

Formulation and Evaluation of Sodium Hyaluronate – Triphala Ophthalmic Emulsion for Treatment of Computer Vision Syndrome

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ABSTRACT

Background: Computer vision syndrome is a eye condition linked to long periods of screen use. Early signs include irritation, redness and dryness. Chronic cases can lead to serious issues like high myopia, vitreous floaters and retinal detachment. The study is aimed to create ophthalmic emulsion based on *Triphala Ghrita* that has antioxidant, anti-inflammatory and tissue healing properties. **Materials and Methods:** The emulsion was made using *Triphala Ghrita* (oil phase), 0.1% sodium hyaluronate (aqueous phase), Tweens and spans (surfactant) and ethanol (co-surfactant). Six formulations were assessed physicochemical properties. Stability studies found that E3 was the most effective formulation. The pharmacological effects using antioxidant and anti-inflammatory assay were analyzed. *In vitro* permeation using eggshell membrane and cornea tissue was performed and finally histopathological studies were conducted to confirm the therapeutic ability of the formulation. **Results:** E3 formulation showed stable physicochemical properties and clear morphology. Antioxidant tests indicated the formulation has better antioxidant potency than the references used, also it was a potent anti-inflammatory agent as well. Permeation studies showed oil phase did not permeate eggshell membrane but using goat corneas the aqueous phase showed a CDR of 9.7% at 30 min and also the oil phase did permeate. Histological analysis confirmed rejuvenation of corneal tissue after treatment with emulsion. **Conclusion:** The optimized ophthalmic emulsion (E3) showed strong anti-oxidant, anti-inflammatory effects, effective corneal penetration and rejuvenation of corneal tissue which highlights its potentials as a treatment for ocular damage due to CVS

Keywords: Computer Vision Syndrome (CVS), Sodium hyaluronate, Triphala, 5-retinal disease, Cornea, Ocular damage, Screen, Emulsion.

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INTRODUCTION

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. Computer vision syndrome as the name suggests is not a disease but a syndrome which means it is a combination of multiple diseases resulting in a syndrome.¹ Computer vision syndrome 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.²

The treatment for these conditions does exist in allopathy, but are limited in providing temporary symptomatic relief, with addressing the root cause that is to prevent screen induced ocular damage. Practically the ophthalmologist will not classify the symptoms under computer vision syndrome or may misdiagnose due to the similarity of symptoms with other common diseases like dry eye or blepharitis,^{3,4} hence the treatment limits to symptomatic relief and doesn't address the origin of the condition. There are treatments in Ayurvedic medicine as well for the treatment of computer vision syndrome however the treatment procedure contains some flaws that needs to be addressed. Netra Tarpana is an Ayurvedic procedure that is conducted in cases of screen induced ocular diseases wherein medicated ghee (*Triphala Ghrita*) is poured in the eye and the eye is blinked in the pool of medicated ghee for a few minutes. The main drawback about this procedure is that eventually the ghee cools and solidifies into particular aggregates thus increasing the risk of corneal scratches, hence attempts were made to amalgamate the Allopathic and



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Ayurvedic treatment approach to address the computer vision syndrome in a more practical and therapeutically advanced manner.

Ocular drug delivery is associated with drug delivery to the eye considering all the physiological and formulation barrier. The main challenge in developing an ocular dosage form is to incorporate appropriate therapeutically valid dosage in a simple eye drops as the eye drops are the easiest dosage forms to produce commercially and also patient compliant. The research overall dwells in the development of simple ocular ophthalmic emulsion to further encourage its commercialization.

Sodium Hyaluronate an allopathic polymer molecule and *Triphala Ghrita* were used in the development of the ophthalmic emulsion. Sodium Hyaluronate known for its hydrating properties was considered in the formulation and also sodium hyaluronate being a constituent of the vitreous was a scientifically valid molecule to be used in the formulation.^{5,6} *Triphala Ghrita* which is a famous Ayurvedic polyherbal formulation containing AmLa, Haritaki, and Bibhitaki (in ratio of 1:2:3) infused in ghee is known for its antioxidant and anti-inflammatory properties and is widely used in the Netra Tarpana process hence was considered for this study. The pathophysiology of Computer vision syndrome involves oxidative stress, inflammation, tear film instability and epithelial micro damage therefore *Triphala Ghrita* was considered as it can counter these mechanisms simultaneously. The Ghrita contains *Phyllanthus emblica*, *Terminalia chebula*, and *Terminalia bellirica* which are rich in polyphenols and tannins which help combat and neutralize screen -induced reactive oxygen species and diminish the inflammatory mediators responsible for ocular irritation and other symptoms. The Ghrita base contributes a lipid layer analogous to the outer tear film thereby providing a lubricating effect which helps in combating with ocular dryness, basically it nourishes the eye. Additionally, lipid soluble phytoconstituents promote healing and rejuvenation of damaged tissue making formulation therapeutically relevant for the prophylaxis of digital-eye strain associated ocular damage.⁷

MATERIALS AND METHODS

Materials

A variety of chemicals and equipment's were utilized in this research carried out. *Triphala Ghrita* was purchased from Baidyanath Ayurveda Pvt. Ltd., Sodium Hyaluronate was sourced from Aseschem Chemical Works Jodhpur India, Propylene Glycol, Tween 60, Tween 80, Span 60, Span 80, Ethanol, Methanol, Acetone, 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.

Methods

Formulation of ophthalmic emulsion

Screening of the surfactant

Screening of the surfactant is vital as it helps in the selection of the right surfactant used in the emulsification process, if the active agent is insoluble then it will not involve in the emulsion and will remain as a separate entity.⁸ A surplus quantity of *Triphala Ghrita* (11 mg) was taken in 5 mL vial to which 1 mL the surfactant (Tween 60, Tween 80, Span 60, Span 80) and was placed on an orbital shaker at room temperature for 24 hr. Later the solutions were centrifuged for 15 min at 3000 rpm and were visually analyzed for any visible particles, precipitates and other insoluble materials. Since *Triphala Ghrita* is a polyherbal formulation it cannot be practically determined by UV analysis hence solubility is concluded on basis of visual observations. If the *Triphala Ghrita* is fully dissolved with a clear solution it is assumed to be completely soluble. Similarly, if any cloudiness precipitates or any particles are visible it is either partially soluble or insoluble depending on the magnitude of the cloudiness or precipitate.

Screening of the co-surfactant

A surplus quantity of *Triphala Ghrita* (11 mg) was taken in 5 mL vial to which 1 mL the surfactant (Propylene glycol and ethanol) and was placed on an orbital shaker at room temperature for 24 hr. Later the solutions were centrifuged for 15 min at 3000 rpm and were visually analyzed for any visible particles, precipitates and other insoluble materials. The purpose of screening for co-surfactant is to select the co-surfactant which offers highest solubility for the emulsion which will further influence the emulsification process. Since *Triphala Ghrita* is a polyherbal formulation it cannot be practically determined by UV analysis hence solubility is concluded on basis of visual observations. If the *Triphala Ghrita* is fully dissolved with a clear solution it is assumed to be completely soluble similarly, if any cloudiness precipitates or any particles are visible it is either partially soluble or insoluble depending on the magnitude of the cloudiness or precipitate.

Preparation of the aqueous phase

The aqueous phase for the emulsion is 0.1% sodium hyaluronate solution. 100mg of sodium hyaluronate is dissolved in 100 mL of distilled water in a 250 mL beaker and is stirred over a magnetic stirrer for 15 min to completely dissolve sodium hyaluronate to obtain the solution.

Preparation of the oil phase

Triphala Ghrita which is the active ingredient of the formulation is sourced from a local vendor and is directly used as the oil phase. *Triphala Ghrita* which is the polyherbal ghee formulation, a quantity of 100 mL is taken in a beaker and heated over a water

bath for 15 min (make sure not to boil the ghee) to make it more flowable.

Preparation of the Emulsion

Preparation of emulsions was carried out using different combinations of the surfactant and co-surfactant with different ratios.^{10,11} Here we have used combinations of surfactants further combined with co-surfactants to achieve a proper HLB balance. 6 grams of the oil (*Triphala Ghrita*) is taken in a small beaker and required quantities of the surfactants (Span 60, span 80 and Tween 60) are added to the oil phase with continuous stirring. Span 60 and Span 80 are lipophilic surfactants having HLB values of 4.7 and 4.3 respectively. Tween 60 although hydrophilic it is added in oil phase as tween 60 tends to clump up in aqueous phase. Aqueous phase sufficient enough to make a total volume of 30 mL is taken in another beaker and is mixed with required quantities of Tween 80 and the cosurfactants (propylene glycol and ethanol).

Now the aqueous phase containing surfactant and the cosurfactant is kept on a magnetic stirrer for 5-10 min to homogeneously disperse the solution. In a syringe 6 mL of the oil phase (combined with surfactant) is taken and dropwise the droplets are released in the aqueous phase over the magnetic stirrer and is continuously stirred for 15 min so that a stable emulsion is formed with homogeneously dispersed oil droplets in the aqueous phase. Further the formed emulsion is subjected to ultrasonication for 10 min to homogenize the system further and obtain droplet size as small as possible to achieve maximum stability.

A total of 6 formulations were prepared using different combinations of surfactant and co-surfactant keeping the ratios constant as mentioned in the Table 1 below.

Evaluation of the Emulsion

Physicochemical evaluation

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.¹²⁻¹⁴ pH of the various combination of solution was tested using 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 formulations flowability (in case of solutions) which in turn affects ease of manufacturing, packaging and instillation into the eyes. Viscosity was tested using Brookfield viscometer and the readings were recorded.

Typographical Evaluation

Typographical evaluation is done using Laser Confocal Microscopy (Olympus Corporation FV3000). Typographical evaluation is necessary as it gives an idea about how homogeneously the oil droplets are dispersed in the dispersing medium, which in turn affects the stability of the emulsion system.

At first the slide to be mounted on the equipment is prepared. A drop of emulsion is gently placed on a slide and is covered by coverslip no 1.5. The coverslip is placed carefully to avoid any air bubbles and is allowed to dry for a few seconds. Any excess flowing sample is wiped with a tissue and the slide is ready for use.

The equipment is switched on (FV3000 main unit, PC stage controller and the transmitting light source). Initiate the Olympus FV-OS software and select the objective (20x). Within the software disable the laser and enable only transmitted light path. Next the prepared slide is loaded on the motorized stage and the position of the field view is adjusted using the joystick. Focus on a droplet plane using the coarse and fine adjustments. Now insert the DIC prisms slider in the optical path and align with the objective and adjust the polarizer and the analyzer in a intersected position. Rotate the DIC prism knob to modulate contrast and obtain real life like appearance of the droplets. Switch the camera to non-confocal mode and capture the image.

Based on the results of typographical evaluation the best formulation is selected and subjected to particle size analysis and zeta potential analysis.

Particle size analysis (Dynamic light scattering) and zeta potential

Particle size analysis is done by using NanoPlus with NanoPlus auto-titrator equipment. The advantage of using this instrument is that it is quick and additionally gives information about the polydispersity index as well. The purpose of determining the particle size is to identify the accurate size of each droplet which helps us in understanding the stability of the emulsion on basis of surface area concept as smaller the particle better is the emulsion, similarly zeta potential gives an idea of the electrical barrier formed around each droplet which repels the droplets from each other thus avoiding aggregation of droplets and failure of emulsion system

To determine the particle size that samples are first prepared by diluting the emulsion in deionized water and is subjected to sonication for 2 min to disrupt any aggregates. The equipment is switched on and the titrator is set up by attaching the acid or the base titrants to the auto titrator syringe ports. Next calibrate the pH probe and the equipment is ready for use

The prepared samples are loaded in the curette and the currettes are placed in the measuring chamber of the equipment and the

pH probe and the titrator tubing is attached. DLS mode is selected in the software and start the run, the machine will automatically measure the particle size and polydispersity index. The machine automatically measures three times and an average reading is displayed.

To determine the zeta potential that samples are first prepared by diluting the emulsion in deionized water and is subjected to sonication for 2 min to disrupt any aggregates. The equipment is switched on and the titrator is set up by attaching the acid or the base titrants to the auto titrator syringe ports. Next calibrate the pH probe and the equipment is ready for use.

The prepared samples are loaded in the cuvette and the cuvettes are placed in the measuring chamber of the equipment and the pH probe and the titrator tubing is attached. Select the electrophoretic mobility measurement parameters and run the instrument. The instrument automatically measures the zeta potential and the readings are displayed.

Stability Evaluation

The formulated emulsions were subjected to stability analysis to check how stable the system is and to identify any creaming or cracking in the emulsion system. Stability analysis is vital so as to have an understanding upon the formulation dynamics and also the storage considerations.¹⁵⁻¹⁷

Freeze-Thawing

The obtained emulsions were subjected to free-thawing by keeping them in -5°C for 24 hr and then immediately transferring them in an oven at 35°C. The process is repeated 3 times on each emulsion and any cracking; phase separation or turbidity is observed.

Heat-cool cycle

The emulsions were heated on a water bath at approximately 45°C and then instantly cooled over an ice bath. The process is repeated for 3 times and any cracking, phase separation or turbidity is observed.

Centrifugation

The emulsions were centrifuged at 3000 rpm for 20 min and was observed for any cracking or turbidity.

PHARMACOLOGICAL EVALUATION

Pharmacological evaluations were performed the dosage forms (ophthalmic emulsion) 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.

Antioxidant Assay

ABTS {2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) Radical Scavenging Activity}

ABTS is an oxidative radical involved in the oxidation process which is applicable for the light induced ocular damage seen in case of computer vision syndrome hence evaluating the scavenging activity for the ABTS radicle will help in advocating that the developed formulation is a capable candidate for the prophylaxis of computer vision syndrome.^{18,19} 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°C for 10 min in the dark. The wells without treatment were considered to be as control. After incubation the absorbance is measured of the decolorization at 734 nm using microplate reader (iMark BioRad). Results were presented with respect to negative control. IC₅₀ was calculated using software Graph Pad Prism 6. Graph was prepared between X axis (sample concentration) Vs Y axis (% inhibition w.r.t. control).

Calculations

$$\%RSA = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

RSA = Radical Scavenging Activity.

Abs_{control} = Absorbance of the control.

Abs_{sample} = Absorbance of the sample.

Hydroxyl Free Radical Scavenging Activity

Hydroxyl free radical is an oxidative radical involved in the oxidation process which is applicable for the light induced ocular damage seen in case of computer vision syndrome hence evaluating the scavenging activity for the hydroxyl free radicle will help in proving that the developed formulation is a capable candidate for the prophylaxis of computer vision syndrome 66 µL reagent mixture (10 µL EDTA0.5M) 24.14 mg Deoxyribose, 88 µL ferric chloride (10 mg/mL), 28 µL hydrogen peroxide 6% and water up to 33 mL and 10 µL of the sample, 24 µL of phosphate buffer (50 nM pH 7.4) and 10 µL of ascorbic acid were added in the wells of 96 plate in sequence and mixtures were incubated at 37°C for 1 hr. The wells without treatment were considered as control.^{20,21} Gallic acid was used as standard. After incubation 50 µL of 10% TCA and 50 µL of 1% TBA were added to each well. A pink chromatogram was developed. And absorbance was taken at 540 nm wavelength using microplate reader. IC₅₀ was calculated using software Graph Pad Prism 6. Graph was prepared between X axis (sample concentration) Vs Y axis (% inhibition w.r.t. control).

$$\%RSA = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

RSA = Radical Scavenging Activity.

Abs_{control} = Absorbance of the control.

Abs_{sample} = Absorbance of the sample.

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

COX-II enzyme is a biochemical marker involved in the process of inflammation hence a study used to identify the magnitude of blocking this marker will justify the usage of the formulation for computer vision syndrome as ocular inflammation is a key highlight of this syndrome.^{22,23} Sample dilutions and buffer (Tris chloride buffer 100 mM, pH 8.0) was prepared. Reaction buffer (Enzyme in Tris/heme/phenol; 100 Mm/1 μM) and buffer Bovine Hemin Chloride were placed in 96 well plate. 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 min and the absorbance was taken at 595 nm using ELISA microplate reader (iMark BioRad). Inhibitor, celecoxib (2500 μM final concentration) was used as a positive control. IC₅₀ was calculated using software Graph Pad Prism 6. Graph was prepared between X axis (sample concentration) Vs Y axis (% inhibition w.r.t. control).

Calculations

$$\%inhibition = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Abs_{control} = Absorbance of the control.

Abs_{sample} = Absorbance of the sample.

IN VITRO EVALUATION

The *in vitro* permeation of the formulation was conducted using Franz Diffusion cell using eggshell membrane and goat corneal tissue.^{24,25} The sole purpose of this study to understand the permeation behavior of the formulation across the biological membranes. The membranes (eggshell and corneal tissue)

were used as a barrier to separate the donor and the receptor compartment. The receptor compartment contained phosphate buffer 7.4. The donor compartment was filled with 2 mL of the emulsion. At 0, 15 and 30 min the 5 mL of aliquots were withdrawn and buffer was used to replenish the receptor compartment. Individually the aliquots were withdrawn the analyses the oil phase and the aqueous phase of the emulsion.

To detect the oil phase of the emulsion, 2 mL of the sample was extracted using acetone -methanol solution and was filtered. The filtered solution was scanned in the UV region using a spectrophotometer and the spectrum was compared with the spectrum of the oil phase that was taken previously.

To detect and quantify the aqueous phase of the emulsion the withdrawn samples were directly analyzed by UV spectrophotometer at 202 nm that is specific for sodium hyaluronate.

HISTOPATHOLOGICAL STUDY

Histopathology study involves the study of the biological tissues at cellular levels, the importance of this study here is to identify if they is any formulation related damage occurring in the eye at cellular scale and most importantly to identify the difference between the light induced damaged ocular tissue and the formulation treated damaged tissue.²⁶ Understanding histology will help in identification of the repair and or rejuvenation of the damaged tissue. 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 a 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 is allowed to dry. The petri dish is then placed in a UV chamber on a heightened platform such that the tissues in the petri dish are just a few inches away from the UV chambers light source. The tissues are exposed to UV light for a period of 15 min to induce

Table 1: Surfactant-surfactant ratio, surfactant-cosurfactant ratio, oil-S_{mix} ratio used in formulations.

Formulation code	Surfactant - surfactant ratio	Surfactant: co-surfactant ratio	Oil: S _{mix} ratio	Total volume
E1	2:1	2:1	7:12	9.5
E2	2:1	2:1	7:12	9.5
E3	2:1	2:1	7:12	9.5
E4	2:1	2:1	7:12	9.5
E5	2:1	2:1	7:12	9.5
E6	2:1	2:1	7:12	9.5

UV induced damage to the tissue. After 15 min the tissues were immersed in the formalin solution to preserve the tissue.

The tissues are dehydrated by passing it through a series of ethanol of various strength (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 slides and is dried at 50-60°C.

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 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.

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 emulsion) was sterilized using drug heat sterilization. Sterilization is vial in case of ophthalmics so as to ensure its free from any sort of pathogens which may infect the site after the application of the dosage form.

The dosage forms were sealed in a glass bottle and were sterilized using an autoclave at 121°C for 15 min.²⁷

Sterility Testing of Finished Products

The sterility testing of both ophthalmic suspension and ophthalmic emulsion is done by the direct inoculation method.²⁸ Sterility testing is vital as it is a confirmative step to confirm the sterilization process. 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°C for 5 days to check for any turbidity. For ophthalmic emulsion 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 laminar air flow unit to maintain sterile conditions.

RESULTS

Formulation of Ophthalmic Emulsion

Screening for surfactant

Screening for surfactant is done by conducting saturation solubility of *Triphala Ghrita* is various surfactants. Here Tween 60, Tween 80, Span 60 and Span 80 were used as surfactants and the solubility of *Triphala* was checked. In tween 60 and tween 80 exactly 8.50 g and 9.75 g of *Triphala Ghrita* was dissolved similarly in span 60 and span 80, 8 g and 8.75 g of *Triphala Ghrita*

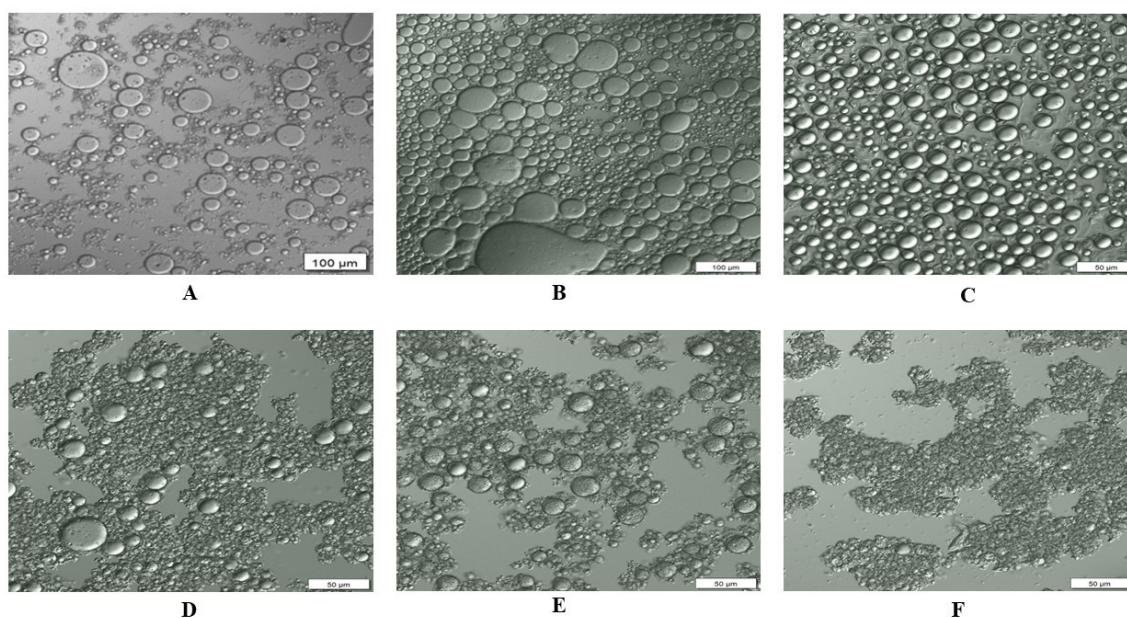


Figure 1: Laser Confocal Microscopy (LCM) images of formulation E1-E6. A LCM of E1 formulation, B LCM of E2 formulation, C LCM of E3 formulation, D LCM of E4 formulation, E LCM of E5 formulation and F LCM of E6 formulation. Formulation E2 and E3 were considered to be typographically satisfying and hence were considered for subsequent evaluations.

was dissolved respectively. From the observations, it is found that *Triphala Ghrita* in Tween 80 exhibits highest solubility.

Screening of the co-surfactant

Screening for co-surfactant is done by conducting saturation solubility of *Triphala Ghrita* in various co-surfactants. Here Propylene glycol and Ethanol were used as co-surfactants and the solubility of *Triphala* was checked. In propylene glycol exactly 8.5 g of *Triphala Ghrita* was soluble and in ethanol 7.3 g of *Triphala Ghrita* was soluble thus, it is found that *Triphala Ghrita* in Propylene glycol exhibits highest solubility.

Preparation of the aqueous phase

100 mL of 0.1% sodium hyaluronate solution was prepared. The prepared solution was found to be mildly viscous in nature, contained no particulate matter and was a clear solution.

Preparation of the oil phase

100 mL *Triphala Ghita* was treated over heat to dissolve all the solidified ghee particles. The resultant solution was a slightly reddish colored oily transparent liquid which was further used as oil phase for the emulsion.

Preparation of the emulsion

Six formulations of emulsions were prepared as per the procedure. The obtained emulsions were milky and slightly viscous in nature. The master formula used in preparation of the formulations are shown in Table 2.

The emulsions were further evaluated for its physicochemical properties to select the most appropriate formulations.

Evaluation of the emulsion

Physicochemical evaluation

Test for pH (and Modification) and test for viscosity

The pH of all 6 formulated emulsions was evaluated. Formulation E1, E4, E5 and E6 required modifications to adjust pH while formulation E2 and E3 require any modifications as

depicted in Table 3. Modifications to adjust pH was done by using triethanolamine or citric acid depending on the pH. The formulations were evaluated to test the viscosity. All the formulations showed viscosity ranging from 75-100 centipoise and were within the range of ophthalmic requirement.

Typographical Evaluation

The typography of formulation E1 (Figure 1A) shows that the oil droplets are unevenly sized and are dispersed in the aqueous media. It is also seen that the oil droplets are not closely dispersed and there exists a lot of inter-globule distance which is not ideal for emulsion stability. It is also seen that some amount of particulate matter is present between the inter-globule space that exists.

The typography of formulation E2 (Figure 1B) shows a combination of spherical small and medium sized oil globules dispersed in the aqueous phase. The smaller globules are seen to fill the inter-globule spaces of medium sized oil globules. Overall, there is a very mimical inter-globule space and shows that the oil globules are homogenously distributed in the aqueous media.

The typography of formulation E3 (Figure 1C) shows that the oil globules are small, equally sized and homogenously dispersed in the aqueous phase. The inter-globule spaces are also well defined and the oil globules are compactly dispersed. The typography represents an ideal stable emulsion system.

The typography of formulation E4 (Figure 1D) revealed a poorly dispersed oil globules in the aqueous phase. The number of oil globules seen were also less in number as compared to other formulations. It is also seen a large amount of particulate matter surrounds the oil globules which can make the system unstable.

The typography of formulation E5 (Figure 1E) revealed the oil globules are evenly sized and are dispersed distant to each other in the aqueous media. The distribution is not homogeneous and a large amount of inter-globule space is seen. It is also seen that particulate matter surrounds the oil globules and is freely dispersed in the aqueous media.

Table 2: Quantities of ingredients taken for preparation of the emulsion.

Ingredients (g)	Formulations					
	E1	E2	E3	E4	E5	E6
Tween 80	1.5				0.75	0.75
Tween 60		1.5	1.5	0.75		
Span 60	0.75			1.5	1.5	
Span 80		0.75	0.75			1.5
Propylene Glycol	1.25		1.25		1.25	
Ethanol		1.25		1.25		1.25
Oil (<i>Triphala Ghrita</i>)	6					
Aqueous phase (0.1% Sodium Hyaluronate solution)	20.5 mL (total volume 30 mL)					

The typography of formulation E6 (Figure 1F) revealed there is no presence of oil globules dispersed in the aqueous phase. It is also seen that there is large quantity of particulate matter present in aqueous phase.

Particle size analysis and zeta potential

Formulation E2 showed an average particle size of 654 nm and polydispersity of 0.345. Formulation E3 showed an average particle size of 3492 nm and polydispersity of 1.414. The zeta potential of E2 and E3 were -0.31mV and -90mV respectively (Table 4).

Stability Evaluation

Stability evaluation was conducted on E1 and E2. E3 has qualified all the stability tests which included freeze thawing, heat cool cycles and centrifugation. E2 failed to pass the centrifugation test, and through these results it is considered that E3 is the most optimized formulation.

The emulsion was formulated and was subjected to various physicochemical evaluation and through the results obtained from the evaluation formulation E3 is considered to be the most optimized formulation and hence is used for all other pharmacological and *in vitro* assessment

PHARMACOLOGICAL EVALUATIONS OF OPHTHALMIC EMULSION

The pharmacological evaluation was conducted for the prepared emulsion 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

Antioxidant Assay

ABTS { 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)} Radical Scavenging Activity

Based on the results obtained from the experimental work (Figure 2A and Figure 2B) Antioxidant activity (ABTS Radical Scavenging Assay) was estimated in sample and 50% inhibitory concentration. Sample E3 was found to be equivalent to 26.65 µg of standard Ascorbic acid.

In case of reference standard (ascorbic acid) the absorbance values increased based on concentration from 0 to 50 µg/mL. The mean values ranged from 0.00 (blank) to 73.39 at 50 µg/mL. Statistical analysis showed more variation at lower concentrations which stabilize at higher concentrations (SD=149 at 50 50 µg/mL). Non-linear regression analysis showed an IC₅₀ value of 26.65 µg/mL with a hillslope of 14 and a high degree of fit (R²=0.9912). The 95% confidence interval for IC₅₀ was 23.41 µg/mL.

The emulsion sample showed a concentration- dependent increase in absorbance from 0-5 µg/mL with mean values ranging from 0.00 (blank) to 102.75 at 5 µg/mL. The statistical variation was low at higher concentrations (SD=0.85-4.09). Nonlinear regression analysis found a notably lower IC₅₀ of 0.7051 µg/mL with a Hillslope of 1.882 and good curve fitting (R²=0.9786). The 95% confidence interval for IC₅₀ was 0.5456-0.9113 µg/mL.

The comparison between the Emulsion (E3) and the reference showed that E3 has a much stronger ABTS radical scavenging capacity than ascorbic acid. This is shown by the nearly 38 folds lower IC₅₀ value (0.7051 µg/mL compared to 26.65 µg/mL). Both the samples had strong fitting model (R²> 0.97) confirming the reliability of the regression analysis.

Hydroxy Free Radical Scavenging Assay

Based on the results obtained from the experimental work (Figure 2C and Figure 2D), Antioxidant activity (Hydroxy Free Radical

Table 3: pH and viscosity measures of formulation E1-E6.

Formulation	pH		Viscosity
	Before Modification	After Modification	
E1	6.9	7.25	75
E2	7.2	7.2	75
E3	7.44	7.44	75
E4	6.8	7.56	100
E5	7.95	7.45	75
E6	8.2	7.66	75

Table 4: Average particle size, Polydispersity and Zeta potential of formulation E2 and E3.

Formulation	Average particle size (nm)	Polydispersity	Zeta potential
E2	654.5	0.345	-0.31mV
E3	3492	1.414	-90mV

Scavenging Assay). 3.504 µg of sample E3 was found equivalent to 7.515 µg of standard Gallic acid.

The reference compound (Gallic Acid) showed a clear increase in scavenging activity that is dependent on concentration across the tested range of 0 to 100 µg/mL. The mean absorbance rose from 0.00 blank to 79.63 at 100 µg/mL. The variability between replicates was low at higher concentration (SD=3.38 TO 5.56). Nonlinear regression analysis indicated an IC₅₀ of 7.515 µg/mL with a Hillslope of 0.5464. This pointed to a gradual concentration-response curve. The model fit was excellent (R²=0.9975) and the 95% confidence interval for IC₅₀ ranged from 6.902 to 8.183 µg/mL.

The emulsion sample E3 showed a concentration dependent increase in the hydroxyl radical scavenging activity across the concentration range of 0-5 µg/mL. The mean absorbance values increased from 0.00 (blank) to 53.03 at 5 µg/mL. The variability was minimal at higher concentrations (SD=1.70 TO 2.02). Nonlinear regression analysis provided an IC₅₀ of 3.504 µg/mL with a Hillslope of 0.4115. The regression fit was strong (R²=0.9827), and the 95% confidence interval for IC₅₀ was 2.585 to 4.751 µg/mL.

In comparison to gallic acid the emulsion had greater hydroxyl free radical scavenging activity as shown by its significantly lower IC₅₀ value (3.504 µg/mL vs 7.515 µg/mL). While the concentration response slope of emulsion (Hillslope= 0.4115) was slightly shallower than that of gallic acid (Hillslope=0.5464) both compounds displayed strong regression fits (R² >0.98) confirming reliability of the results.

Anti-Inflammatory Analysis (Cyclo-oxygenase-II Inhibition Assay)

Based on the results obtained from the study (Figure 2E and Figure 2F), Enzyme Inhibition Activity (COX-II) was estimated in all samples and 50% inhibitory concentration. Sample- E3 was found active. 1.562 µL of the sample – E3 was found equivalent to 1732 µM of the standard Celecoxib. Lower is the IC₅₀, higher be inhibition activity.

The reference compound celecoxib showed a concentration-dependent inhibition. The mean absorbance values ranged from 0.64 at 0.78 µg/mL. to 58.26 at 2500 µg/mL. The variability across concentration was moderate (SD=5.15 to 9.86). Non-linear regression analysis showed a high IC₅₀ of 1.732 µg/mL. and a Hillslope of 0.8576. The regression fit was strong

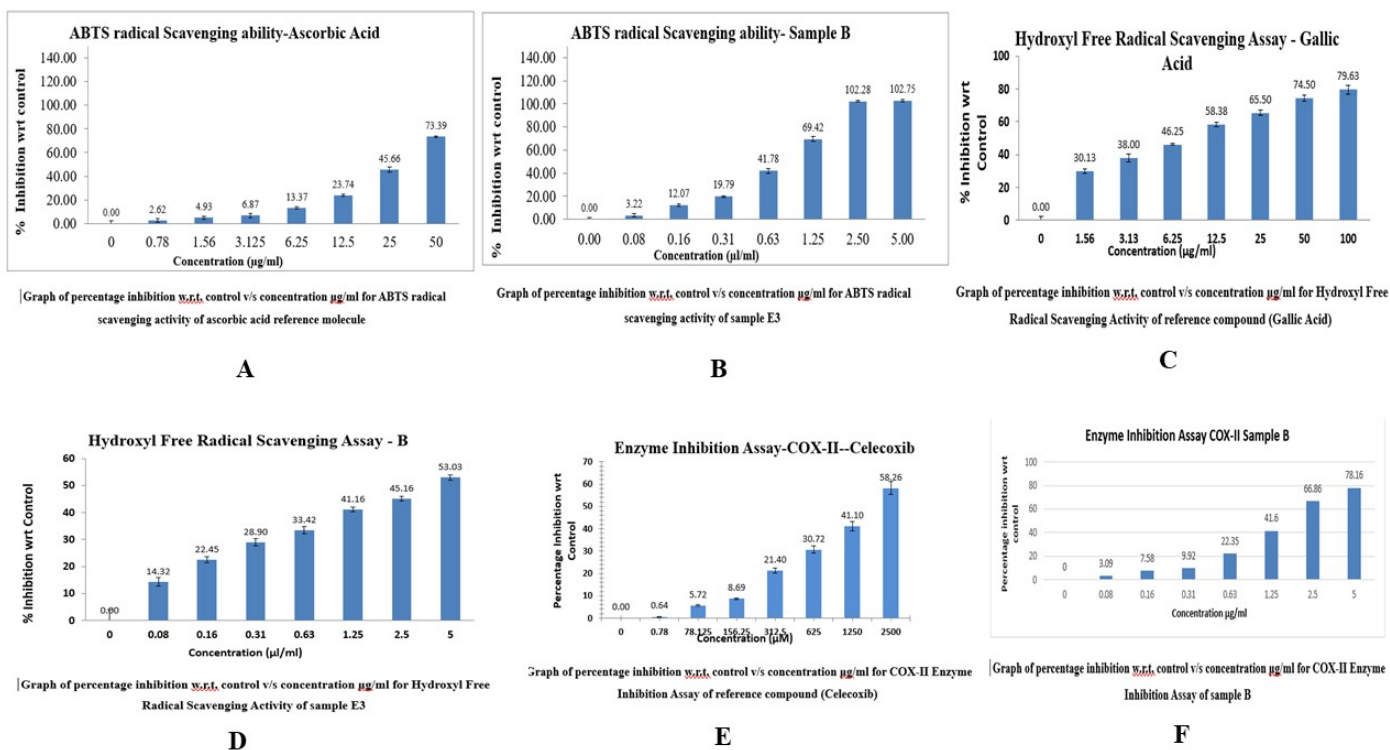


Figure 2: Graph of percentage inhibition w.r.t. control v/s concentration µg/mL, (A) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for ABTS radical scavenging activity of ascorbic acid reference molecule, (B) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for ABTS radical scavenging activity of sample E3, (C) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for Hydroxyl Free Radical Scavenging Activity of reference compound (Gallic Acid), (D) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for Hydroxyl Free Radical Scavenging Activity of sample E3, (E) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for COX-II Enzyme Inhibition Assay of reference compound (Celecoxib), (F) Graph of percentage inhibition w.r.t. control v/s concentration µg/mL for COX-II Enzyme Inhibition Assay of sample.

($R^2=0.9922$). The curve indicated a gradual inhibition profile at increasing concentrations.

The emulsion sample E3 demonstrated stronger inhibitory effect at much lower concentrations. The absorbance values steadily increased from 0.00 (blank) to 78.16 at 5 $\mu\text{g/mL}$. There was minimal variability across the replicates ($SD=0.09$ to 2.27). Non-linear regression analysis revealed a much lower IC_{50} of 1.33 $\mu\text{g/mL}$ with a steeper Hill slope of 1.722. The goodness of fit was excellent ($R^2=0.9984$) suggesting that the inhibition curve is highly reliable.

A clear difference was observed between the reference and emulsion. Celecoxib showed inhibitory activity only at very high concentrations while the emulsion achieved effective COX-II inhibition at significantly lower concentration ($IC_{50}=1.333 \mu\text{g/mL}$). The steeper the slope for the emulsion suggests a sharper inhibitory response compared to celecoxib which clearly indicate emulsion is more potent than the reference celecoxib in inhibiting COX-II enzyme.

IN VITRO EVALUATION

Permeation study was conducted for ophthalmic emulsion using eggshell membrane as the permeating membrane. Percentage cumulative drug release is calculated for the aqueous phase (sodium hyaluronate solution). It is observed that the percentage cumulative drug release at the 15th min is -18.909% and at the 30th min it is -9.62843 % (Table 5). Both the cumulative drug release values are in negative which is suggestive that sodium hyaluronate did not permeate through the eggshell membrane this is mainly due to proteinaceous structure of egg membrane which acts as a physical barrier and also carrier mediated transport system is not seen in eggshell membrane which prevents the transport of high molecular weight substances.

The permeation study was also conducted for the oil phase and directly spectra is taken by UV-spectrophotometer and is compared with the spectra of *Triphala Ghrita*. It is observed that the obtained spectra are different from the spectra of *Triphala Ghrita* however a peak at 277 nm is observed in spectra taken for the aliquot at 30th min which is similar to that of a peak observed at 270nm in the spectra of *Triphala Ghrita* (Figure 3A). This difference in spectra is suggestive that the oil phase has not permeated through the eggshell membrane or has partly permeated.

Permeation study for the ophthalmic emulsion was also conducted using freshly excised goat cornea as the permeating membrane and percentage cumulative drug release was calculated for sodium hyaluronate (aqueous phase) and the presence of oil phase is determined directly by taking UV spectra at the 30th min. It is observed that sodium hyaluronate as aqueous phase in emulsion permeated through the corneal tissue and showed a cumulative drug release of 3.10043% at the 15th min and 9.70762% cumulative drug release at the 30th min (Table 6). The presence of the oil phase i.e., *Triphala Ghrita* is confirmed by comparing the spectra taken from the aliquot at 30th min (Figure 3B) with the spectra of pure *Triphala Ghrita* (Figure 3C). The spectra of oil phase showed peaks at 362.40 nm, 352.40 nm, 338.60 nm, 276 nm, 269.40 nm, 231.40 nm, 204 nm. In contrast with the pure spectra of *Triphala Ghrita* which showed peaks at 362 nm, 350.60 nm, 316.80 nm, 270.20 nm, and 204.20 nm. Upon comparison the two spectra there are similar peaks seen in both the spectrums which confirms the presence of the original component (*Triphala Ghrita*) permeating through the corneal tissue.

HISTOPATHOLOGICAL ANALYSIS

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

Figure 4A shows H&E-stained tissue of normal goat cornea. It is seen that the normal goat cornea shows stratified squamous non-keratinized epithelium (a) 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 (b). The Bowmans layer is thin and acellular. Endothelium (c) is 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 4B, is the pathology induced goat corneal tissue. The epithelium shows thickening, loss of stratification and ulceration. (a) It is also evident that there is presence of dense infiltration of inflammatory cells in the stroma. (b) Stroma is edematous and contains disorganized collagen fibers. These characteristics are suggestive of neovascularization indicating angiogenesis due to inflammation.

Figure 4C is the histological slide of ophthalmic emulsion treated goat cornea tissue which was previously induced UV damage.

Table 5: Cumulative Drug Release of Sodium Hyaluronate in ophthalmic emulsion (aqueous phase).

Time	Absorbance	Concentration mcg	Conc mg	5 mL	200	Cumulative Drug Release (CDR)	% CDR
0	0	0	0	0	0	0	0
15	0.029	-0.53207517	-0.00053208	-0.00266	-0.10642	-0.10908	-18.9696
30	0.035	0.253952867	0.000253953	0.00127	0.050791	0.05206	9.053972

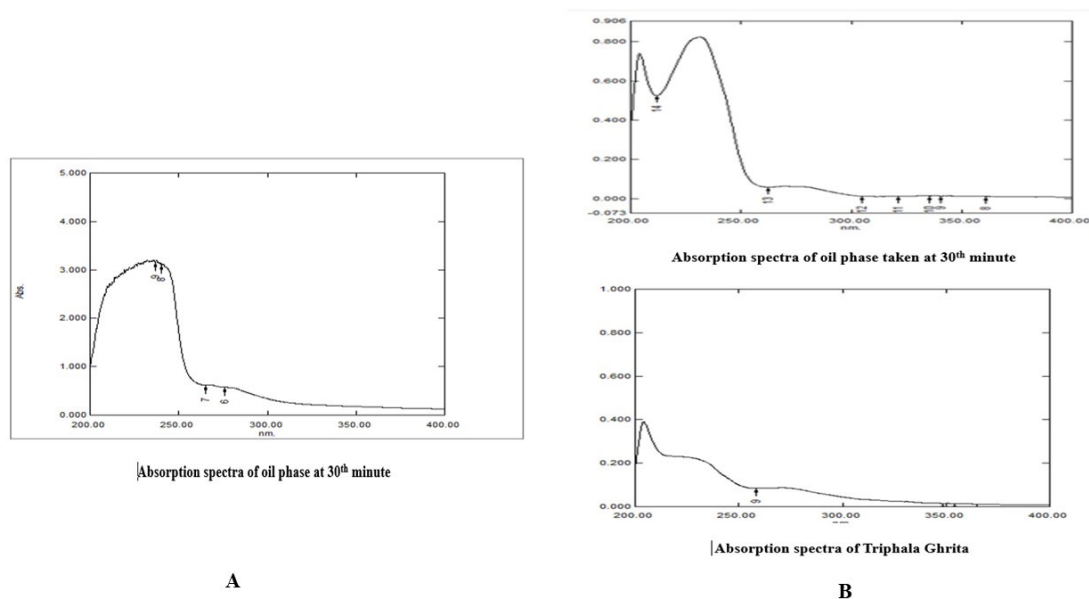


Figure 3: UV absorption spectra (A) Absorption spectra of oil phase at 30th min, (B) Absorption spectra of oil phase taken at 30th min, (C) Absorption spectra of *Triphala Ghrita*.

The histology of the tissue shows that the epithelium is near to complete restoration, (a) Multilayered that is close to normal morphology. The stroma shows minimal inflammatory cells along with well-organized collagen fibers and also the edema is resolved. (b) Overall tissue structure is similar to that of normal cornea which is a clear indication of superior healing.

Sterilization of Dosage Forms

The developed dosage forms (ophthalmic emulsion) were 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 after incubation for 7 days. The test tube 1 contains ophthalmic emulsion and the thioglycolate media, although the test tube looks turbid in nature but it is due to the milky nature of the emulsion. However, the intensity of turbidity remains the same as before the incubation period. Test tube 2 is the control group.

DISCUSSION

The formulation of ophthalmic emulsion began with the solubility screening of *Triphala Ghrita* (oil phase) in different surfactants and cosurfactants. *Triphala Ghrita* showed highest solubility in tween 80 (surfactant) and propylene glycol (co-surfactant). The emulsion was prepared using 0.1% solution of sodium hyaluronate as aqueous phase and *Triphala Ghrita* as oil phase. Different combinations of surfactant and co-surfactant along

with fixed quantity of oil and aqueous phase were used to prepare six emulsion formulations.

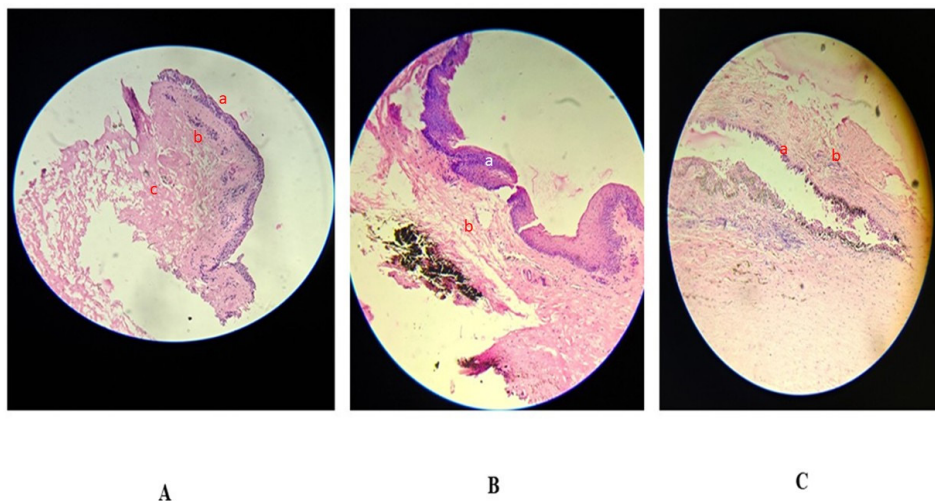
The developed emulsions were subjected to physicochemical evaluations, tests like pH, viscosity Zeta potential, particle size were determined. Typographical evaluation was conducted for all the five-emulsion formulation using laser confocal microscopy and all the five formulations presented different typography. Stability testing was also conducted on emulsions and subsequently E3 formulation was selected to be most optimized based on the evaluations.

Further pharmacological evaluations were conducted E3 (Emulsion). Antioxidant assays, anti-inflammatory was performed to determine the therapeutic ability of the formulations. In anti-oxidant assay ABTS, Hydroxyl free radical activity, studies were performed and sample (E3) showed higher antioxidant activities than the individual reference standards used. Similarly, both sample E3 showed anti-inflammatory responses (COX-II enzyme inhibition).

In vitro permeation evaluation of emulsion was performed using Franz diffusion cell using eggshell membrane as permeation membrane. The percentage cumulative drug release of sodium hyaluronate showed a negative value indicating that the sodium hyaluronate didn't permeate through the eggshell membrane which is mainly due to its higher molecular weight and also the oil phase was determined by comparing the spectral scan of pure *Triphala Ghrita* with the scan of extracted permeating medium after 30th min. The two scans were different indicating that oil phase did not permeate through the eggshell membrane. Similarly, *in vitro* permeation study using goat corneal tissue

Table 6: Cumulative drug release of Sodium hyaluronate (aqueous phase) using cornea as permeation membrane.

Time	Absorbance	Concentration mcg	Conc mg	5 mL	140	Cumulative Drug Release (CDR)	% CDR
0	0	0	0	0	0	0	0
15	0.034	0.122948194	0.000122948	0.000615	0.017213	0.017827	3.100433
30	0.036	0.384957539	0.000384958	0.001925	0.053894	0.055819	9.707625

**Figure 4:** Microscopic images of histology slides (A) Normal goat corneal tissue, (B) Pathology induced goat corneal tissue, (C) Ophthalmic emulsion treated goat cornea tissue.

as permeating membrane was conducted for the emulsion. The aqueous phase (0.1% sodium hyaluronate solution) showed a percentage Cumulative drug release of 9.70765% at the end of 30th min and also the spectral scan of fluid from reservoir chamber at the end of 30th min was similar to the spectral scan of pure *Triphala Ghrita* which exhibited peaks at 262.4, 352.4, 338.6, 276, 269.4, 231.4 and 204 nm which subsequently confirmed the permeation of oil phase through the cornea.

Histopathological analysis was conducted to study the practical effect of the formulation on cornea. Goat eyes were sourced from local slaughterhouse and corneas were extracted. The corneas were divided into four groups for the study. Firstly, the normal cornea was preserved using formaldehyde and remaining three corneas were subjected to UV damage using UV chamber. The 2nd cornea was preserved after inducing UV damage and 3rd cornea ophthalmic emulsion for 1 hr and were later preserved. H&E staining was done and the corneal tissues were examined on an optical microscope. The 3rd cornea presented features of healing wherein the epithelial and stromal cells had begun to rearrange themselves as seen in normal cornea. Overall, the ophthalmic

emulsion treated corneas presented rejuvenation after cellular damage and restoration of normal cellular histology.

CONCLUSION

The research on prophylaxis of Computer vision syndrome was successfully conducted. The gaps that existed in the available allopathic and Ayurvedic treatment approaches were identified and were addressed successfully. Formulation of ophthalmic emulsion were formulated and evaluated. The ophthalmic emulsion was aimed for the treatment of chronic conditions.

The developed dosage form was evaluated for its physicochemical characteristics, pharmacological effects and *in vitro* permeation characteristics and were found to be in par with the scientific specifications.

Histopathological analysis confirmed the effectiveness of the formulation on corneal tissue subsequently concluding that ophthalmic Emulsion (E3) is highly effective in prophylaxis of chronic conditions of CVS.

Ultimately the developed formulations (ophthalmic emulsion) were found to be pharmacologically active and physically stable formulations for the treatment of Computer Vision Syndrome.

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ABBREVIATIONS

CVS: Computer vision syndrome; **UV:** Ultra violets; **HLB:** Hydrophilic lipophilic balance; **DLS:** Dynamic light scattering; **ABTS:** 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **RSA:** Radical scavenging activity; **TCA:** Trichloroacetic acid; **TBA:** Thiobarbituric acid; **CDR:** Cumulative Drug Release; **SD:** Standard Deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Computer Vision Syndrome is a growing condition caused by long periods of screen exposure. Early symptoms include redness, irritation and dryness of the eyes. In severe cases, it can lead to problems like high myopia, vitreous floaters, posterior vitreous detachment, and retinal detachment.

To tackle this issue, we developed an ophthalmic emulsion that used *Triphala Ghrita* as the oil phase and 0.1% sodium hyaluronate as the aqueous phase. Our solubility test showed that Tween 80 (surfactant) and ethanol (co-surfactant) worked best as solubilizers. We created six formulations with different surfactant and co-surfactant ratios. We assessed these formulations based on pH, viscosity, zeta potential, and particle size. Lazer confocal microscopy revealed different morphologies, and stability tests found formulation E3 to be most effective.

Pharmacological tests showed strong therapeutic potential. Antioxidant assays (ABTS and hydroxyl radical scavenging) indicated that E3 had a higher activity than the reference standards. The anti-inflammatory activity, measured by COX-II inhibition, also demonstrated that E3 was more effective than celecoxib.

We conducted *in vitro* permeation studies Franz diffusion cells. With eggshell membrane, the sodium hyaluronate initially showed no permeation due to its high molecular weight, but became detectable after 30 min. Yet, spectral scans indicated that the oil phase did not permeate through this membrane. Using freshly excised goat cornea, the aqueous phase achieved a Cumulative Drug Release (CDR) of 9.7% within 30 min. Spectral scans of the reservoir medium showed characteristic peaks of *Triphala Ghrita*, confirming that the oil phase penetrated the corneal tissue.

Histopathological analysis further supported the therapeutic potential. We divided goat corneas into control, UV-damaged, and treatment groups. UV exposure caused significant disruption to epithelial and strong cells, while corneas treated with the ophthalmic emulsion showed signs of healing with epithelial and strong cell rearrangement that resembled normal corneal architecture. The tissues treated with the ophthalmic emulsion showed restoration of normal history, confirming corneal repair after damage.

In conclusion, formulation E3 demonstrated excellent physiochemical stability, strong antioxidant and anti-inflammatory properties, effective corneal permeation, and histological evidence of tissue repair. These results highlight its potential as a treatment for CVS related eye damage.

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