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Formulation and Evaluation of a Herbal-based Ophthalmic Solution Containing Sodium Hyaluronate and Triphala for the Treatment of Computer Vision Syndrome

Hrithik Satish Revankar*, Shilpa Bhilegaonkar

Department of Pharmaceutics, PES Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi, Goa, India. *Corresponding Author's Email: hrithikrevankar2000@gmail.com

Abstract

Computer Vision Syndrome is an emerging ocular condition resulting from prolonged and continuous exposure to screen. Despite its increasing prevalence in today's digital league, the condition goes underdiagnosed as symptoms such as irritation of the eye, redness and dryness can be managed symptomatically, while the underlying light -induced ocular damage remains largely overlooked. Existing therapeutic options in Allopathy and Ayurveda provide temporary or non-specific relief, highlighting the need for a more specific approach. To address this gap, through this study a novel integrated approach merging Allopathic and Ayurvedic principles of healing. The formulation utilizes Triphala, recognized for its antioxidant, anti-inflammatory and rejuvenating properties, combined with sodium hyaluronate, an established ocular lubricant and a natural ocular component to specifically manage and treat computer vision syndrome. The dosage form was designed with an emphasis on enhanced therapeutic efficiency, improved patient compliance, ease of manufacturing and strong potential for large scale commercialization. Detailed physicochemical, stability and pharmacological evaluations confirmed the reliability of the formulation. *In vitro* permeation studies using Franz diffusion cells demonstrated efficient and favorable ocular penetration. Further histopathological findings carried out on goat corneal tissue, serving as a validated human corneal model, showed significant healing, regenerative and protective effects against light induced ocular damage. Overall, the findings strongly uphold the formulations potential as a targeted, novel and commercially viable therapy for computer vision syndrome

Keywords: Cornea, Screen, Sodium hyaluronate, Triphala, Vitreo-retinal disease

Introduction

The extensive integration of digital technologies into occupational educational and personal environments has markedly increased daily exposure to visual display terminals. Prolonged interaction with computers, smartphones and other digital devices has led to the emergence of Computer Vision Syndrome, a prevalent yet under-recognized ocular condition. CVS is not a discrete pathological entity but a multidimensional syndrome encompassing a spectrum of functional, visual and ocular surface disturbances resulting from sustained near-work and screen related visual stress. Clinically CVS manifests initially as symptoms such as ocular fatigue, burning sensation, redness, irritation, dryness foreign body sensation and intermittent blurred vision. These manifestations are primarily associated with reduced blink rate, tear film instability, accommodative stress and prolonged exposure to high energy visible light emitted from screens. If left unaddressed, chronic exposure may exacerbate ocular strain and has been associated with more serious visual complications, including progressive myopia, vitreous floaters, posterior vitreous detachment and in severe cases retinal complications. These outcomes highlight the progressive nature of CVS and underscore the need for early and mechanistically targeted interventions (1, 2).

Current allopathic management of CVS predominantly emphasizes symptomatic alleviation, employing artificial tears, lubricating eye drops, and anti-inflammatory agents. While these approaches provide transient relief, they do not sufficiently mitigate the underlying biochemical and cellular mechanisms, particularly oxidative stress and chronic low-grade inflammation induced by prolonged screen exposure. Additionally, the symptomatic overlap of CVS with other ocular surface disorders such as

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dry eye disease, allergic conjunctivitis or blepharitis often leads to misdiagnosis thereby limiting treatment strategies to nonspecific symptomatic management rather than addressing the etiological basis of the condition. Accumulating evidence suggests that oxidative stress plays a central role in the pathophysiology of CVS with excessive screen exposure promoting the generation of reactive oxygen species (ROS) that damage corneal and conjunctival epithelial cells. This oxidative vandalism contributes to tear film instability, epithelial disruption, and inflammatory cascades. Despite this understanding most commercially available ophthalmic formulations lack antioxidant components thereby restricting their effectiveness in preventing or reversing screen-induced ocular damage. This represents a critical limitation in existing therapeutic approaches (3, 4).

Traditional Ayurvedic medicine describes various herbal preparations for ocular disorders and visual fatigue, many of which exhibit antioxidant and anti-inflammatory properties. However, conventional Ayurvedic ophthalmic therapies often involve elaborative procedures, limited formulation standardization, and challenges related to sterility and patient compliance, which restrict their acceptance in contemporary ophthalmic practice. Consequently, there is increasing interest in integrating herbal bioactive into modern pharmaceutical dosage forms to enhance therapeutic efficacy while ensuring safety and reproducibility. Sodium hyaluronate, a naturally occurring glycosaminoglycan and a physical constituent of the vitreous humor and tear film, is widely employed in ophthalmic formulations due to its viscoelastic, mucoadhesive and hydrating properties. It improves ocular surface lubrication, enhances tear film stability and promotes epithelial repair. Owing to its biocompatibility and presence in ocular tissues, sodium hyaluronate represents a scientifically justified excipient for ophthalmic use. However, its therapeutic action is primarily supportive, and lacks inherent anti-oxidant or anti-inflammatory activity (5, 6).

Triphala, a classical Ayurvedic polyherbal formulation consisting of *Emblica officinalis, Terminalia chebula* and *Terminalia bellirica* has been extensively reported to possess potent antioxidant, anti-inflammatory and free radical scavenging activities. Attributed to its rich content of phenolic compounds, flavonoids, tannins and gallic acid. Previous studies have demonstrated its protective effects against oxidative stress-mediated tissue damage, thus suggesting its potential utility in conditions where oxidative mechanisms predominate. Nevertheless, its applications in sterile, standardized ophthalmic formulations remains insufficiently explored. Despite the individual therapeutic relevance of lubricating polymers and herbal antioxidants there exists a notable research gap in the development of an ophthalmic formulation that simultaneously provides ocular lubrication, antioxidant protection and anti-inflammatory effects in the management of CVS. To date, limited studies have investigated the synergistic integration of sodium hyaluronate with standardized herbal extracts in ophthalmic solutions designed specifically for screen induced ocular damage (7).

The aim of this study was to develop and evaluate a novel ophthalmic solution incorporating sodium hyaluronate and Triphala extract for the management of Computer Vision Syndrome. The specific objectives included formulation development, physicochemical and analytical characterization, qualitative and quantitative evaluation of active constituents, assessment of antioxidant and anti-inflammatory activity using established *in vitro* models, evaluation of *in vitro* drug release, histopathological examinations of corneal tissues for protective effects, and sterility testing to ensure ophthalmic suitability. The novelty of this work lies in the rational combination of a well-established ophthalmic polymer with a traditional polyherbal formulation into a single, sterile and standardized ophthalmic dosage form. This integrative approach is designed not only to provide symptomatic relief but also to address key pathogenic mechanisms of CVS, thereby offering a scientifically validated strategy that bridges traditional medicine and modern pharmaceutics

Methodology

A variety of chemicals and equipment were utilized in this research. Triphala powder was purchased from Baidyanath Ayurveda Pvt Ltd, Sodium Hyaluronate was sourced from Aseschem Chemical Works Jodhpur India, Sodium hydroxide, Sodium dihydrogen Phosphate, Sodium chloride,

Sodium bicarbonate, D-Glucose, Potassium chloride, Magnesium chloride, Calcium chloride was purchased from Molychem Laboratory Reagents and Fine Chemicals.

Formulation

a) Preparation of Aqueous Extract of Triphala

Triphala powder sourced locally was accurately weighed for six grams and was transferred in a round-bottom flask. 90 mL of distilled water and 10 mL of ethanol was added to the round bottom flask and the mouth of the round bottom flask was sealed using aluminium foil and was left undisturbed in a dry environment for two days. The rationale behind leaving the powder soaked in hydro-alcoholic mixture is to completely extract all the water soluble and alcohol soluble active components from the Triphala powder (8).

After soaking it completely for 2 days the round bottom flask along with the contents were attached to a reflux system and was heated at 60-70 degrees Celsius for about 30 minutes, after cooling it down the contents were heated on a medium flame on a water bath for 5 minutes to evaporate the ethanol, now the contents of the RBF were filtered using Whatman filter paper and the obtained liquid is stored in a sterile glass container. The whole process beginning with weighing of ingredients to filtration is carried out in a sterile environment to avoid any contamination.

b) Preparation of 0.1% Sodium Hyaluronate Solution

Sodium hyaluronate powder was weighed accurately for 100 mg on an analytical balance and was transferred in a clean and dry 250 mL beaker. To this 100 mL of distilled water was added and stirred over a magnetic stirrer for 15 minutes until the sodium hyaluronate powder completely dissolves leaving no visible particles.

c) Preparation of the Final Solution

The aqueous extract of Triphala and the sodium hyaluronate solution were mixed in ratios of 1:1, 2:3, 3:1, 3:2 and 1:3 to prepare a total volume of 10 mL. The resultant solutions were stored in sterile containers and were left undisturbed for 24 hours. Further, these solutions were evaluated for their physicochemical attributes.

Evaluation Of Triphala Extract

Chemical evaluation of the Triphala extract is done to detect the presence of alkaloids, flavonoids, tannins etc. in the extract. These are the pharmacologically active components of the extract.

Test for Alkaloids

Dragendroffs Test

2 mL of the Triphala extract was mixed with 2 mL of dilute hydrochloric acid and was heated for a few minutes. The resultant was cooled and filtered and Dragendroffs reagent was added. An orange or reddish-brown precipitate indicates the presence of alkaloids (9).

Mayers Test

2 mL of the Triphala extract is mixed with 2 mL of Mayers reagent, a creamy or white colored precipitate indicates presence of alkaloids (9).

Wagners Test

2 mL of the Triphala extract is mixed with Wagners reagent, a reddish-brown color indicates presence of alkaloids (10).

Test for Tannins (Ferric Chloride Test)

2 mL of the Triphala solution is mixed with 10 % ferric chloride solution, a dark bluish color change indicates presence of tannins/phenols (10).

Test for Flavonoids

Shinoda Test

2 mL of the Triphala extract is taken and a small quantity of magnesium turnings or powder is mixed. To this add a few drops of concentrated hydrochloric acid and shake. A pink or red color indicates presence of flavonoids (11).

Lead Acetate Test

2 mL of Triphala extract is mixed with 10 % lead acetate solution, a yellow-colored precipitate confirms the presence of flavonoids. The presence or absence of the above components hints about the efficiency of Triphala extraction further confirming the presence of active components in the solution (12).

Evaluation of Ophthalmic Solution to Test for Sodium Hyaluronate

2 mL of the solution is mixed with 3 mL Sulphuric acid and is heated over a water bath for 10 minutes, cool and add 1 mL of carbazole reagent and heat again for 15 minutes, a purple or violet color confirms presence of uronic acid. Sodium hyaluronate contains glucuronic acid which reacts with carbazole reagent in presence of Sulphuric acid to give the purple color (13).

Qualitative Test

Molisch Test

2 mL of the solution is mixed with 3 mL of the Molisch reagent, now add 2 mL concentrated Sulphuric acid along the side of the test tube to form a layer, a violet or purple ring confirms the presence of sodium hyaluronate. This is an indirect test to confirm the presence of sodium hyaluronate. Sodium hyaluronate contains sugar derivatives that give positive results due to furfural ring reacting with alpha naphthol.

Carbazole test

2mL of the solution is mixed with 3mL Sulphuric acid and is heated over a water bath for 10 minutes, cool and add 1mL of carbazole reagent and heat again for 15 minutes, a purple or violet color confirms presence of uronic acid. Sodium hyaluronate contains glucuronic acid which reacts with carbazole reagent in presence of Sulphuric acid to give the purple color (14).

Quantitative Test

Method Development

An analytical method was developed to carry out drug release studies thereafter.

Test for Solubility

Solubility of sodium hyaluronate was checked with different solvents such as water, phosphate buffer 7.4 and methanol.

a) Preparation of phosphate buffer 7.4

Phosphate buffer 7.4 was prepared by accurately weighing 27.218 g of potassium dihydrogen phosphate in 1000 mL of distilled water to prepare solution A. 4 g of sodium hydroxide pellets were separately weighed and dissolved in another 1000 mL of distilled water to prepare solution B. 250 mL of solution A was mixed with 195.5 mL of solution B to prepare the phosphate buffer.

b) Preparation of Stock Solution

Sodium hyaluronate stock solution of 1000 µg/mL was prepared by accurately weighing 100 mg of the drug on analytical balance and transferring to 100 mL volumetric flask and then phosphate buffer 7.4 was added to make up the mark. Further 10 mL from this stock was pipetted into another 100 mL volumetric flask and a buffer was added to make up the volume to get working stock of 100 µg/mL.

c) Determination of λ_{max}

Sodium hyaluronate solution of 100 µg/mL was scanned in the UV region ranging from 200-400 nanometers to get the spectral scan and determine the maximum wavelength of absorbance.

Method validation

Method validation is crucial as it confirms the developed method is correct and can be repeated to get the same results. Linearity is a validatory attribute and is tested by preparing standard stock solution (100 µg/mL) of sodium hyaluronate was diluted using phosphate buffer 7.4. From the above solution aliquots of 1.0, 2.0, 3.0, 4.0, 5.0 mL were pipetted out into individual 10 mL volumetric flasks and the volume was made up using phosphate buffer 7.4 to get concentration ranging from 10-50 µg/mL. Absorbance of these solutions were measured at 202 nanometers using UV spectrophotometer against blank (buffer) (15).

Drug Content

A measured volume of the ophthalmic formulation was diluted with phosphate buffer pH 7.4, sonicated and filtered using Whatman filter paper. Sodium hyaluronate content was quantified by UV spectrophotometry at 202 nm.

Physicochemical Evaluation of the Solution

Test for pH

pH is vital to maintain the physical stability of the formulation, as sodium hyaluronate is stable in neutral to very mildly acidic media hence maintaining pH is vital. The pH of the various combinations of solutions was tested using the Aquasol digital pH meter and the readings were recorded. Further modifications were done to the formulation to adjust the pH.

Test for Viscosity

Viscosity is an essential attribute that defines any formulation's flowability (in case of solutions) which in turn affects ease of manufacturing, packaging and installation into the eyes. Viscosity was tested using Brookfield viscometer and the readings were recorded.

Clarity

Clarity defines that a solution is absolutely free from any particulate matter that might irritate the ocular tissue. Clarity is primarily checked with the naked eye to check the presence of any particles further; a beam of light is also focused on the walls of the container in which the solutions are stored and checked for any particles. The results are recorded as a clear solution or not.

Pharmacological Evaluation

Pharmacological evaluations were performed using the dosage form (ophthalmic solution) to evaluate the efficiency of the dosage forms and to confirm that the developed dosage forms exhibit pharmacological activities that are required to treat the computer vision sample. Sample A refers to the Ophthalmic solution.

Antioxidant Assay (ABTS Radical Scavenging Activity)

ABTS (SRL-Chem-Cat no- 28042) radicals were prepared by mixing APS (Ammonium persulfate-2.45 mM) and ABTS (7 mM) solution which was diluted 100 X to prepare ABTS free radical reagent. 10 µL of different stock of the standard (Ascorbic acid) concentration as per in data and samples were added to 200 µL of ABTS free radical reagent in 96 well plate and was incubated in at 37^0C for 10 minutes in the dark. The wells without treatment were considered to be in control. After incubation the absorbance is measured of the decolorization at 734 nm using a microplate reader (iMark BioRad). Results were presented with respect to negative control. % inhibition was calculated using Equation [1]. IC_{50} was calculated using software Graph Pad Prism 6. Graph was prepared between X axis (sample concentration) Vs Y axis (% inhibition of control) (16, 17).

$$\%\text{RSA} = \left(\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right) \quad [1]$$

Where, Absorbance $_{control}$=Absorbance of the control,
Absorbance $_{sample}$= Absorbance of the sample and
%RSA= Percentage Radical Scavenging Activity

Anti-inflammatory Assay (COX-II enzyme inhibition assay)

Sample dilutions and buffer (Tris chloride buffer 100mM, pH 8.0) was prepared. The reaction buffer (Enzyme in Tris/heme/phenol;100Mm/1µM) and buffer Bovine Hemin Chloride were placed in 96 well plates. The reaction was initiated by adding 5 µL TMPD solution and then the plate was incubated (Basil Scientific Corp India incubator) at room temperature for 10 minutes and the absorbance was taken at 595 nm using ELISA microplate reader (iMark BioRad). Inhibitor, celecoxib (2500µM final concentration) was used as a positive control. % inhibition was calculated using Equation [2]. IC_{50} was calculated using software Graph Pad Prism 6. Graph was prepared between X axis (sample concentration) Vs Y axis (% inhibition of control) (18, 19).

$$\%\text{inhibition} = \left(\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}\right) \text{X } 100 \quad [2]$$

Where, Absorbance $_{control}$=Absorbance of the control and
Absorbance $_{sample}$= Absorbance of the sample

In vitro Evaluation

In vitro drug release from the ophthalmic solution was estimated using the Franz Diffusion cell. Here, the Franz Diffusion cell with dialysis membrane is employed so as to obtain a controlled and reproducible *in vitro* drug release profile. It also serves as an inert consistent barrier suitable for preliminary and comparative evaluations while avoiding biological variability.

Dialysis membrane was soaked previously for 24 hours in phosphate buffer 7.4 and was used for the study. The donor compartment of the diffusion cell contained the formulated ophthalmic solution and the dialysis membrane. The membrane acted as a barrier between donor and the receptor compartment. The donor compartment contained phosphate buffer 7.4. Aliquots were withdrawn at 0, 15 and 30 minutes from the donor compartment and were analyzed spectrophotometrically. 5mL of the aliquots were withdrawn every time and were replaced with buffer media in the donor compartment immediately. The withdrawn samples were analyzed by a spectrophotometer at 202 nm (quantification of sodium hyaluronate) and 258 (quantification of gallic acid) and percentage of cumulative drug release was estimated (20, 21).

Histopathological Analysis

The eyes of the goat were procured from a local slaughterhouse. The extracted eyes were immediately immersed and stored in prepared Tyrode's solution to transport the eyes to the laboratory from the slaughterhouse. The eyes were kept in an upright position and using a sterile

scalpel, the eye was cut along the transverse plane and the transparent corneal tissue was extracted. The extracted tissue was washed with distilled water and was immediately stored in 10% formalin solution to preserve the histology of the tissue. Further the tissues were processed for the test and control groups. The extracted tissues are kept on a clean petri dish and are allowed to dry. The petri dish is then placed in a UV chamber on a heighted platform such that the tissues in the petri dish are just a few inches away from the UV chamber's light source. The tissues are exposed to UV light for a period of 15 minutes to induce UV induced damage to the tissue. After 15 minutes the tissues were immersed in the formalin solution to preserve the tissue. Similarly, another set of corneas were induced by UV damage and then immersed in the developed formulation for 30 minutes and after 30 minutes they were rinsed and preserved in formalin solution. The tissues are dehydrated by passing it through a series of ethanol of various strengths (70%, 80%, 90%, 100%). Further ethanol is replaced with xylene (cleansing agent) and the tissue is then infiltrated with molten paraffin wax. The tissue is placed in a mold with molten paraffin using a holding station and is oriented precisely for desired sectioning angle. A microtome is used to cut thin sections (4-6 µm) and the cut sections are made afloat on warm water to remove any wrinkles and are mounted on glass sides and dried at 50-60 degree Celsius. The slide is now stained with hematoxylin stain which stains the nucleus and other elements a reddish-purple color. The slide is rinsed in tap water and the section is treated with an alkaline solution which converts the hematoxylin to a dark blue color. Excess background stain is eliminated using a weak acid alcohol and eosin counterstain is applied and now the slide is ready for microscopic examination (22, 23). The prepared slides are mounted on an optical microscope and slides are observed under 10X magnification, subsequently photos of the sections are captured using a high-resolution camera.

Sterilization of Dosage Forms

The developed dosage form (ophthalmic solution) was sterilized using drug heat sterilization. The dosage forms were sealed in a glass bottle and were sterilized using an autoclave at 121 °C for 15 minutes (24, 25).

Sterility Testing of Finished Products

The sterility testing of ophthalmic solution is done by the direct inoculation method. The test is performed using thioglycolate agar media. Thioglycolate Agar media is prepared using quantities as per manufacturer's instructions. The prepared media is poured into test tubes and a drop of the formulations are directly added into the media and are kept in the incubator for 37-45 ^{o}C for 5 days to check for any turbidity. For ophthalmic solution a control is also maintained. The control group does not contain any formulation and consists of only the media. Ideally there should not be any turbidity seen in the medium after 5 days. The whole procedure is performed under a laminar air flow unit to maintain sterile conditions (26, 27).

Results

Formulation

Triphala extract of 75 mL was obtained and solution of sodium hyaluronate was prepared and a total of 5 different formulations as shown in Table 1. A total volume of 10 mL was prepared using different combinations of Triphala extract to sodium hyaluronate ratios.

Table 1: Formulation of Ophthalmic Solution

Formulation Number	Ratio of Triphala Extract to Sodium Hyaluronate	Volume of Triphala Extract (mL)	Volume of Sodium Hyaluronate (mL)	Quantity of Excipients (Benzalkonium Chloride) (mg)
OPS1	1:1	5	5	
OPS2	2:3	4	6	1 equivalent to 0.01%
OPS3	3:1	7.5	2.5	
OPS4	3:2	6	4	
OPS5	1:3	2.5	7.5	

Table 1 presents the composition of the ophthalmic solution including the active ingredients and excipients used. The formulation was designed to ensure ocular safety, stability and therapeutic efficacy. Different excipients were used to keep the system stable and free from bacterial contamination.

Evaluation of Triphala Extract

The chemical evaluation of the extract was performed to check the presence of various active components as shown in Table 2. Table 2 summarizes to confirm the presence of various formulation components. These evaluations ensured the identity and compatibility of the ingredients used.

Table 2: Chemical Evaluation to Check Presence of Different Components

Test	Results	Inferences
Test for Alkaloids		
Dragendroffs Test	Reddish Brown Precipitate	Alkaloids Present
Mayers Test	Creamy Precipitate	Alkaloids Present
Wagners Test	Red-Brown Precipitate	Alkaloids Present
Test For Tannins	Bluish Black Color	Tannins Present
Test For Flavonoids		
Shinoda Test	Pink Color	Flavonoids Present
Lead Acetate Test	Pale Yellow Precipitate	Flavonoids Present

Table 3: Qualitative Chemical Tests for Sodium Hyaluronate

Test	Results	Inferences
Molisch Test	Purple ring seen at the surface	Sugar derivative present, Molisch test is positive hence sodium hyaluronate is detected
Carbazole Test	Dark purple color observed	Gluconic acid detected. Carbazole test is positive and sodium hyaluronate is detected

Evaluation of Ophthalmic Solution to Test for Sodium Hyaluronate

Qualitative test

The qualitative tests were performed and the results obtained are depicted in Table 3. Table 3 lists the qualitative chemical tests employed to confirm the presence of sodium hyaluronate. Positive test results verified its successful incorporation into the formulation.

Quantitative Test

Method Development

The prerequisites for the method development i.e., preparation of buffers, solubility tests and preparation of stock solution was successfully completed and was subjected to UV analysis The spectral scan of Sodium Hyaluronate shown in Figure 1 - 3 λ_{max} at 202, 252 and 306 nm. The absorption spectrum of sodium hyaluronate was recorded to identify its characteristic absorbance pattern. The spectrum confirms suitable analytical behavior under selected experimental conditions. The absorption maxima at 202nm were however considered as it showed its presence even at low concentrations. Maximum absorbance of sodium hyaluronate was seen at 202 nm using phosphate buffer 7.4 as solvent of choice.

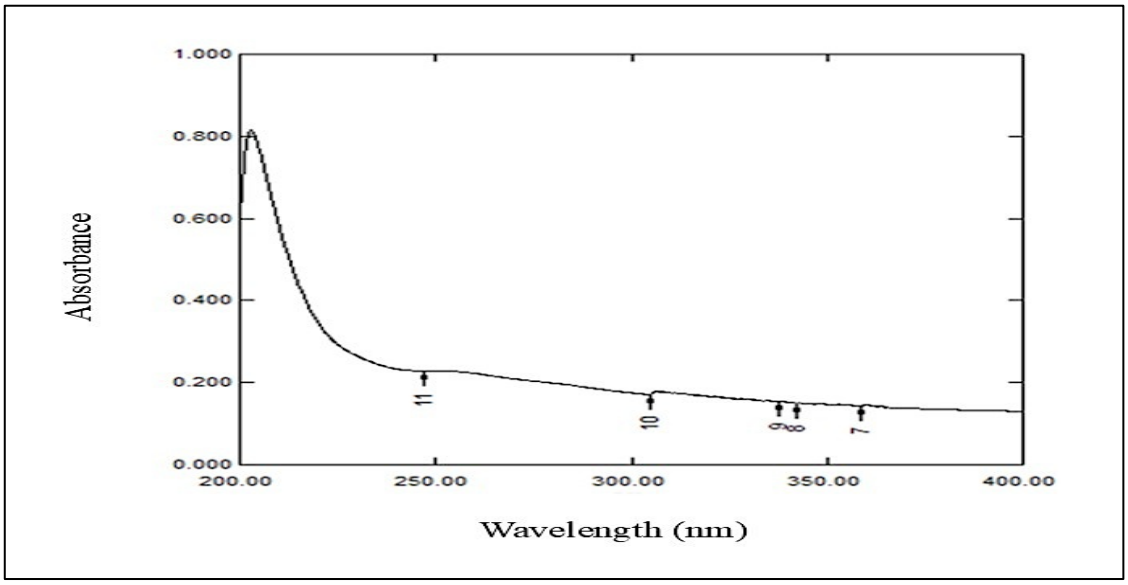

Figure 1: Absorption Spectra of Sodium hyaluronate in Phosphate Buffer 7.4

Method Validation

The prepared dilutions of 20, 40, 60, 80, 100 µg/mL were scanned at the determined wavelengths and a standard calibration plot is developed which is seen in Figure 2, to exhibit linearity. The linearity graph demonstrates the relationship between concentration and absorbance of sodium hyaluronate. The plot confirms compliance with Beer-Lamberts law over the studied concentration range.

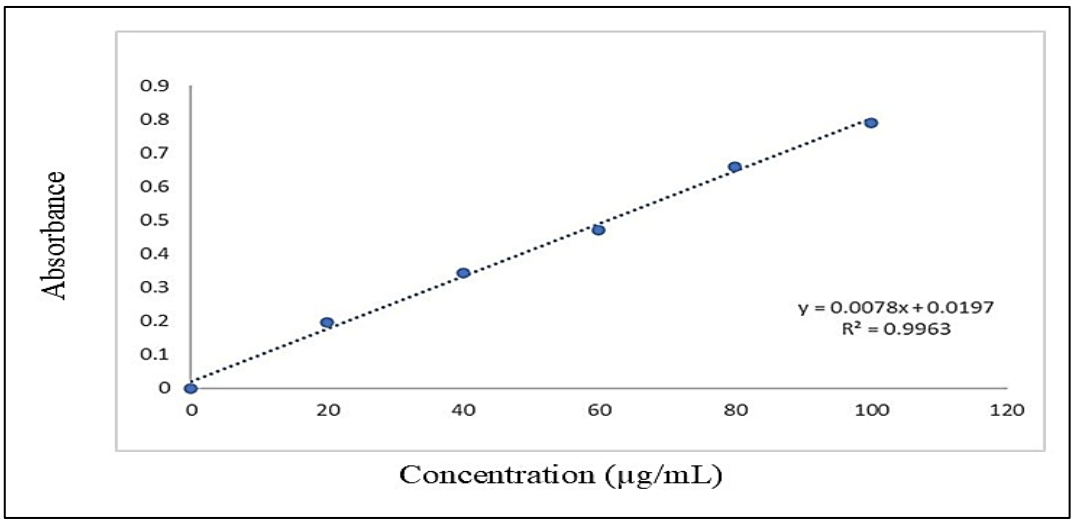

Figure 2: Linearity Graph of Sodium Hyaluronate in Phosphate Buffer 7.4

Table 4: Comparative Physicochemical Parameters of Ophthalmic Solution Formulations

Formulation	pH	Viscosity	Clarity
OPS1	7.6	20	Clear
OPS2	7.4	20	Clear
OPS3	6.8	18	Clear
OPS4	7.1	16	Clear
OPS5	7.9	14	Clear

Drug Content

The drug content for the optimized formulation was found to be 34.9%.

Physicochemical Evaluation of The Ophthalmic Solution

All the 5 formulations were tested for pH using Aquasol digital pH meter, followed by viscosity testing which ranged between 14-20 cP and lastly test for clarity where in it was observed all the formulations were free from any particulate matter. The obtained observations are recorded in Table 4 which reports the values for pH, viscosity and clarity which is vital in maintaining the formulations characteristics for ophthalmic applications.

OPS2 was considered to be the most optimum based on the physicochemical evaluations and was further used for other analysis.

Pharmacological Evaluations of Ophthalmic Solution

The pharmacological evaluation was conducted for the prepared ophthalmic solutions to check the pharmacological activity of the prepared formulations. Various physiological processes that are seen in computer vision syndrome pathologies were analyzed by conducting biochemical assays and the effect of the formulation was observed in these assays.

Throughout the following pharmacological evaluation sample A refers to ophthalmic solution.

Antioxidant Assay (ABTS Radical Scavenging Activity)

Based on the results obtained from the experimental work depicted by Table 5-9, the sample A exhibited elevated antioxidant activity than the reference.

The absorbance values of the reference, standard and sample A were determined using microplate reader as represented in Table 7 and 9 respectively. Statistical analysis of both datasets, including mean, standard deviation and %RSD as shown in Table 8 and 10, confirmed the precision and reproducibility of the measurements. Non - linear regression analysis as shown in Table 11 enabled the determination of IC_{50} values, indicating concentration dependent radical scavenging activity.

Ascorbic acid was taken as reference.

Table 5: Absorbance Values of the Reference

	Final Replicate Values			
Sample Conc. (µg/mL)	**1**	**2**	**3**	**4**
0	-0.562149	5.683948	-4.80949	-0.3123
0.78	7.4328545	1.93629	2.186134	-1.06184
1.56	6.6833229	6.933167	3.935041	2.186134
3.125	11.6802	8.182386	5.18426	2.435978
6.25	15.178014	14.17864	11.43036	12.67958
12.5	24.921924	22.67333	21.67395	25.67146
25	50.03123	47.78264	42.28607	42.53592
50	75.015615	71.5178	73.01686	74.01624

Table 6: Statistical Calculations for Reference Sample

Sample Conc. (µg/mL)	Mean	SD	SEM	N
0	0.00	4.314782	2.157391	4
0.78	2.62	3.529643	1.764822	4
1.56	4.93	2.280752	1.140376	4
3.125	6.87	3.973355	1.986678	4
6.25	13.37	1.649411	0.824705	4
12.5	23.74	1.873829	0.936914	4
25	45.66	3.862492	1.931246	4
50	73.39	1.492107	0.746053	4

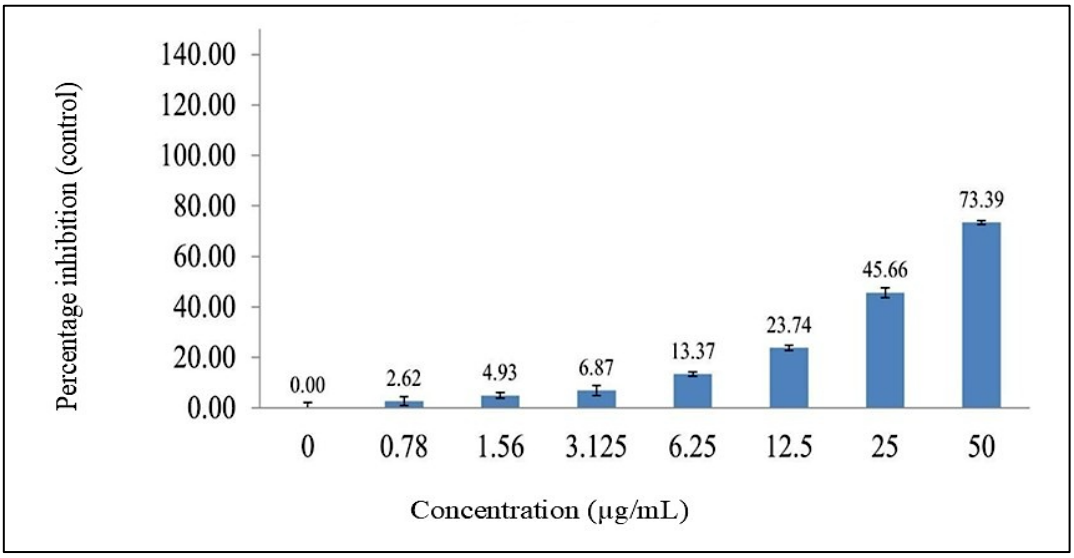

Figure 3: Percentage inhibition of control vs concentration (µg/mL) for ABTS radical scavenging activity of ascorbic acid reference molecule

According to Figure 3 and Figure 4, Sample A was found to be highly active compared to 26.65 µg of standard Ascorbic acid. The ABTS radical scavenging activity of the reference compound ascorbic acid and sample A is depicted in Figure 3 and 4. Both exhibited an increase in the percentage inhibition with rising concentration indicating effective radical scavenging activity. Comparative evaluation demonstrated appreciable anti-oxidant potential of sample A relative to the reference and control.

Table 7: Absorbance Values of Sample A

	Final Replicate Values			
Sample Conc. (µg/mL)	**1**	**2**	**3**	**4**
0	-0.643777	-0.64378	-1.50215	2.7897
0.078125	8.3690987	6.437768	8.798283	8.154506
0.15625	14.592275	15.02146	14.59227	13.9485
0.3125	31.11588	29.82833	30.4721	31.54506
0.625	56.11588	56.54506	55.4721	57.18884
1.25	91.523605	92.38197	91.95279	93.24034
2.5	94.527897	93.88412	91.7382	93.66953
5	93.776824	94.20601	95.49356	92.70386

Table 8: Statistical Calculations for Sample A

Sample Conc. (µg/mL)	Mean	SD	SEM	N
0	0.00	1.90331	0.951655	4
0.078125	7.94	1.036579	0.51829	4
0.15625	14.54	0.442393	0.221197	4
0.3125	30.74	0.751073	0.375536	4
0.625	56.33	0.722425	0.361213	4
1.25	92.27	0.732972	0.366486	4
2.5	93.45	1.201206	0.600603	4
5	94.05	1.153953	0.576977	4

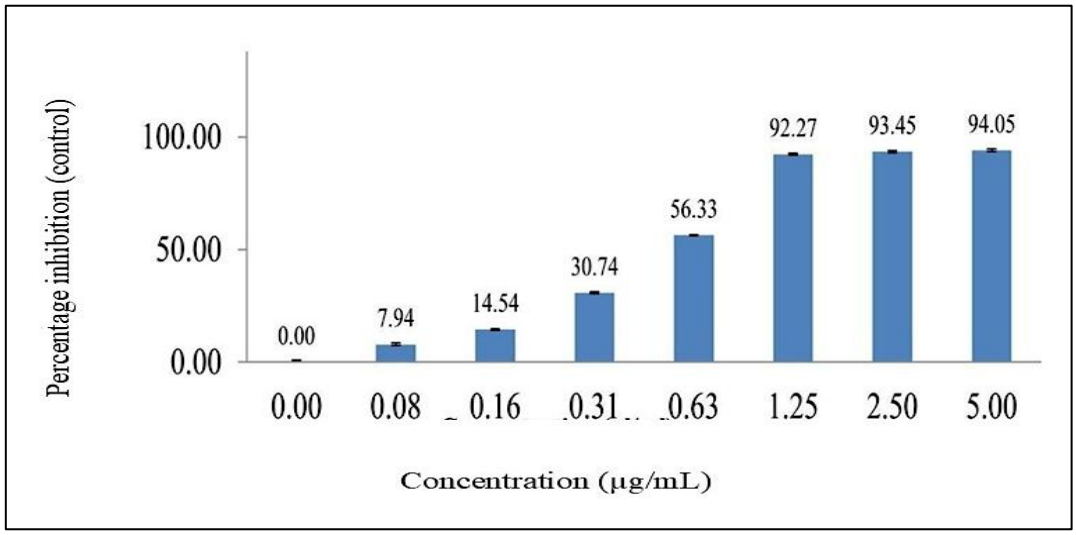

Figure 4: Percentage inhibition with respect to control vs concentration (µg/mL) for ABTS radical scavenging activity of sample A

Table 9: Non-linear Regression Analysis of ABTS Radical Scavenging Activity

Parameter	Ascorbic Acid	Sample A	Sample B
LogIC50	1.426	-0.316	-0.1518
Hillslope	1.4	1.756	1.882
IC50	26.65 ± 0.021	0.483 ± 0.03	0.7051 ± 0.043
Std. Error			
LogIC50	0.02194	0.0344	0.04333
Hillslope	0.1029	0.2173	0.3129
95% Confidence Intervals			
LogIC50	1.369 to 1.482	-0.4045 to -0.2276	-0.2632 to -0.04035
Hillslope	1.135 to 1.664	1.197 to 2.314	1.077 to 2.686
IC50	23.41 to 30.35	0.3940 to 0.5922	0.5456 to 0.9113
Goodness of Fit			
Degrees of Freedom	5	5	5
R square	0.9912	0.985	0.9786
Absolute Sum of Squares	36.45	132.3	225.1
Sy.x	2.7	5.144	6.71
Number of points Analyzed	7	7	7

Anti-Inflammatory Analysis (COX-II Inhibition Assay)

Enzyme Inhibition Activity (COX-II) was estimated in all samples Sample- A was found active. 1.562 µL of the sample - A was found equivalent to 1732 µM of the standard Celecoxib as shown in Figure 5 and Figure 6. Lower is the IC50, higher is inhibition activity. Figure 5 and 6 illustrate the COX-II enzyme inhibition activity of the reference compound Celecoxib and sample A respectively. Both showed an increase in percentage inhibition with increasing concentration., indicating concentration dependent enzyme inhibition. Comparative assessment demonstrated that sample A exhibited notable COX-II inhibitory activity relative to control.

Based on the results obtained from the study as shown in Table 10-14 the absorbance values of the reference compound celecoxib and sample A obtained from the COX-II enzyme inhibition assay are presented in Tables 12 and 14 respectively. Statistical evaluation of both datasets as shown in Table 13 and 15 demonstrated acceptable precision and reproducibility of the measurements. Comparative analysis confirmed the inhibitory response of sample A relative to the reference. Non-linear regression analysis represented by Table 16 was employed to determine inhibitory potency and IC_{50} values indicating concentration-dependent COX-II inhibition.

Celecoxib was used as reference compound.

Table 10: Absorbance Values of Reference Compound (Celecoxib)

	Final Replicate Values			
Sample Conc. (µg/mL)	**1**	**2**	**3**	**4**
0	-5.932203	3.389831	-0.84746	3.38983
0.78	0	5.932203	-1.69492	-1.69492
78.125	2.9661017	13.13559	-2.11864	8.89831
156.25	-0.423729	11.44068	19.9153	3.81356
312.5	10.169492	17.79661	29.661	27.9661
625	38.135593	28.81356	29.661	26.2712
1250	47.033898	49.57627	27.5424	40.2542
2500	52.118644	53.81356	57.2034	69.9153

Table 11: Statistical Values of Reference Compound (Celecoxib)

Sample Conc. (µg/mL)	Mean	SD	SEM	N
0	0.00	4.43062	2.21531	4
0.78	0.64	3.62034	1.81017	4
78.125	5.72	6.68633	3.34316	4
156.25	8.69	8.95195	4.47598	4
312.5	21.40	9.13723	4.56861	4
625	30.72	5.14908	2.57454	4
1250	41.10	9.85872	4.92936	4
2500	58.26	8.05085	4.02542	4

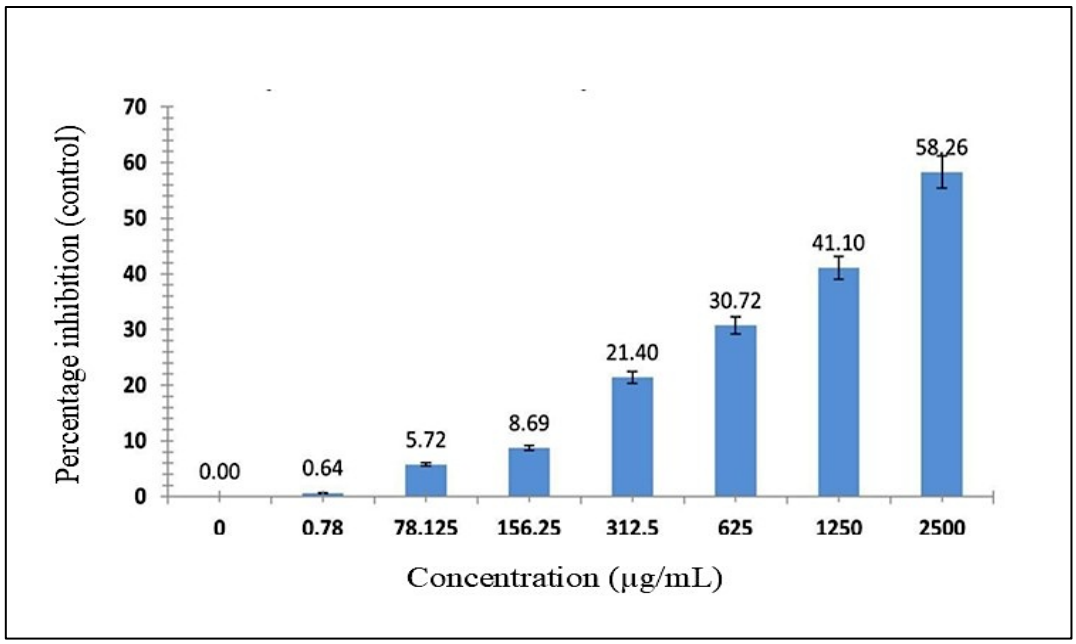

Figure 5: Percentage inhibition of control vs concentration (µg/mL) for COX-II Enzyme Inhibition Assay of reference compound (Celecoxib)

Table 12: Absorbance Values of Sample A

	Final Replicate Values			
Sample Conc. (µg/mL)	**1**	**2**	**3**	**4**
0	-16.441006	0.580271	10.2515	5.60928
0.08	6.76982592	0.580271	1.74081	3.2882
0.16	5.80270793	7.736944	8.89749	7.73694
0.31	13.7330754	7.930368	10.2515	7.15667
0.63	18.3752418	26.11219	29.207	17.2147
1.25	35.7833656	45.84139	38.8781	47.0019
2.5	67.5048356	65.18375	70.9865	68.2785
5	75.2417795	79.11025	81.0445	82.205

Table 13: Statistical Values of Sample A

Sample Conc. (µg/mL)	Mean	SD	SEM	N
0	0.00	11.6505	5.82523	4
0.08	3.09478	2.68945	1.34472	4
0.16	7.54352	1.28303	0.64151	4
0.31	9.76789	2.95248	1.47624	4
0.63	22.7273	5.853	2.9265	4
1.25	41.8762	5.41931	2.70966	4
2.5	67.9884	2.39252	1.19626	4
5	79.4004	3.05217	1.52609	4

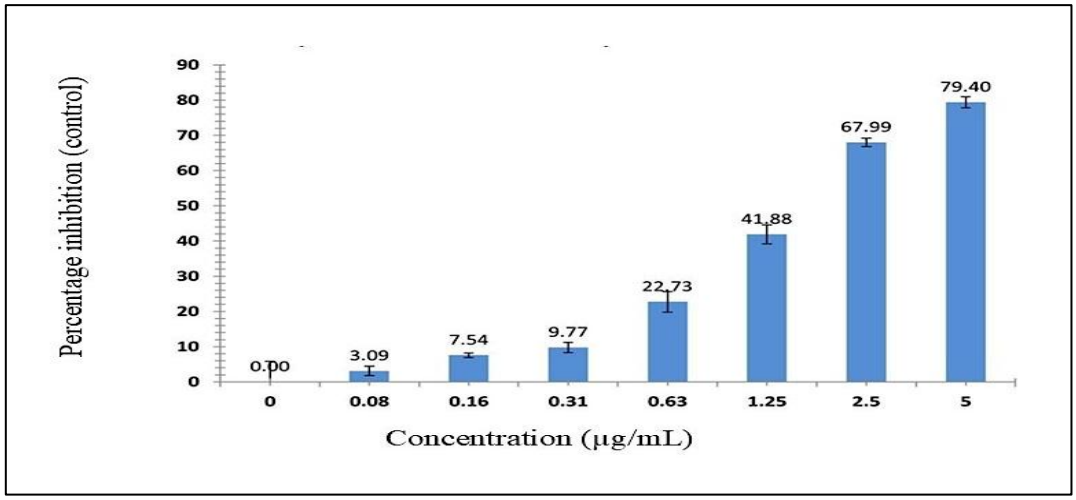

Figure 6: Percentage inhibition of control vs concentration (µg/mL) for COX-II Enzyme Inhibition Assay of sample A

Table 14: Non-Linear Regression Analysis of COX-II Enzyme Inhibition Assay

Non-Linear Regression Parameters	Celecoxib	A	B
LogIC50	3.239	0.1937	0.125
Hillslope	0.8576	1.292	1.722
IC50	1732 ± 0.027	1.562 ± 0.018	1.333 ±0.104
Degrees of Freedom	5	5	5
R square	0.9922	0.9952	0.9984
Absolute Sum of Squares	20.49	27.08	8.182
Sy.x	2.025	2.327	1.714
Number of points Analyzed	7	7	7

In vitro Evaluation

In vitro drug release was conducted for the ophthalmic solution (OPS2). The test was conducted in phosphate buffer 7.4 for a total time period of 30 minutes. Aliquots withdrawn at 0, 15 and 30 minutes were analyzed by UV spectrophotometer to detect the amount of gallic acid and sodium hyaluronate diffused in the receptor chamber and subsequently cumulative drug release was calculated.

It is observed that the cumulative drug release of Gallic acid at 15th minute was 43.124 % and at 30th minute the cumulative drug release was 70.49 % as shown in Table 15. Table 15 presents cumulative drug release data of gallic acid from the ophthalmic formulation over the study period. The results demonstrate the extent of drug release and confirm the consistency of the formulation It is seen that at 30th minute the cumulative drug release almost doubled in contrast with the cumulative drug release at 15th minute which is suggestive of increased residence time in the ophthalmic sac will enhance the drug release to the eye.

It is also observed that the cumulative drug release of Sodium hyaluronate at 15th minute was 4.200 % and at the 30th minute it was 31.05 % In contrast with the cumulative drug release of gallic acid it is seen that the drug release of sodium hyaluronate is less than that of gallic acid, which is mainly due to the high molecular weight of sodium hyaluronate as shown in Table 16. Table 16 depicts the cumulative release of the sodium hyaluronate from the ophthalmic formulation over time which indicates formulation stability and uniform drug release behavior.

Table 15: Cumulative Drug Release of Gallic Acid in Ophthalmic Solution

Time (min)	Absorbance	Concentration (mcg)	Conc (mg)	5 mL	200 mL	CDR	CDR %
0	0	0	0	0	0	0	0
15	0.238	52.59059226	0.052590592	0.262952961	10.51812	10.78107	43.12429
30	0.361	85.97222396	0.085972224	0.42986112	17.19444	17.62431	70.49722

Note: CDR = Cumulative Drug Release

Table 16: Cumulative Drug Release of Sodium Hyaluronate in Ophthalmic Solution

Time (min)	Absorbance	Concentration (mcg)	Conc (mg)	5 mL	200 mL	CDR	CDR %
0	0	0	0	0	0	0	0
15	0.034	0.122948194	0.000122948	0.000614741	0.02459	0.025204	4.20073
30	0.04	0.908976231	0.000908976	0.004544881	0.181795	0.18634	31.05669

Note: CDR = Cumulative Drug Release

Histopathological Analysis

All the four slides were examined using an optical microscope under 10X magnification and the following histopathological analysis was concluded.

Figure 7 shows H and E-stained tissue of normal goat cornea. It is seen that the normal goat cornea shows stratified squamous non-keratinized epithelium which is tightly arranged with uniform cell layers that is seen as dark purple color layer in the slide. The Bowmans Layer is present immediately below the epithelium that is seen as dark pink color. The Bowmans layer is thin and acellular. Descents Membrane and Endothelium which are the undermost cellular layers are seen to be intact with endothelial cells showing uniform cell morphology. These features are suggestive of normal tissue histology.

Figure 8 is the pathology induced goat corneal tissue. The epithelium shows thinning, loss of stratification and ulceration. It is also evident that there is presence of dense infiltration of inflammatory cells in the stroma. Stroma is edematous and contains disorganized collagen fibers. These characteristics are suggestive of neovascularization indicating angiogenesis due to inflammation.

Figure 9(A) and 9(B) is the histological slide of ophthalmic solution treated goat corneal tissue. The goat corneal tissue was previously induced with UV damage. The histology of the tissue presents with epithelium that shows partial restoration and increased stratification (Figure 9A) and also shows enhanced thickness of epithelium nearing normalcy in Figure 9B. Stroma shows moderate reduction in edema and inflammatory cell infiltration. Also, the collagen fibers have begun to reorganize, overall, the histology shows early signs of tissue recovery.

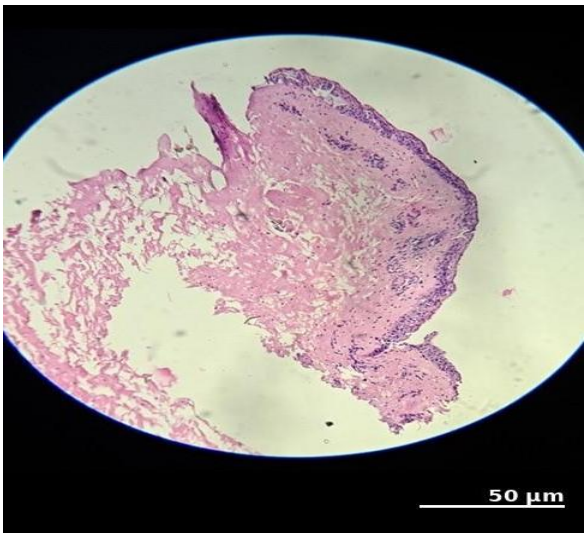

Figure 7: Normal Goat Corneal Tissue

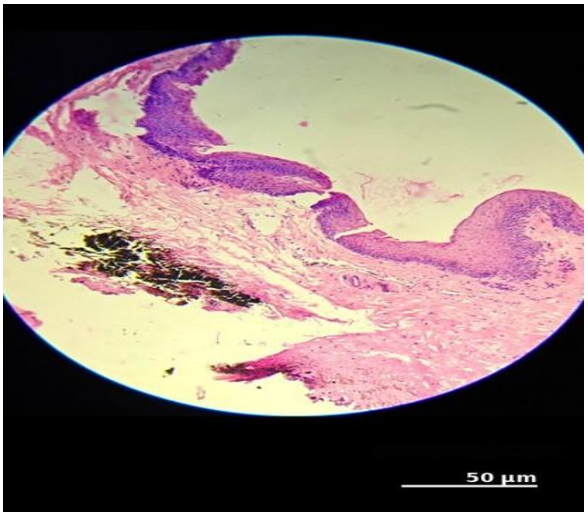

Figure 8: UV Pathology Induced Goat Corneal Tissue

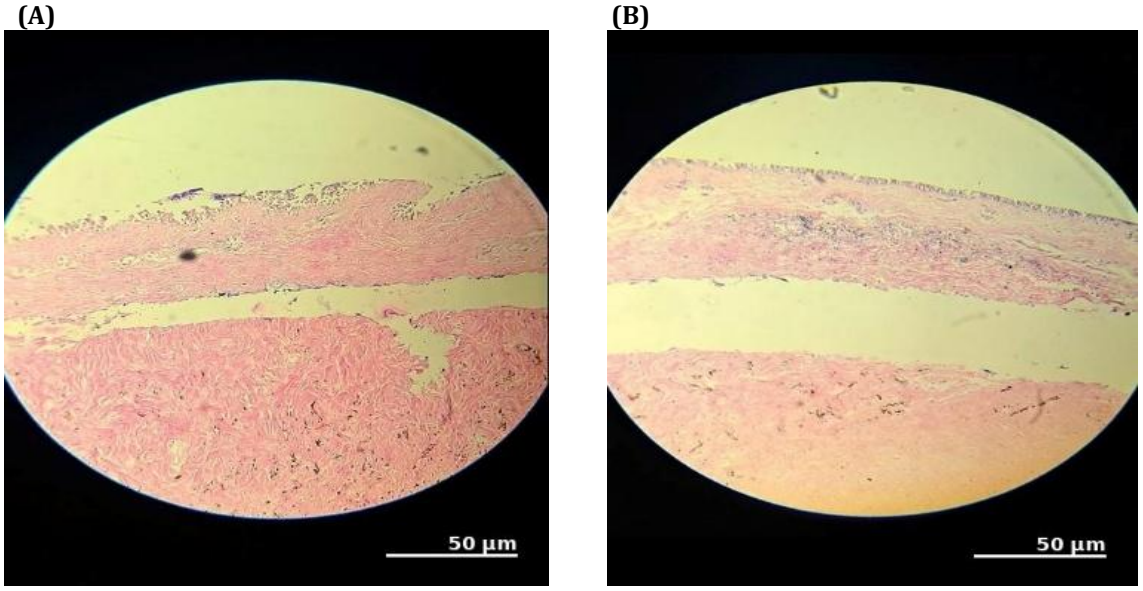

Figure 9: Histological slides of ophthalmic solution treated goat corneal tissue. A. Ophthalmic solution-treated goat cornea tissue of field 1 microscopic view, B. Ophthalmic solution treated goat cornea tissue of field 2 (adjacent to field 1) microscopic view

Sterilization of Dosage Forms

The developed dosage form (ophthalmic solution) was successfully sterilized as per the procedure. The dosage forms appeared to be stable post-sterilization.

Sterility Testing of Finished Product

The sterility testing of the finished product is done by the direct inoculation method. The finished dosage forms showed no signs of microbial growth as seen in Figure 10. It presents the sterility testing results of the formulated dosage forms. The test tube 3 contains ophthalmic solution and the thioglycolate media, which shows no sign of turbidity. Test tube 4 is the control group.

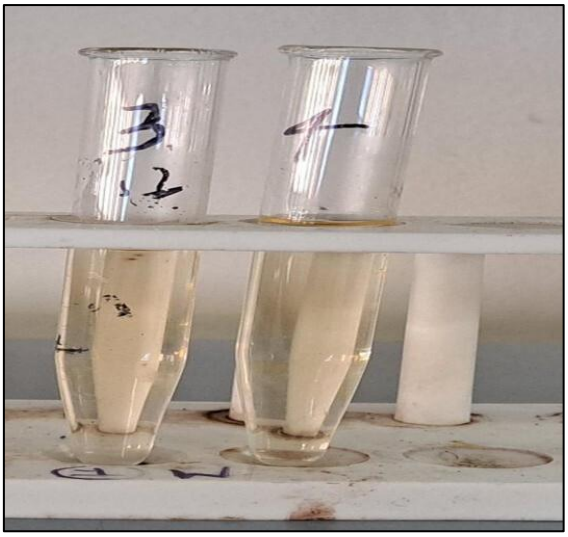

Figure 10: Sterility Test of Dosage Forms

Discussion

Computer vision syndrome is an eye condition that arises due to excessive exposure to the screen. Computer vision syndrome is a newly emerging disease that shows normal like negligible symptoms but is associated with chronic effects if ignored. CVS as the name suggests is not a disease but a syndrome which means it is a combination of multiple diseases resulting in a syndrome. CVS is a combination of retinal, vitreo-retinal ocular disease. It presents clinical symptoms like redness of the eye, irritation of the eye, eye dryness in acute cases, but as the conditions aggravate, symptoms like higher associated myopia exceeding -6 diopters, vitreous floaters, posterior vitreous detachment, retinal detachment are seen. Recent epidemiological studies consistently report a significant rise in CVS incidence across all age groups, particularly among young adults and professionals with prolonged screen exposure reinforcing the clinical relevance of early intervention strategies (28). The delayed manifestations of severe symptoms often result in underestimation of CVS allowing cumulative oxidative stress, inflammation and tear film instability to progressively damage ocular tissues, thereby emphasizing the need for early prophylactic and therapeutic intervention. Contemporary mechanistic studies have attributed these changes to sustained oxidative stress, blue light induced photochemical damage and low-grade damage to ocular tissues which aligns with the pathophysiological rationale adopted in recent studies (29), while earlier reports primarily focused on symptomatic relief using lubricants alone.

Ophthalmic solution was prepared using 2 key ingredients: sodium hyaluronate 0.1% solution and aqueous extract of Triphala. The selection of sodium hyaluronate was based on its viscoelastic, mucoadhesive and corneal soothing properties while Triphala was incorporated for its well documented antioxidant , anti-inflammatory and rejuvenative effects, making the combination suitable for addressing the multifactorial pathology of CVS This combinatorial strategy is consistent with recent formulation based studies that advocate the integration of bioactive phytoconstituents with conventional polymers to achieve synergistic ocular protection (30). Five formulations of ophthalmic solutions in ratio of 1:1, 2:3, 3:1, 3:2, 1:3 was prepared, mixing different ratios of sodium hyaluronate solution and Triphala aqueous extract. Evaluation of sodium hyaluronate and Triphala extract was also performed. Chemical tests like Molisch test and Carbazole test confirmed the presence of sugar derivative and glucuronic acid thus confirming the presence of sodium hyaluronate in the formulation, similarly specific tests for tannins, alkaloid and flavonoids confirmed Triphala.

Physicochemical evaluation of the ophthalmic solution was conducted to test the viscosity, pH and clarity of all the five formulation and based on the results Formulation 2 was considered to be the most optimized formula as its physicochemical parameters exactly matched with the ideal

characteristics of an ophthalmic solution. Optimal viscosity is essential for enhancing precorneal residence time without causing visual blurring while physiological pH and clarity ensure ocular comfort and patient compliance justifying the selection of formulation 2 for further evaluations Comparable studies have demonstrated that maintaining the optimal viscosity is critical for prolonged residence time without inducing visual disturbances, a finding which directly supports the selection of Formulation 2 in the present investigation (31).

Quantitative spectrophotometric method development for sodium hyaluronate was successfully established using phosphate buffer Ph 7.4 which provided clear solutions and stable pH conditions suitable for ocular drug analysis. Sodium hyaluronate exhibited maximum solubility in aqueous polar solvents, particularly water and phosphate buffer, while remaining insoluble in organic solvents such as methanol confirming the appropriateness of the selected medium. UV spectral scanning revealed characteristic absorption peaks at 202,252 and 306nm with 202nm selected as the analytical wavelength due to its higher sensitivity and reliable detection even at low concentrations. Method validation demonstrated good linearity in the concentration range of 20-100 µg/mL as confirmed by the calibration curve, indicating the suitability of the method for quantitative analysis. The drug content of the optimized formulation was found to be 34.9% reflecting uniform distribution and adequate incorporation of sodium hyaluronate within the ophthalmic formulation. This further supports the formulation's consistency, stability and reliability for therapeutic application in CVS which are the critical attributes highlighted in a recent regulatory-oriented formulation - evaluation study (32).

The pharmacological evaluations were conducted Antioxidant assays (ABTS Assay) and anti-inflammatory (COX-II enzyme inhibition) was performed to determine the therapeutic ability of the formulations (table 7-16). In anti- oxidant assay (ABTS) the formulation showed higher antioxidant activities than the individual refence standards used. Similarly, the formulation showed anti-inflammatory responses (COX-II enzyme inhibition). The enhanced ani-oxidant activity suggests a synergistic interaction between Triphala phytoconstituents and sodium hyaluronate which is particularly significant in reducing light-induced oxidative damage associated with prolonged screen exposure. COX-11 inhibition further indicates the formulation's ability to suppress inflammatory mediators responsible for ocular irritation and surface inflammation in CVS.

In comparison with earlier studies conducted, the present study is largely consistent highlighting the role of antioxidants and anti-inflammatory agents in management of computer vision syndrome and other ocular surface disorders (33). Previous studies have demonstrated that sodium hyaluronate improves ocular surface lubrication, enhances tear film stability and reduces ocular irritation, while Triphala's phenolic constituents exhibit anti-oxidant activities (34). The enhanced antioxidant and anti-microbial effect observed in optimized formulation suggests a synergistic effect which is also previously reported in scientific literature for polyherbal systems in ocular applications (35). Overall, the present results reinforce existing evidence while extending it by demonstrating the combined therapeutic potential of sodium hyaluronate and Triphala in addressing the multifocal pathology of CVS.

In vitro permeation evaluation of the ophthalmic solution was performed using Franz diffusion cell wherein dialysis membrane was used as the permeating membrane and percentage CDR was calculated for both the components of the solution i.e., sodium hyaluronate and Triphala. Triphala is estimated as gallic acid as Triphala itself cannot be estimated as it is a polyherbal formulation. It was seen that at 30th minute gallic acid showed a percentage CDR of 70.497 % and sodium hyaluronate showed a percentage CDR of 31.05 %. (Table 15 and 16). Further histopathological analysis (Figure 7-9B) on light induced damaged goat cornea as human models showed significant rejuvenation after being subjected to treatment with the developed formulation The higher permeation of gallic acid can be attributed to its low molecular weight and rapid diffusibility, providing immediate antioxidant protection, while the comparatively lower release of sodium hyaluronate supports sustained ocular lubrication and surface protection which is desirable for conditions like CVS .

Conclusion

The present research on the prophylaxis of Computer Vision Syndrome was successfully conducted with the objective of addressing the unmet needs and gaps existing in both conventional allopathic and traditional Ayurvedic treatment approaches. The formulation of an ophthalmic solution integrating sodium hyaluronate and aqueous extract of Triphala was systematically designed and evaluated to target the multifactorial pathology of CVS, particularly focusing on ocular surface protection and early-stage prophylaxis. The developed ophthalmic solution was primarily aimed at preventing and managing acute manifestations associated with prolonged digital screen exposure. The two developed dosage forms were evaluated for their physicochemical characteristics; pharmacological activity and *in vitro* permeation behavior and the findings were found to be in accordance with established scientific requirements for ophthalmic preparations. Among the formulations, OPS2 demonstrated optimal pH, viscosity, clarity, and uniform drug content thereby ensuring ocular compatibility, patient comfort and enhanced precorneal residence time. This optimized physicochemical profile is particularly significant for prophylactic ophthalmic applications, where long term tolerability and compliance are critical. Pharmacological evaluations revealed that the optimized formulation exhibited significant anti-oxidant and anti-inflammatory activity, attributable to the synergistic interaction between sodium hyaluronate and the bioactive phytoconstituents of Triphala. The combined therapeutic action offers a comprehensive approach by simultaneously mitigating oxidative stress, suppressing inflammatory mediators, and improving tear film stability; a key pathological mechanism implicated in CVS progression. *In vitro* permeation studies further demonstrated a desirable release pattern wherein rapid diffusion of gallic acid provided immediate anti-oxidant protection while sustained release of sodium hyaluronate ensured prolonged ocular surface lubrication. Histopathological analysis confirmed the effectiveness of the formulation on UV-induced damaged corneal tissue, demonstrating notable rejuvenation and structural restoration. These findings substantiate the protective and reparative potential of the developed ophthalmic solution, thereby concluding that OPS2 is highly effective in the prophylaxis of acute conditions associated with Computer Vision Syndrome. The use of an *ex vivo* goat corneal model further strengthens the translational relevance of the findings, as it closely mimics human corneal tissue response.

Overall, the developed ophthalmic formulation was found to be pharmacologically active, physically stable, and therapeutically promising for the management of Computer Vision Syndrome. The study contributes novel evidence supporting the integration of polyherbal antioxidants with biocompatible polymers in ophthalmic drug delivery, thereby extending current knowledge beyond symptomatic relief toward mechanism-based prophylactic intervention. Despite these encouraging outcomes, certain limitations of the study should be acknowledged. The investigation was limited to *in vitro* and *ex vivo* evaluations, and *in vivo* pharmacokinetic and clinical efficacy studies were not conducted. Additionally, long term stability studies and patient- based tolerability assessments remain to be explored. Future research should focus on *in vivo* validation, clinical trials, and long - term safety evaluation of the formulation in CVS patients. Further optimization using advanced ocular delivery systems and assessment of its efficacy in chronic and severe stages of CVS may also expand its clinical applicability. In conclusion, the present work lays a strong foundation for the development of a safe, effective and integrative ophthalmic prophylactic therapy for Computer Vision Syndrome in the context of increasing global digital screen dependency.

Abbreviations

ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), CDR: Cumulative Drug Release, COX-II: Cyclooxygenase II, CVS: Computer Vision Syndrome, H and E: Hematoxylin and Eosin, IC_{50}: Half Maximal Inhibitory Concentration, UV: Ultra-Violet (or Ultra-Violette)

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Author Contributions

Hrithik Satish Revankar: conceptualization, methodology, investigation, Shilpa Bhilegaonkar: supervision, statistical analysis.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Experimental data.

Declaration of Generative AI and AI Assisted Technologies in The Writing Process

The authors declare to have not used any sort of artificial intelligence assistance.

Ethics Approval

Not applicable.

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