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STUDY TITLE: A PRECLINICAL INVESTIGATION TO EVALUATE THE EFFECTIVENESS OF AYURVEDIC INTERVENTION IN KAPHAJA LINGANASHA (CATARACT)

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ABSTRACT

Background: This study investigates the potential anticataract effects of Netraum Anjan eye drops as an alternative treatment for cataracts, a leading cause of blindness worldwide. The study evaluates the efficacy of Netraum Anjan eye drops in a controlled rat model, providing insights into its therapeutic potential for cataract management.

Objective: The objective is to evaluate the cataract preventive action of the test substance Netraum Anjan against Naphthalene-induced experimental Rats.

Materials and Methods: Forty albino Rats in five groups were used to study anticataract activity. Netraum Anjan Eye Drop (single and double application) and Vitamin E (50 mg/kg) served as standards, while normal saline was the control. The test substance was administered with naphthalene for 42 days to induce cataracts (0.5 g/kg for 3 days, then 1 g/kg for 42 days).

Results: Forty Wistar albino rats were divided into five groups to assess the anticataract effect of Netraum Anjan Eye Drop against naphthalene-induced cataracts. Naphthalene administration for 42 days depleted glutathione (GSH) levels, causing oxidative stress and cataract formation. Treatment with Vitamin E (50 mg/kg) and low- and high-dose Netraum Anjan Eye Drop significantly restored GSH levels compared to the pathological control.

Conclusion: This study concludes that Netraum Anjan eye drops significantly inhibit cataract development. The findings suggest that the test substance offers promising cataract prevention and management potential.

Keywords: Cataract, Anjan, Naphthalene-induced cataracts, Eye drop

INTRODUCTION

Modern medical science offers several solutions for medical cures, with eye lens surgery being the most popular. Three main types of cataract treatment are available: Phacoemulsification, femtosecond laser-assisted cataract surgery, and extracapsular cataract extraction. [1] Phacoemulsification, a cutting-edge surgical technique, is highly favored due to its high success rate. However, cases have been reported where this surgery can lead to permanent damage to the eye lens, posing a significant risk of permanent blindness if not properly administered. Additionally, other post-surgery issues such as permanent blindness and unclear vision have been identified. [2]

Currently, several eye diseases affect living organisms, including astigmatism, color blindness, dry eye, floaters, amblyopia, diabetic retinopathy, cataracts, and others. Of these, cataracts pose a significant concern, with their prevalence on the rise. According to the National Institute of Health (NIH), cataracts account for 50–80% of cases of bilateral blindness. The primary symptom of cataracts is cloudiness in the eye lens, leading to impaired vision and the inability to see objects clearly. [3]
In the human anatomy, various structures serve specific functions to ensure the proper functioning of the body. Among these, the eye stands out as a major component, providing vision by capturing reflections of objects through light. [4] In the realm of Ayurvedic medicine, alternative solutions for treating cataracts are emerging, with effective eye drops being among them. [5] Consequently, the human brain can form a clear picture of the object and store it for future recognition. However, eye-related diseases are increasingly common in today's society. While traditionally associated with older individuals, these issues are now prevalent among younger generations and children as well. [6]
The study focuses on the utilization of Netraum Anjan eye drops for treating cataracts. The experiments were conducted under controlled conditions, using rats as experimental subjects. The rats were divided into specific groups, and their diets were carefully measured and administered according to the experimental protocols. The treatment of the eyes was conducted under various conditions, forming a significant aspect of this study.

MATERIALS AND METHODS

Materials: Netraum Anjan Eye Drop has been used to treat cataract issues, and this item was received from Netraum Ayurveda. The ingredients stated are Cleome viscose, Peppermint, and camphor are sourced from Oorja Firm, Raipur. Rewrite the sentence: The water and food used to feed the test subjects are sourced from standard cow ghee and obtained from the local market.
Husbandry: The experiments on the test objects were done highly precisely and in an organized manner, and those characteristics were discussed in subsequent sections.
Animal Welfare: The animal experiment was conducted according to the guidelines of a committee for the control and supervision of experiments on animals (CPCSEA Registration Number-1803/PO/RcBi/S/2015/CPCSEA).
Animal Housing Conditions: Animals were housed under a temperature of 22 ± 3°C, relative humidity of 30-70%, 12 hours light and 12 hours dark cycle. Animals were housed in a standard polypropylene cage with a stainless-steel top grill having facilities for pelleted food and drinking water in bottles. Sterile Corncob was used as bedding material and changed every day.
Feed and Water: A normal cow diet (Purina Lab diet 5L79 Rat 18%) (PMI Nutrition International) was provided to all the animals throughout the experiment. Fresh water was provided ad libitum. Animals were provided access to fresh, potable, and uncontaminated drinking water. Periodic monitoring of microbial contamination of water was done. Drinking water bottles and their tubes were examined routinely to ensure their proper operation.
Randomization: Each animal was marked with picric acid, and numbering was given individually to each animal. Each cage was numbered separately to identify the group. All animals were randomized based on their body weight.
Animal Care: All procedures involving animals were conducted humanely and were performed by or under the direction of an expert supervisor. The study commenced after the protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of *Radiant Research Services Pvt. Ltd., Bangalore, India*.
Pain or Distress Category: The animals were treated or euthanized according to professional judgment, and these have already been discussed in previous literature. Treatment or euthanasia was based upon the circumstances and the CPCSEA Pain, Distress, and Protocol Guidance.
Dose, Route, number and Species: The rat species are accepted by regulatory authorities for this type of study. The number of animals to be used in this study is the minimum needed for this type of memory-enhancing study. The number of animals to be used in the study is also appropriate for statistical analyses (ANOVA, Dunnett’s Test. etc.) of the data generated from the study. The dose

levels were selected based on requirements (Table 1). The ocular route is the intended clinical route of administration.

Table 1. Analysis parameters of the study

Sl. No.	Factors	Analysis Parameters
1	Species and strain	Rat Swiss Albino Wistar
2	Sex	Male
3	Age	8 to 10 weeks
4	Diet	Normal cow diet

Test items, vehicle and formulation details: Several factors were highly important during the performance of the experimental protocols. These particular factors are mentioned in Table 2. Moreover, five major factors are here that are responsible for the variation of the results, such the test item, dose, route, frequency, and vehicle. However, the vehicle (normal saline) played a major role here because normal saline was universally accepted and routinely used as an oral route for animal studies. The test substance forms a uniform suspension in normal saline, as evidenced by the in-house suspend ability test.

Table 2. Major factors that are applied in the experimental protocol.

Sl. No.	Factor	Effective Parameter
1	Test item	Netraum Anjan Eye drop
2	Dose	Single and Double Application
3	Route	Ocular
4	Frequency	Daily
5	Vehicle	Normal Saline

Grouping and Allocation: An experimental model of cataracts was induced in rats by feeding naphthalene at a dose of 0.5 gm/kg/day p.o for the first three days and 1 g/kg/day p.o thereafter for a period of 42 days. All male rats (a total of 40 rats) were randomly divided into five groups consisting of eight rats in each group. The normal control group (G1) received normal saline, the Pathological control group (G2) received naphthalene solution 0.5 gm/kg/day p.o for the first three days and l g/kg/day p.o thereafter, Reference standard group (G3) was received Vitamin E 50 mg/kg/day p.o, along with naphthalene Group 4 was treated with the test substance at single application by ocular route along with naphthalene and group 5 will be treated with the test substance at double application by ocular route along respectively simultaneously with naphthalene. All the groups were treated for 42 days. On day 42 cataract was examined under a slip lamp (Table 3). On day 43 lenses were removed from the eyes of all the animals for estimation.

Table 3. Grouping and allocation of the animals

Group	Group descriptions	Dose level	No. of animals
G1	Normal control	Normal saline	8
G2	Pathological control	Normal saline	8
G3	References standard (vitamin - E)	50 mg/Kg	8
G4	Test substances Low dose	Single application	8
G5	Test substances High dose	Double application	8

Observations

Body Weight: Individual body weights were recorded at the receipt, on the day of randomization, on the first day of treatment before dosing (Day 1) and weekly thereafter. The body weight changes for all the animals were calculated arid reported along with the body weight data.

Examination of the Eye: The eyes of the rats were examined using an ophthalmoscope for morphological changes in the lens, on the first day of treatment before dosing (Day 1) and weekly thereafter.
Statistical analysis: All data including body weight and biochemical parameters were statistically analyzed using Graph-Pad Prism Software, version 5.01. All va1ues were expressed as Mean ± SD. The significant difference between the treatment and control group was estimated using one-way ANOVA with Dunnett's test. All results of the statistical analysis were summarized in separate tables. In any case, the values were considered statistically significant at $P<0.05$.
Biochemicals: Naphthalene has been utilized as a biochemical substance that helps to create cataract conditions in the selected test objects. Additionally, five other biochemicals have been utilized to analyze object observation conditions: lens protein, lens water content, glutathione, reductase, and nitric oxide. This substance was analyzed in the all-five object group condition.

RESULTS

Body Weight: As shown in Figure 1, when the time was moving ahead, the body weights of all the object groups were seen in increasing order. In the initial time, the weights of all the test group average weights were minimum, and at the end of the final weights, the weights of all the test group average weights were maximum. This can be possible due to the natural growing condition of the test objectives, and the second most important reason is to provide the optimum amount of diet materials so that the weights of the body of the objects have been affected by consuming diet.

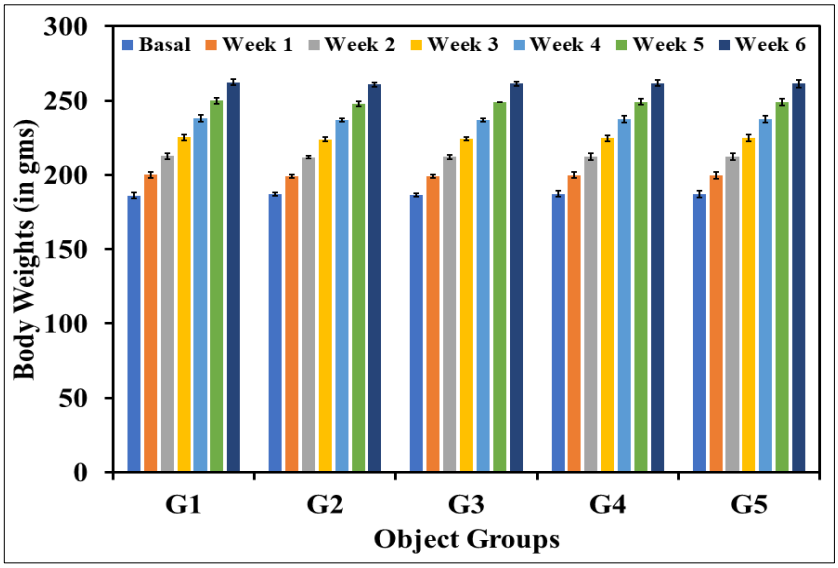

Figure 1: Effect of test substance on rat body weight

Analysis of Biochemical: The result of this inhibitory action of test substances against naphthalene-induced cataract rats. The treated group's body weight did not show significant changes from basal body weight to week 6 body weights when compared to the Pathological control group shown in Figure 1. The effect of the test substance on lens protein and water content on naphthalene-induced cataracts in rats is shown in Figure 2. The lens protein and lens water content of pathological control animals showed a significant decrease when compared to the normal control group. Group 3 (vitamin E (50 mg/kg)), Group 4 (Low dose) and Group S (High dose) showed a significant increase in the protein and lens water content as compared to the pathological control group. The result indicates that there was a reduction in the glutathione levels in the pathological control group when compared to that of the normal control group.

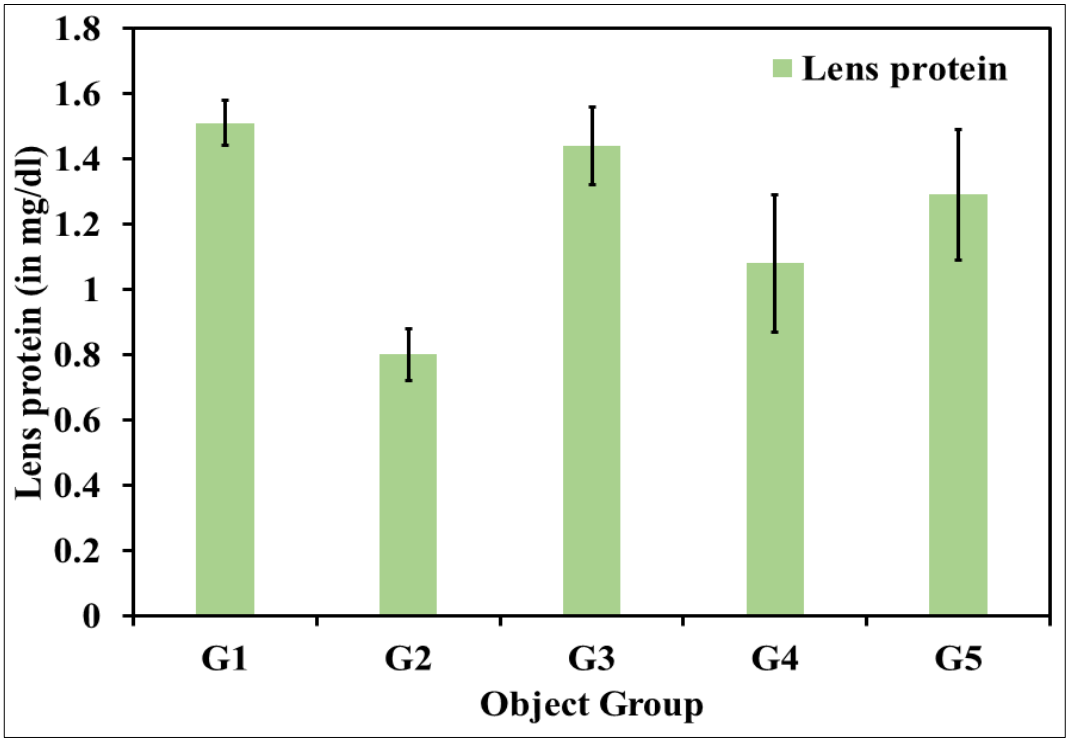

Figure 2: Lens Protein in pathological control vs normal control group

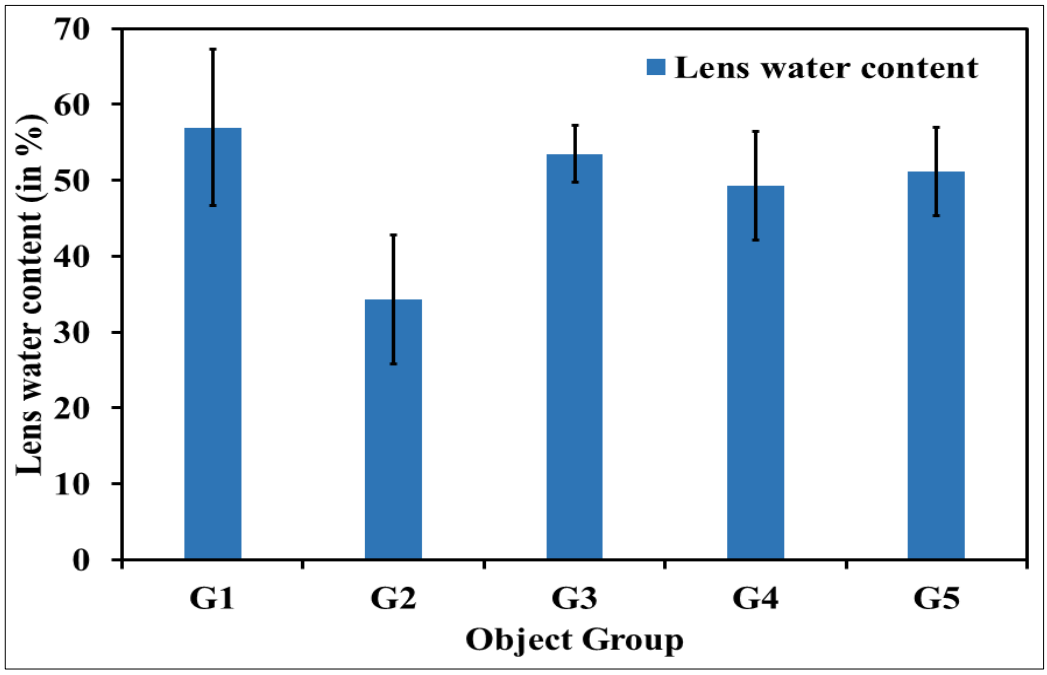

Figure 3: Lens Water Content in pathological control vs normal control group

Group 3 (vitamin E (50 tug/kg)), Group 4 (Low dose) and Group S (High dose) showed a significant increase in the glutathione as compared to the pathological control group as shown in Figure 4.

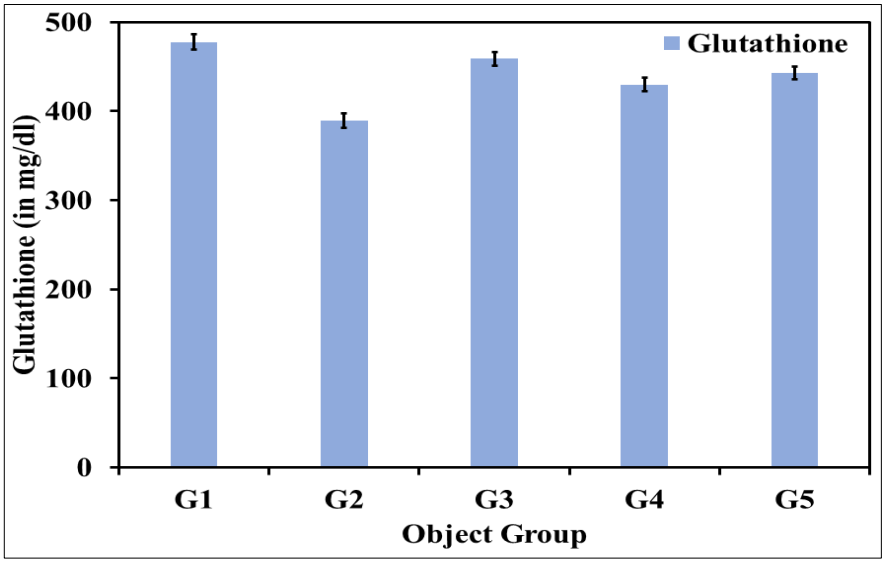

Figure 4: Glutathione in pathological control vs normal control group

Elevated level of aldose reductase in pathological control group rats compared to the normal control group. Group 3 (Vitamin E 50 mg/kg), Group 4 (Low dose) and Group 5 (High dose) showed a significant decrease in the aldose reductase as compared to the pathological control group as shown in Figure 5.

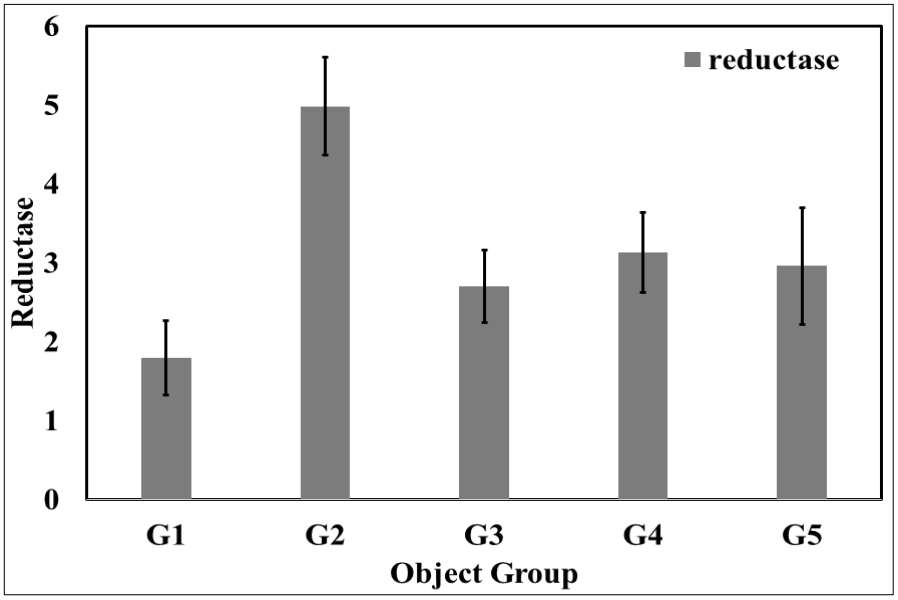

Figure 5: Aldose reductase in pathological control vs normal control group

An elevated level of nitric oxide in normal control group rats compared to the pathological control group. Group 3 (Vitamin E 50 mg/kg), Group 4 (Low dose) and Group 5 (High dose) showed a significant increase in the nitric oxide as compared to the pathological control group as shown in Figure 6.

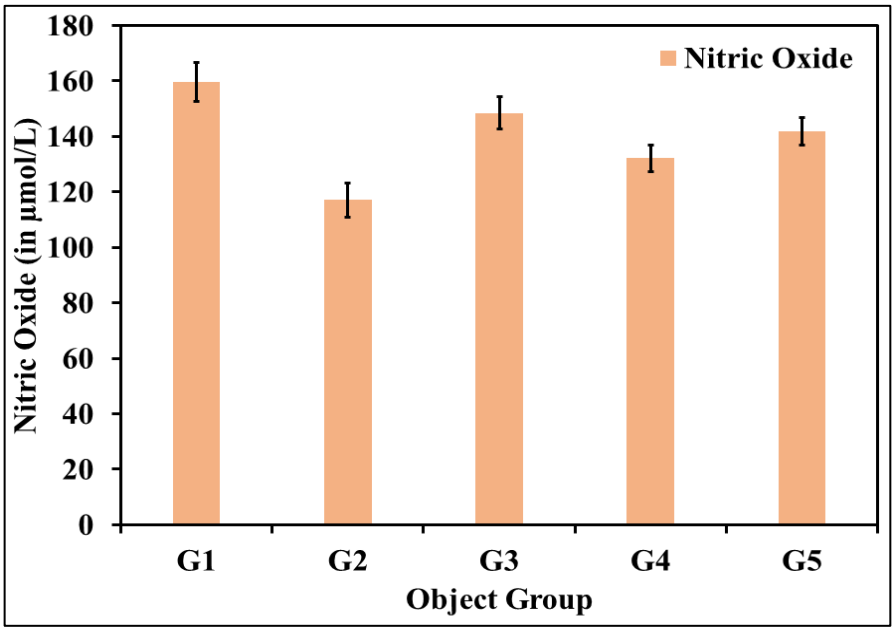

Figure 6: Nitric Oxide in pathological control vs normal control group

DISCUSSION

Naphthalene-induced cataracts have been extensively used to test potential anti-cataract drugs.[7] Because the morphology as well as toxic manifestations of naphthalene induced cataract is reported to be similar to that of age-related cataracts, naphthalene cataract genesis in rats has been used as a valuable animal model to study the etiology of age-related cataracts in humans.[8] Ingested naphthalene is metabolized in the liver to the stable compound naphthalene-1,2- dihydrodiol and it is further metabolized to NQ by an enzyme dihydrodiol dehydrogenase that can quickly react with glutathione or protein sulfhydryl groups and cause its alkylation.[9] This led to the formation of disulfide bridges causing precipitation of high molecular weight protein, hence opalescence in the lens. This NQ formation is considered the underlying mechanism of cataract development in naphthalene-fed animals.

Aldose reductase is the key enzyme for the metabolism of naphthalene-1,2-dihydrodio1 in the process of naphthalene cataract development.[10] Oxidative stress is caused by an imbalance between the production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.[11] Glutathione is an extremely important cell antioxidant. It is known to directly quench reactive hydroxyl free radicals and other oxygen-centered free radicals. In healthy cells and tissue, more than 90% of the total glutathione is in the reduced (GSH) state and less than 10% exists in the disulfide form (GSSG).[12] In this study the reduced activity of reduced GSH content implicated in damage to the lens during cataract development resulting from overconsumption of naphthalene.

The lens is affected by an increase in oxidative stress around the lens protein which gradually loses in transparency and turns it opaque. The aggregation of lens protein and disruption of normal lens cell structure occurs due to post-translation protein modification that leads to lens opacification. An increase in the protein content indicates an improvement in the cataract genic condition and dehydration of the lens in association with increasing hardness. Water in the crystalline lens may be in a free state or be bound to the protein molecules. The relative dehydration demonstrated by this study likely reflects the loss of both proteins bound and free water within the nucleus. Reduced water content is probably associated with changes in the interaction between protein chains as well as increased protein concentration resulting in hardness.

CONCLUSION

It can be concluded that the test substance (Netraum Anjan Eye Drop) at the dose levels Low and high dose exhibited a significant effect of inhibitory action on cataracts when compared to pathological control (Group 2). This indicates that the test substance provides a favourable effect in preventing or inhibiting cataracts.

REFERENCES

1. Hatch KM, Schultz T, Talamo JH, Dick HB (2015) Femtosecond laser-assisted compared with standard cataract surgery for removal of advanced cataracts. J Cataract Refract Surg 41:1833–1838.
2. Lin Q, Lim JYC, Xue K, et al (2021) Polymeric hydrogels as a vitreous replacement strategy in the eye. Biomaterials 268:120547.
3. Lohia K, Soans RS, Agarwal D, et al (2023) Stereopsis following surgery in children with congenital and developmental cataracts: A systematic review and meta-analysis. Surv Ophthalmol 68:126–141
4. Moreno-Arjonilla J, López-Ruiz A, Jiménez-Pérez JR, et al (2024) Eye-tracking on virtual reality: a survey. Springer London
5. Pokkalath AS, Sawant A, Sawarkar SP (2022) Herbal medicine for ocular diseases: An age-old therapy and its future perspective. J Drug Deliv Sci Technol 68:102979.
6. Wolffsohn JS, Lingham G, Downie LE, et al (2023) TFOS Lifestyle: Impact of the digital environment on the ocular surface. Ocul Surf 28:213–252.
7. Khadka A, Brashier DB, Sharma AK, Dahiya N, Gupta AK, Sharma S. Am Experimental Study to Evaluate Protective Effects of Naproxen and Valdecoxib on Naphthalene induced Cataract in Albino Rats. J. Drug Delivery Ther. 2014 Jul. 14; 4(4):34-7.
8. Xu GT, Zigler JS Jr, Lou MF. The possible mechanism of naphthalene cataract in rat and its prevention by an aldose reductase inhibitor (ALO1576). Exp Eye Res. 1992;54(1):63-72.
9. Tomoyuki, Terada. (2005). Role of Glutathione S-Transferases in Lens under Oxidative Stress. Journal of Health Science - J HEALTH SCI. 51. 263-271. 10.1248/jhs.51.263.
10. Sugiyama K, Wang TC, Simpson JT, Rodriguez L, Kador PF, Sato S. Aldose reductase catalyzes the oxidation of naphthalene-1, 2-dihydrodiol for the formation of ortho-naphthoquinone. Drug Metab Dispos. 1999;27(1):60-67.
11. Henkler, F., Brinkmann, J., & Luch, A. (2010). The role of oxidative stress in carcinogenesis induced by metals and xenobiotics. Cancers, 2(2), 376–396.
12. Appala RN, Chigurupati S, Appala RV, Krishnan Selvarajan K, Islam Mohammad J. A Simple HPLC-UV Method for the Determination of Glutathione in PC-12 Cells [retracted in: Scientifica (Cairo). 2022 Feb 24;2022:9781919. doi: 10.1155/2022/9781919]. Scientifica (Cairo). 2016;2016:6897890.