., Degott, C., Berson, A., Fromenty, B., Delaforge, M., ... & Pessayre, D. (1990). Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice: evidence that silymarin acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochemical pharmacology, volume 39, issue 12.
[9] Maggs, D. J., Miller, P. E., Ofri, R., Slatter, D. H. (2008). Slatter's Fundamentals of Veterinary Ophthalmology. Switzerland: Saunders Elsevier.
[10] Mourelle, M., Muriel, P., Favari, L., & Franco, T. (1989). Prevention of CCl4-induced liver cirrhosis by silymarin. Fundamental & clinical pharmacology, volume 3, issue 3.
[11] Nautrup, C. P., Tobias, R. (2000). An Atlas and Textbook of Diagnostic Ultrasonography of the Dog and Cat. Germany: Manson Publishing Limited.
[12] Scott Luper, N. D. (1998). A review of plants used in the treatment of liver disease: part 1. Alternative medicine review, volume 3, issue 6.
[13] Varma, S. D., Devamanoharan, P. S., & Morris, S. M. (1995). Prevention of cataractss by nutritional and metabolic antioxidants. Critical Reviews in Food Science & Nutrition, Volume 35, issue 1-2.
[14] Vică, M. L., Delcea, C., Dumitrel, G. A., Vușcan, M. E., Matei, H. V., Teodoru, C. A., & Siserman, C. V. (2022). The influence of HLA alleles on the affective distress profile. *International Journal of Environmental Research and Public Health*, *19*(19), 12608.
[15] Vogel, G., Trost, W., Braatz, R., Odenthal, K. P., Brüsewitz, G., Antweiler, H., & Seeger, R. (1975). Pharmacodynamics, site and mechanism of action of silymarin, the antihepatoxic principle from Silybum mar.(L) Gaertn. 1. Acute toxicology or tolerance, general and specific (liver-) pharmacology. Arzneimittel-forschung, Volume 25, issue 1.
[16] Williams, D. L. (2017). Effect of oral alpha lipoic acid in preventing the genesis of canine diabetic cataracts: a preliminary study. Veterinary sciences, Volume 4, issue 1.