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FORMULATION, DEVELOPMENT AND EVALUATION OF ADVANCE HERBAL OPHTHALMIC PREPARATION (EYE DROP) CONTAINING ACTIVE CONSTITUENT FROM VARIOUS MEDICINAL PLANT SOURCE OF DIFFERENT REGION OF NORTH INDIA

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ABSTRACT

The aim of the present study was to formulate, develop and evaluate ophthalmic eye drop. In this research work we have formulated an herbal alternative for ophthalmic disorders. The number of ocular disorders is increasing daily due to rapid development of human civilization although there are synthetic formulations available still the demand for herbal alternative is growing daily, as people are getting more health oriented and the knowledge about the potential hazard of synthetic product is well known. Ayurvedic literatures recount potential ophthalmic drugs for the management of surface inflammatory conditions of eye such as dry eye syndrome. This demand is enough for the development of formulation. We have used herbs like Tamarix gallica, Badruj (Ocimum basilicun) L, Jadvar (curcuma zedoaria Rose) and Komon (Curum carvi L). as their use is well known for reducing inflammation and other underlying issues. The pre-clinical ocular toxicity studies also revealed safety of this formulation on topical use. The formulation was prepared in sterile condition and all the necessary practices were followed.

KEYWORDS: Tamarix gallica, Badruj (Ocimum basilicun l), eye drops, dry eye syndrome, formulation development, evaluation of formulation,Stability studies.

INTRODUCTION

Eye drops and ophthalmic products are the pharmaceutical products instilled in eye that are used to treat and prevent several ophthalmic disorders. Eyes are one of the main sensory organs of the living world. Delivery of medication to the human eye is an integral part of medical treatment.[1] ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology, and biochemistry of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy.[2]

The modern world is so much depended on screens and the number of screens is increasing daily, but the effects of same can be seen as people with ophthalmic disorder is increasing and the effectiveness of ophthalmic product is promising in these cases. The alternative of using herbal products in eye drops is one of the best substitutes as herbs are easy to cultivate and contain several active pharmaceutically important constituents. Ophthalmic preparations are specialized dosage forms designed to be instilled onto the external surface of the eye (topical), administered inside (intraocular), adjacent to the eye (periocular) or used in conjunction with any special device.

The preparation may have any several purposes like therapeutic, prophylactic or palliative. The versatility of dosage form enables therapeutic agent to be suitable for function of preparation. Therapeutically active formulation may be designed to provide extended action for either convenience or reduction in dose frequency, improved bioavailability of an agent or improved delivery to target tissue. The residence time of an ocular preparation may range from few seconds (ophthalmic solutions) to hours (gel, ointments), two months or years (intra ocular or periocular dosage forms).Ophthalmic preparations are similar to parenteral dosage form in theirrequirements for sterility as well as consideration for osmotic pressure (tonicity), preservation, and tissue compatibility, avoidance of pyrogens and particulate matter and suitable packaging.

On the ground of anatomy and physiology eye is a complex and incomparable structure guarded by a number of defensive attitude machineries. The

framework, biochemistry, and physiology of the eye set down this organ tremendously impervious to strange entities4. Assorted adaptations guarding the eye from noxious entities and agents such as lacrimation, reflex blinking, rapid tear turnover, drainage, and pre-corneal loss concludes in.

The hazardous effects of synthetic products, provides the rationale behind the development of such an herbal product that provides least toxicity and can treat the disorders in an efficient and cost-effective manner as the ophthalmic preparations containing herbal Nutraceuticals are low and provides a better market to the formulation, as well as comes up as the better alternatives than the synthetic ones. Various pain mediators like prostaglandins and Cox enzyme can be inhibited using the active constituents found in herbs and decrease the inflammation. The herbs can stimulate the trabecular meshwork and increase the tear production and provide moisturization to eye and cornea of the patient. Drugs are administered to the eye for local effects such as bacterial infection, miosis, mydriasis, or to reduce intraocular pressure.[3,4,5]

Delivery route for ophthalmic preparation

Conventionally, many ocular diseases are treated with either topical or systemic medications. Topical application of drug has remained the most preferred method due to ease of administration and low cost. Topical application is useful in the treatment of disorders affecting the anterior segment of the eye.

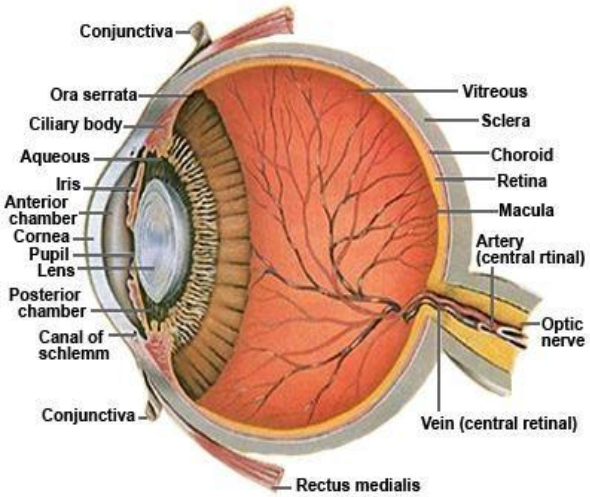
Anatomical and physiological barriers hinder drugs from reaching posterior segment of eye mainly choroid and retina. A major fraction of drug following topical administration is lost by lacrimation, tear dilution, nasolacrimal drainage, and tear turnover. Such precorneal losses result in very low ocular bioavailability.

Typically, less than 5% of total administered dose reaches aqueous humor. So in order to maintain minimum inhibitory concentrations, the agents need to be frequently dosed. Upon topical instillation drugs are absorbed by corneal route (cornea → aqueous humor → intra ocular tissue) or non corneal route (conjunctiva → sclera → choroid/ retinal epithelial pigment). The preferred route depends mainly on the corneal permeability of drug molecules. Unlike topical

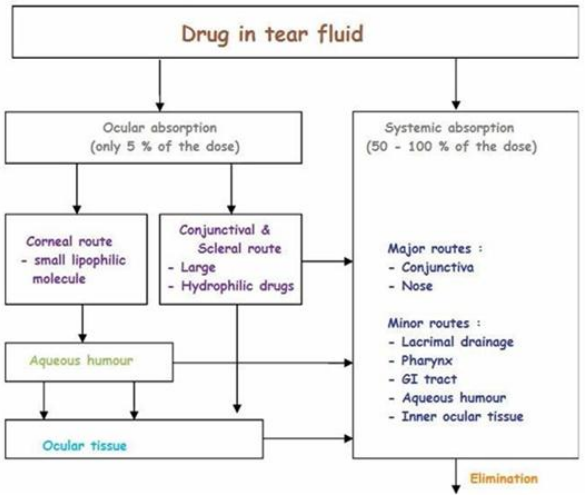
(**Human eye Anatomical structure**)

Administration, systemic dosing helps in the treatment of disease affecting posterior segment of the eye.

A major drawback associated with systemic administration is only 1-2% of administered drug reaches to vitreous cavity. Blood retinal barrier which is selectively permeable to more lipophilic molecules mainly governs the entry of drug molecules into posterior segment of the eye.[6,7,8,9]



[Mechanism of action Flow chart]



MATERIALS AND METHODS

Some of the herbs used for formulation of our product are Athl (Tamarix Gallca L), Badruj (Occimum Basilicum L), Jadvar(Curcuma Zedoaria Rose), Komon (Curum Carvi L) the reason for using them is very simple as they all show some sort of beneficial properties to humans to reduce inflammation, kill microbes and avoid synthetic toxicities.

Tamarix gallica is a deciduous, herbaceous, twiggy shrub or small tree reaching up to about 5 meters high. It has fragile, woody branch lets that drop off in autumn along with the small, scale-like leaves that cover them. The pink flowers are tiny, hermaphroditic, and are borne on narrow, feather-like spikes. They frequently bloom earlier than the leaves, first in May, and sometimes a second time in August.]In its native range the plant grows in moist areas such as riverbanks, especially in saline soils. It has been used medicinally for rheumatism, diarrhea, and other maladies. Obtain by crushing the leaves in mortar and using fine powder after passing it from Mesh #14.

Badruj (Occimum Basilicum L) Basil is an annual, or sometimes perennial, herb used for its leaves. Depending on the variety, plants can reach heights of between 30 and 150 ft. Its leaves are richly green and ovate, but otherwise come in a wide variety of sizes and shapes depending on cultivar. Leaf sizes range from 3 to 11 cm long, and between 1 and 6 cm wide. They are of particular interest for applications in the areas of functional foods and Nutraceuticals because they act as preserving agents to protect the human body system against degenerative diseases caused by oxidative damage. Among the aromatic and medicinal plants available to the food industry, basil (Occimum Basilicum L.) has promising beneficial properties. Obtain by crushing the leaves in mortar and using fine powder after passing it from Mesh #14.

Jadvar (curcuma zedoaria Rose) many medicinal plants and their preparations are practiced at home as remedies for treating and preventing various diseases and disorders. For example, medicinal plants and their crude parts such as Tulsi, Neem, turmeric and ginger are used to cure or treat several common ailments, out of which Curcuma zedoaria Rosc commonly known as white turmeric is one of the important crude drugs belonging to Zingibaraceae family and genus Curcuma. Traditionally, it has been reported to possess many biological activities been Obtained by crushing the leaves in mortar and using fine powder after passing it from Mesh #14.

Komon (Curum carvi L*)* Carum carvi is used in a number of different ways worldwide depending on region. In the Nordic countries it is a well-known spice for example in bread and cheese. It is also used in different dishes with cabbage as sauerkraut and cabbage soup. Another typical products including caraway is Scandinavian Aquavit, including Icelandic Brennvin, and several liqueurs.Obtained by crushing the leaves in mortar and using fine powder after passing it from Mesh #14.

Sr. No.	Plant name	Family	Image
1	Tamarix gallica	Tamaricaceae	
2	Badruj (Ocimum basilicun L	Lamiaceae	
3	Jadvar (curcuma zedoaria Rose	Zingiberaceae	
4	Komon (Curum carvi L	Apiaceae	

All raw powders were passed through mesh #14 to ensure same powder size.

0.9% NaCl was prepared and used as the vehicle to ensure is tonicity of the product and phosphate buffer was used to reduce the P^h changes; also rose water was used to provide essence to the product. Phenyl ethyl alcohol was used as a preservative as the product contained herbs, so is prone to microbial contamination thus to avoid such deterioration it was used.

Experimental work

Selection of herbs

More than 59 herbs show actions useful in the treatment of ophthalmic disorders but there are only a few suggested by the literature that is beneficial and compatible with different excipients to be used. It was an interesting yet challenging task to select the ingredients of best therapeutic effect and compatible parameters.

Thus Athl (Tamarix Gallca L), Badruj (Occimum Basilicum L), Jadvar (Curcuma Zedoaria Rose), Komon (Curum Carvi L) were selected.

Selection and Procurement of ingredients

Raw ingredients Athl (Tamarix Gallca L), Badruj (Occimum Basilicum L), Jadvar (Curcuma Zedoaria Rose), Komon (Curum Carvi L) powder was procured. The identity was confirmed with compliance of microscopic, Macroscopic parameters of ayurvedic pharmacopoeia of India (API) through Pharmacognostic study. The purity and strength were also confirmed through physico- chemical studies done as per various official literatures.

Pharmacognostic and Phytochemical evaluation of herbs.[10,11,12]

Extraction

The method employed was Maceration method, the drugs were allowed to be soaked in 250 ml distilled water for overnight and then they were extracted using methanol or water via distillation apparatus.[7,12]

Evaluation by preliminary tests

(A) Preliminary tests for primary constituents

(a)Tests for proteins

1-Million's test: - Mix 3 ml test solution with 5 ml Million's reagent. White and warm ppt change in to red or the ppt dissolves giving red colored solution.

2-Biuret test: - To 3 ml test solution add 4 % NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink color appear

(b)Tests for amino acids

1-Ninhydrin test: - Heat 3 ml extract, add 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. purple or bluish color appears.

(B) Preliminary tests for secondary metabolites

Tests for alkaloids

- **Hager's test**: - To 2-3 ml of filtrate add few drops of Hager's reagent (sodium picratesolution).
- **Dragendroff test:** - To 2-3 ml filtrate add 2-3 drops of Dragendroff reagent (Potassium bismuth iodide solution).
- **Wagner test:** - To 2-3 ml of filtrate add 2-3 drops of Wagner's reagent (potassium mercuric iodide solution).

Tests for tannins

- Take 2-3 ml of extract and add few drops of 5% ferric chloride solution.
- Take 2-3 ml of extract and add a few drops of lead acetate solution.
- Take 2-3 ml of extract and add a few drops of potassium permanganate solution.

Tests for flavonoids

- Take 2-3 ml of sample and add dilute sulphuric acid solution.
- Take 2-3 ml of sample and add few drops of Sodium hydroxide sample.
- Take 2-3 ml of sample and add a few drops of lead acetate solution.

1- Thin layer chromatography

Thin layer chromatography of the herbs was done to check the quality and standard of the drugs and to identify the active constituents in the herbs.

- **Sample preparation:** - The powdered drugs were allowed to soak in water overnight andafter 24 hours extraction was done using methanol. The extract was then filtered and then 1 ml of sample was taken, and methanol was added to make it up to 10 ml.
- **Preparation of stationary phase:** - TLC plates were taken; Silica gel G was dissolved and poured on the plate then the plate was dried in oven at 105 ^{o}C for 30 minutes.
- **Mobile phase preparation:** - Toluene: ethyl acetate: Diethyl amine was took in the ratio (7:2:1) for alkaloid and Toluene: acetone: Formic acid (4.5:4.5: 1) was took for tannins.

(C)) Tests for carbohydrates

(a) For reducing sugars

- **Benedict's test**: - Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. in boiling water bath and cool. Red ppt is observed.
- **Fehling's test:** - Mix 1 ml Fehling A and Fehling B solution, boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt is observed.[13]

(b) For non- reducing sugar

- Test solution does not give response to Fehling's and Benedict's test.
- Hydrolyse test solution. Fehling's and Benedict's test are negative.

(D)Evaluation by physico-chemical tests

1-**Determination of ash value**

Weigh and ignite flat, thin porcelain dish or silica crucible

Take 2 gm of sample in it weigh it again.

Keep it on tripod stand and ignite it at 2 cm height and keep the dish 7cm above the flame, heat till all vapours have evolved and all the carbon is burnt off. cool it indesiccator and weigh again.

Then ash value is determined using the formula

$$\%ash = \frac{wt\ of\ crucible\ with\ ash - wt\ of\ empty\ crucible}{wt\ of\ sample} \times 100$$

The % ash value found must be under standard values given in the literature if not so then itis concluded that the product or herb is of substandard category.

2-Determination of water-soluble ash

Total ash + add 10 ml water and boil for 5 minutes.
Filter through ash less filter paper and ignite it at 450 ^{o}C
Cool it and weigh again.

$$Wt\ of\ water\ soluble\ ash\ =\ weight\ of\ total\ ash\ -\ weight\ of\ water\ insoluble\ ash$$

$$\%\ water\ souble\ ash = \frac{wt\ of\ water\ soluble\ ash}{wt\ of\ sample} \times 100$$

3-Determination of acid insoluble ash

- The ash is dissolved in 25 ml dilute hydrochloric acid.
- Then it is filtered through ash less filter paper and thoroughly washed with water.
- The filter paper is then ignited in the original dish, cooled in a desiccator and thenweighed.
- Then determine the acid insoluble ash using the formula

$$\%\ acid\ insouble\ ash = \frac{m2 - m1}{m1 - m} \times 100$$

Where, m_2 = lowest mass in gm of dish with acid insoluble ashm_1 = Mass in gm of empty dish
m = Mass in gm of dish with dried material

Formulation and Development of herbal eye drop

Three different batches were prepared for the formulation containing different concentration of the ingredients.

Formulation batches.

Ingredients	Formulation 1 - F1 Conc. in gm	Formulation 2 – F2 Conc. in gm	Formulation 3 – F3 Conc. in gm
Athl	2.8	2.4	**3**
Badruj	2.4	2	**2.8**
Jadvar	2.4	1.8	**2.2**
Komon	**2**	**1.6**	**2.4**

Formulation development

- The step wise development of eye drops encompasses the preparation of distillate, making of the distillate isotonic to lachrymal fluid and adjustment of pH, addition of preservative and packing under sterile conditions.
- All coarsely powdered drugs were soaked in rose water for overnight in a beaker.
- The mixture was refluxed at 60 ^{o}C for a span of 3 hours.
- The mixture was then cooled, filtered and Alum was added, and solution was filtered again. The resulting filtrate was collected.
- And then transferred to a distillation unit. Distillate was obtained by adjusting the temperature to 40 ^{o}C for 15 minutes and raising the temperature slowly to 80 ^{o}C The first450 ml. of distillate was collected at the rate of 20 drops per minutes in an airtight container.
- 0.9 % NaCl was selected as vehicle and 2.5% of extract was added.
- The distillate was made isotonic to lacrimal fluid by adding 0.9% NaCl to distillate and dissolving properly and adding isotonic phosphate buffer viz.0.16 gm of monobasic Sodium phosphate and 0.76 gm of dibasic Sodium phosphate.
- Finally, the pH of the eye drops was adjusted to 6.9-7.30. Phenyl ethyl alcohol was added as preservative and pH was again checked and found within the specified range of the ophthalmic drops (pH 6.9-7.30).
- Test for sterility was performed after addition of preservative the preparation was observed for 48 hours and found sterile.
- The packing was made in autoclaved sterilized amber glass containers of 10 ml capacity**.**
- The finished product was tested for quality assurance and safety and the analyticalspecifications complied specified parameters of Indian pharmacopeia for ophthalmic preparations.[13,14]

Evaluation of finished product[15]

Various evaluation tests were performed on the product as suggested by the literature using proper standards given in specified condition so as the product obtained was sterile and estimated to be beneficiary to the consumer.

1- Organoleptic properties

To cheeks the various parameter like Color, Odor, Appearance and Texture.

2 -Physico-chemical parameters of finished product

1- Determination of P^{H}

- The pH meter was calibrated using acetate and phosphate buffer.
- First the acetate buffer was dissolved having pH around 3.6 and checked under theequipment if not found then knob was adjusted.
- Then it was cleaned using distilled water.
- Then phosphate buffer was dissolved in water and pH of 6.8 was obtained.
- Then the indicator electrode was cleaned using distilled water.
- Then the machine was calibrated and then product was checked.

2-Determination of density

- 10 ml density bottle was taken and the weigh balance was calibrated.
- Empty bottle was weighed(w_1)
- Then product was taken, and its weight was determined (w_2)

- Then weight of product was determined $(w2 - w1)$
- Then density was determined by given formula

3-Determination of viscosity

- Ostwald viscometer was used, clean viscometer was mounted in vertical position
- Water was filled up to G mark; time required to flow from A mark to B mark was countedin seconds.
- Same process was repeated 3 times
- Viscometer was rinsed and product was filled.
- Then viscosity was determined by formula.

$$n2 = \frac{p2t2}{p1t1} \times n1$$

Where,
n_1 = viscosity of std liquidp_1 = Density of std liquid
t_1 = Time required by std liquid n_2 = Viscosity of test liquid
p_2 = Density of test liquid
t_2 = time required by test liquid.

4- Determination of Surface tension

- Stalagmometer and drop count method was used for determination of surface tension.
- Clean the Stalagmometer and mount it in vertical position.
- Fill with water up to mark A; drops were counted till it reached mark B.
- Then it was filled with sample and surface tension was determined using formula

$$r2 = \frac{p2n1}{p1n2} \times r1$$

Where,
n_1 = Number of drops of std liquidn_2 = number of drops of test liquidp_1 = density of std liquid
p_2 = density of test liquid
r_1 = surface tension of std liquid r_2 = surface tension of test liquid.

5-Isotonicity[13,14]

- The product was checked for isotonicity on Red Blood Cells.
- The finger was pricked, and sample was collected. Blood sample was mixed with RBC fluid and observed under microscope for shape and size.
- Then formulation was added in blood sample and observed for shrinkage or swelling ofthe cells.

6-Sterility testing

- The product was made sterile in autoclave at 121 ^{o}C and 115 psig for 15 mins.
- The product was filtered using membrane filter and the membrane was cut in 2 halves and direct inoculation was done for presence of any microbial growth.
- The product was observed under microscope for growth of any viable bacteria after 48 hrs in incubation.

7- Stability testing

- Stability tests were performed after 1 month under ICH guidelines for ophthalmic preparation.
- Various tests including physico-chemical tests, organoleptic property screening, and UV spectrometry studies were conducted on the formulation to study the stabilitycharacteristics.

RESULT AND DISCUSSIONS

Result of preliminary tests performed on raw powders.

Sr.no	Powder	Test for Proteins	Test for Amino acid	Test for Carbohydrate
1	Athl	Positive	Positive	Negative
2	Badruj	Positive	Positive	Negative
3	Jadvar	Negative	Negative	Positive
4	Komon	Negative	Negative	Positive

Result of secondary metabolites test.

Sr.no	Powder	Test for Flavonoids	Test for Tannins	Test for alkaloids
1	Athl	Positive	Positive	Positive
2	Badruj	Positive	Positive	Negative
3	Jadvar	Negative	Negative	Positive
4	Komon	Negative	Negative	Positive

Thin Layer Chromatography Results.

1	**Sample preparation**	1 ml sample in 10 ml methanol
2	Stationary phase	Silica gel G
3	Mobile phase for alkaloid	Toluene: ethyl acetate: diethylamine(7:2:1)
4	Mobile phase for tannin	Toluene: acetone: Formic acid(4.5 :4.5: 1)

6	Development time	22 mins
7	Spraying reagent for alkaloid	Ethanol
8	Spraying reagent for tannin	5% Ferric chloride solution
9	Rf value alkaloid	0.39
10	Rf value tannin	0.21

Reports of Organoleptic properties.

Sr No.	Characters	Observations
1.	Colour	**Transparent**
2.	Odour	**Pleasant**
3.	Texture	**Smooth**
4.	**Appearance**	**No turbidity free from viable particles**

Result of physico chemical testing.

Formulations	pH	Density gm/ml	Viscosity cp	Tension(surface)
F1	7.01 ± 1	1.15 ± 0.5	1.74 ± 2	52.1 ± 1
F2	6.5 ± 0.5	0.9 ± 0.1	1.05 ± 0.1	48 ± 0.3
F3	7.9 ± 2	1.04 ± 0.9	2.9 ± 1	Surface 65 2

Sterility testing

Sterility test was performed using the membrane filter through which it was filtered, the filter was cut in 2 and directly incubated in fluid thioglycolate media and no visible Microbial Growth was seen

Reports of Organoleptic test after 1 month.

1.	**Colour**	**Transparent**
2.	Odour	**Pleasant**
3.	Texture	**Smooth**
4.	**Appearance**	**No turbidity free from viable particles**

Stability testing

The product was kept for 1 month and tested for various parameters as per Various ICH guidelines at Temp 40 ^{o}C and RH 75 ± 5%.

- ICH Q1A – Stability testing of new drug substances and Products
- ICH Q1B – Photo stability testing of new drug substances and products[17,18,19,20,21,22,23,24,25]

CONCLUSION

In present research work eye drops were formulated from herbs like Athl and Badruj, Jadvar and Komon shows promising action in reduction of strain and inflammation of eyes. Ophthalmic solutions mainly used in the Topical administration for the treatment of eye disease The eye drops were prepared and evaluated as per various literature sources available. The herbal extracts were obtained and tested for their purity and strength ensuring good and safe product formulation. Safe and effective concentration was determined for the herbs by batch production. Different tests for constituents were performed showing promising results. Ophthalmic preparations are similar to parenteral dosage form in their requirements for sterility as well as consideration for osmotic pressure (tonicity), preservation, and tissue compatibility, avoidance of pyrogens and particulate matter and suitable packaging As per the recommendation from various literature the drops were made sterile and isotonic to support the formulation as well as treat the disorder. All the specifications provided were tried to be met to gain beneficial as possible from the product. It is estimated to show beneficial health effects on eyes. The product formulation F1 is concluded to be safe and effective as various standards were met during the preparation of the product. It can be concluded from the results that the formulation prepared is of standard quality with least possible deterioration and hazard. The product was checked for stability and sterility to avoid any risk or chance factor. Ophthalmic preparations are specialized dosage forms designed to be instilled onto the external surface of eye (topical), administered inside (intraocular), adjacent to the eye (periocular) or used in conjunction with any special device.

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