Formulation And Evaluation Of Opthalmic Ointment Using Foeniculum Vulgare Miller Seeds Extract

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Abstract:
The present investigation was conducted with the objective of formulating and evaluation of herbal ophthalmic ointment formulation using aqueous decoction of Foeniculum vulgare Miller (Fennel) seeds. Inflammation of eye, commonly referred to as ocular inflammation, results from several conditions like infection, allergy, trauma, oxidative stress, autoimmune conditions, and even dry eyes, resulting in symptoms like redness, irritation, pain, and poor vision. Traditionally, ophthalmic medications, such as corticosteroids and non-steroidal anti-inflammatory drugs, have been employed successfully to combat inflammation, but the continuous usage of these medicines may cause side effects such as increased intraocular pressure, formation of cataracts, nephrotoxicity, and gastric irritation. Thus, the need for an effective and safe herbal ophthalmic medication emerged.
The primary reason behind choosing fennel seed as the main raw material was due to its antioxidant, anti-inflammatory, oculohypotensive, and protective property to ocular tissue. The use of tulsi extract and honey in the formulation was due to their antimicrobial and soothing nature. Preliminary testing for the identification of phytoconstituents in plants resulted in the detection of various phytoconstituents like flavonoids, tannins, alkaloids, carbohydrates, phenols, and saponins.
The aqueous extract of fennel seeds and Tulsi leaves was made through decoction process. Ophthalmic ointment was prepared with fusion process and the base was comprised of beeswax and castor oil. The product was packed in sterile ophthalmic tubes. Evaluation of the formulated product with respect to physicochemical parameters such as appearance, pH, spreadability, viscosity, percentage of drug content, and stability was done. Ointment was found to have smooth, homogenous nature with slight yellow colour and without unpleasant odour. The ointment demonstrated appropriate pH level, spreadability, viscosity and drug content. Anti-inflammatory activity was measured by protein denaturation test with egg albumin and the formulation inhibited protein denaturation effectively.

Keywords: Foeniculum vulgare, Herbal ophthalmic ointment, Ocular inflammation, Anti-inflammatory activity, Antioxidant activity, Fennel seed extract, Phytochemical screening, Protein denaturation assay, Ophthalmic formulation, Tulsi extract.

Chapter1:Introduction

Eye is the most vital sensory organ of the body and very complex too. There are ophthalmological diseases that affect a great number of people and since ancient times, people have been using natural resources like plants and animals to cure eye diseases. WHO says that 80% of the population around the world depend upon herbal medicines for primary healthcare(1)

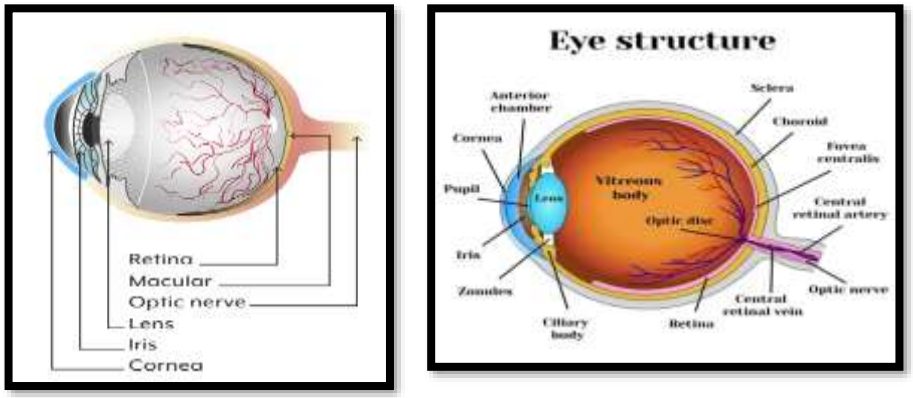


Fig.No:1 Eye Structure(2)

Ocular Vision pertains to the visual capabilities of the eye, which involve image formation through the retina, then subsequent relay of information to the brain. In a healthy state of vision, the process involves ocular motor functions that fixate, stabilize, and keep images firmly on the retina in order to achieve clear vision.(3)

Ocular Inflammation is an immune reaction occurring in one or several portions of the eye. This involves the in filtration of immune cells, release of inflammatory mediators like cytokines, and can vary from minor and self-

limiting forms to those that pose serious threats to vision.[4]

Causative factors of ocular inflammation are bacterial, viral, and fungal infection, trauma, chemical irritation,and inflammatory conditions, particularly autoimmune disorders.The core factor in Dry Eye Disease is Inflammation. The poor quality and quantity of tears lead to continuous injury and inflammation to the ocular surface which is the conjunctiva, cornea, and eyelids.[5]

1:Mechanism of ocular vision:
Light rays enter the eye through the cornea (Bends light rays)

Light rays pass through the pupil (Iris regulates the amount of light that enters)

Light rays hit the lens which focuses light rays onto the retina

An image is formed on the retina (Inverted and upside down; Rods/Cones sense light)

Retina converts light rays to an electrical impulse (Through rods/cones)

Impulse travels through the optic nerve (From ganglion cells)to optic chiasm

At optic chiasm:fibers cross partially

Fibers travel through the optic tract to lateral geniculate nucleus

Fibers then through optic radiations to visual cortex

Visual cortex(Occipital lobe)interprets impulses into perceived[3]

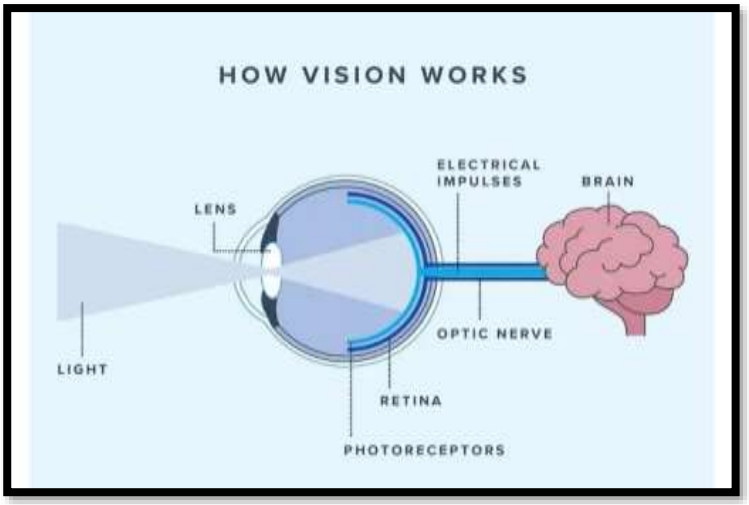


Fig No.2Mechanism of vision[6]

Inflammation is 'an organized response of the body to injury or infection.' This means that inflammation is a process by which the body responds to an irritant such as infection or injury. Inflammation involves the cells and mediators responsible for changes in vascularization, cell migration, and tissue response. With regard to vision, various types of inflammation may occur in various parts of the visual system including the cornea, uvea, or retina. This may present as pain and redness.[7]

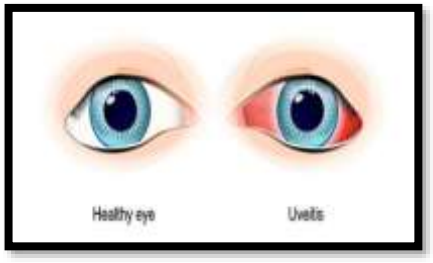


Fig no:3Healthy eye Vs Eye with Uveitis[8]

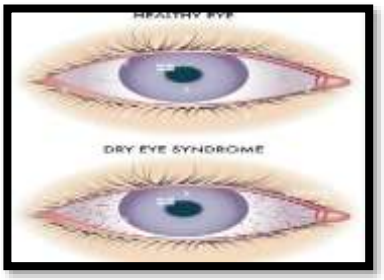


Fig No:4 Healthy Eye with an eye affected by Dry Eye [9,10]

2:Mechanism of Ocular Inflammation:
1. Immune System Activation:

In case of injury, infection, or autoimmune stimuli,the innate immune system cells (macrophages,dendritic cells, and microglial cells) are activated.They secrete pro inflammatory cytokines (for instance,TNF-alpha, IL-1 beta, IL-6), reactive oxygen species(ROS).[11]

2. NF-κB Signaling Pathway:
Nuclear Factor κB (NF-κB) plays an important role intranscription. Activated NF-κB migrates into the cellnucleus where it activates pro-inflammatory gene expression including COX-2 and iNOS.[12]

3. MAPK and STAT Signaling Pathways:
MAPK signaling pathway is involved in inflammation induction and cytokine secretion in the oculocytes.STAT signaling pathway regulates expression of inflammatory genes in epithelial and immune cells.[11]

4. Disruption of Blood-ocular Barriers:
Disruption of Blood-Ocular Barriers Inflammation disrupts the tight junctions in the blood-aqueous and blood-retinal barrier causing disruption.

Table No.1:Common eye conditions that do not typically Cause vision impairment[2]

Sr.No	Eye Condition	Description
1.	**Blepharitis**	Inflammation of the eyelids near the base of the eyelashes characterized by redness and irritation of the eye and eyelid
2.	**Chalazion and hordeolum (stye)**	Common eyelid disorders resulting from a blocked gland or localized infection that can cause pain.
3.	**Conjunctivitis**	Inflammation of the conjunctiva (the clear membrane lining the inside of the eyelids and covers the white part of the eye) most commonly caused by allergy or infection.
4.	**Dry eye**	Due to an inadequate tear production that can result in irritation and blurred vision
5.	**Pterygium and pinguecula**	Abnormal growths on the conjunctiva that can cause pain. In advanced cases, pterygium can encroach on the cornea and cause vision loss.
6.	**Subconjunctival haemorrhage**	Broken blood vessels underneath the conjunctiva.

Table No: 2 Common eye conditions that can cause vision impairment blindness[2].

Sr.No	Eye Condition	Description
1.	**Age-related macular degeneration**	Damage to the central part of the retina responsible for detailed vision leads to dark patches, shadows or distortion of the central vision. The risk of developing macular degeneration increases with age.
2.	**Cataract**	Cloudiness in the lens of the eye, leading to increasingly blurred vision. The risk of developing cataract increases with age.
3.	**Corneal opacity**	A group of conditions causing the cornea to become scarred or cloudy.Opacity is most commonly caused by injury, infection or vitamin A deficiency in children.
4.	**Diabetic retinopathy**	Damage to blood vessels in the retina which become leaky or blocked.Vision loss most commonly occurs due to swelling in the central part of the retina which can lead to vision impairment. Abnormal blood vessels can also grow from the retina, which can bleed or cause scarring of the retina and blindness.

5.	**Glaucoma**	Progressive damage to the optic nerve. Initially, loss of vision occurs in the periphery and can progress to severe vision impairment (this is known as open angle glaucoma, the most common type and the type generally referred to in this report).
6.	**Refractive error**	Due to an abnormal shape or length of the eye ball; light does not focus on the retina resulting in blurred vision. There are several types of refractive error; those most commonly referred to in this report are: – Myopia – difficulty seeing distant objects (near-sightedness). – Presbyopia – difficulty seeing objects at near distance with increasing age(i.e. after 40 years of age).
7.	**Trachoma**	Caused by a bacterial infection. After many years of repeated infections, the eyelashes can turn inwards (known as trichiasis) which can lead to corneal scarring and, in some cases, blindness.

Treatment:

- Vision Enhancement Treatment Options

Non-surgical options include corrective glasses and contacts,
low-vision aids such as magnifiers, and special lenses.
Surgical options include LASIK, PRK, cataract surgery, intraocular lens implants

- Inflammation Treatment Options

Medication Treatment Options
Corticosteroids including Prednisolone and Dexamethasone
NSAIDS such as ketorolac and diclofenac
antibiotics/antiviral medication in cases of infections
antihistamines for allergic conjunctivitis[13]

3:Is herbal preparation better than marketed preparation?

❖ Multi-Targeted and synergistic:

Herbal medicines work through multiple mechanisms, whereas a lot of conventional medicines target only a single mechanism. Plants produce substances like phytochemicals, which together target anti-inflammatory, antioxidant,immunomodulatory, and vasculoprotective mechanisms,useful in the management of complex multi-factorial diseases such as eye inflammation and visual disturbances. Herbal medicines contain more than one active substance,which works in synergy. Conventional medicines usually interact with only one mechanism of action, such as the mechanism of action of NSAIDs, which is an inhibitor of COX enzymes, whereas herbs act as inhibitors of cytokines,ROS, immunological activation, and much more.

❖ Generally Fewer and Milder Side Effects:

Herbal medicines are also considered to be safer than conventional medicines when used alone.

• Prospective clinical trials carried out on humans have shown that patients taking only herbal medicine had a low incidence of systemic adverse events (like no liver involvement in one hospital-based clinical study), but those who combined both herbal and conventional medicines had higher chances of the above mentioned events.

• Marketed pharmaceutical anti-inflammatory drugs, like corticosteroids and NSAIDs, can cause serious ocular and systemic adverse effects, like increased intraocular pressure, cataracts, renal damage, or gastrointestinal mucosal injury when used for a prolonged duration.

❖ Improved Patient Acceptance and Compliance:

Herbal medicines may be more acceptable to the patient. This could be possible when the HER2 positive cancers have to be managed using supportive therapy over an extended period of time.

Additionally, natural medicine is believed to be gentler to the body compared to conventional medicine, and the ease of use may be a factor that will increase patient adherence to the prescribed treatment, especially when managing chronic disorders such as inflammation or age- related eye conditions.

The herbs can also be used in the preparation of herbal medications, and their use leads to greater satisfaction by the patient compared to conventional medications[13].

4:Plant profile:

1.Foeniculum Vulgare Miller:

- Biological source: Fennel consist of dried riped fruit of Foeniculum Vulgare Miller and is a medicinal plant belonging to the Umbelliferae (Apiaceae) family.
- Chemical constituents: It consist of 3 to 7% of volatile oil, about 20%each of fixed oil. The chief active constituent of volatile oil is ketone, fenchone and a phenolic ether anethole.
- Uses: The fruit and root infusions are used as relaxant, estrogenic, analgesic and anti-inflammatory medicines in traditional medicine. Fennel seeds have exhibited estrogenic, antioxidant, and antihirsutism activities; it increases milk secretion, promotes menstruation, facilitates birth and alleviates the symptoms of dysmenorrhea.[1,15]

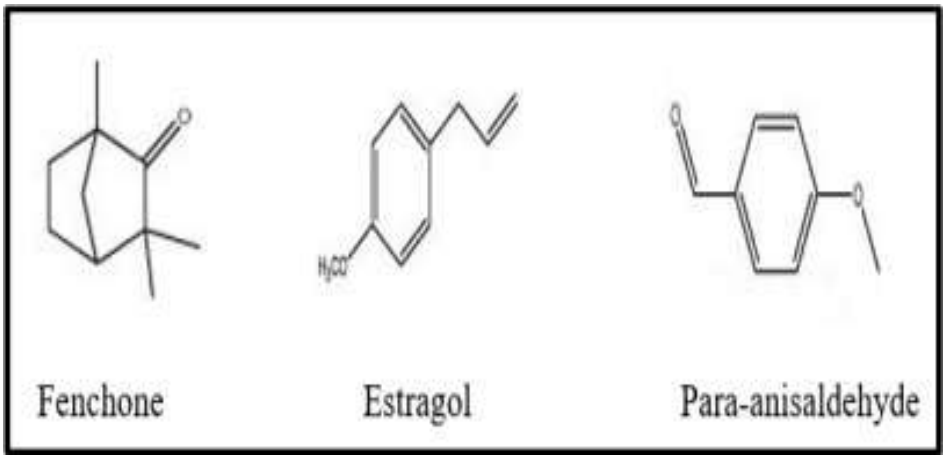


Fig No: 6 Chemical Constituent of Foeniculum Vulgare Miller[1]

Table No.3:Organoleptic properties of Fennel[14]

Colour	**Green to yellowish brown**
Odour	Sweet aromatic
Taste	Strongly aromatic
Size	5-10 x 2-4mm
Shape	Straight or slightly curved
Condition	straight or slightly curved Cremo carp that has been dried
Surface	Glabrous

Table No.4:Microscopic properties of Fennel: [12]

1.	Pericarp: [a] Epicarp	A layer of quadrangular to polygonal cells, with smooth cuticle

	[b]Mesocarp	Reticular, lignified parenchyma surrounding the vascular bundles
	[c]Vascular bundles	Five in number, bicollateral present below ridge (primary ridge)
	[d]Vittae	Schizogenous oil cells, 4 on dorsal side, 2on commissural surface/ventral surface. About 250 micron in maximum width, the walls are brown.
	[e]Endocarp	Consist of narrow elongated cells having a parquetry arrangement
2.	Seed: [a]Testa	Single layered yellowish brown in colour
	[b]Endosperm	Thick walled, polygonal, cellulosic parenchyma containing oil globules, aleurone, grains and rosette crystal of calcium oxalate
	[c]Raphe	A single ridge of vascular strands appears in the middle of commissural surface
	[d]Carpophore	With very thick walled sclerenchyma in 2 strands

Phytochemical Screening of Fennel Seeds Extract:

1.Test For Alkaloid:

a) MAYER'S TEST: To 2ml of extract, few drops of mayer's reagent were added in the side of test tube. White or creamy precipitate confirmed that test is positive for the presence of alkaloids.

2. Test For Flavonoids:

a) LEAD ACETATE TEST: To 2ml of extract, few drops of 10% lead acetate solution was added and well mixed.

3.Test For Tannin:

a) FERRIC CHLORIDE TEST: The extract solution was mixed with few drops of ferric chloride solution. The presence of gallic tannins, blue colour was obtained and green-black for catecholic tannins.

4.Test For Saponins:

a) FOAM TEST: 5ml of extract was shaken with 20ml distilled water and then boiled. Frothing confirms the presence of saponins.

5.Test For Carbohydrate:

a) MOLISH TEST: Firstly 2ml of extract was taken in the test tube, then 1 drop of Molish reagent was added .Then 2ml of concentrated HCL was added from the side of test tube. Violet ring formation was observed in the test tube. Formation of violet ring indicates the presence of carbohydrates:[17].

2.Tulsi:

- Biological Source:Tulsi consists of the dried leaves and flowering top**s** of Ocimum sanctum Linn belonging to Family Lamiaceae.
- Chemical Constituents: Volatile oil (essential oil): Eugenol (main constituent) ,Methyl eugenol ,Caryophyllene Flavonoids: Orientin ,Vicenin. Triterpenoids: Ursolic acid.
- Uses: Antimicrobial, Anti-inflammatory, Antipyretic.[16]

3.Honey:

FigNo:7 Tulsi[16]

- Biological Source: Honey is a sugary secretion deposited in the honey comb by honey bees such as Apis mellifera, Apis dorsata and other species of Apis belonging to family Apidae.
- Chemical Constituents: invert sugar (mainly glucose and fructose), Sucrose, Dextrin and gums ,Organic acids such as formic acid, acetic acid and succinic acid, Enzymes like invertase, diastase and inulase ,Vitamins, pollen grains and beeswax.
- Uses: used as a demulcent,sweetening agent, antiseptic for burns and wounds.[16]

FigNo:.8 Honey[16]

5.ICH Stability Guidelines:

1.ICH Q1A (R2): The principal guideline for the stability testing of new drug substances and products. It establishes testing frequency, batch requirements (typically 3 primary batches), and specific climatic conditions.

Table No:5.1.Recommended Storage Conditions for Stability Studies:

Study Type	Storage Condition	Minimum Time Period
Long-term	25°C ± 2°C / 60% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C / 65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C / 75% RH ± 5% RH	6 months

Table No:6.2.Refrigerated Product:

Study Type	Storage Condition
Long-term	5°C ± 3°C
Accelerated	25°C ± 2°C / 60% RH ± 5% RH

Table No:7.3. Frozen Product:

Study Type	Storage Condition
Long-term	−20°C ± 5°C

2.ICH Q1B: Dictates photostability testing to ensure drug products are not adversely affected by light.

3.ICH Q1C: Covers new dosage forms (e.g., modified-release products) and specifies how they differ from the original approved application.

4.ICH Q1D: Details bracketing and matrixing designs, allowing companies to reduce the number of samples tested by focusing on extremes or subsets of batches.

5.ICH Q1E: Provides guidance on the evaluation of stability data, detailing how to extrapolate shelf-life and retest periods based on regression analysis.Standard Climatic Zones & Storage The stability of data,detailing how to extrapolate shelf-life and retest periods based on regression analysis[51]

Chapter2:Review of Literature

1. Hassan et al.2017,Investigated the protective and therapeutic effects of 0.5% aqueous extract of Foeniculum vulgare (fennel) seed eye drops against sodium selenite-induced cataract in rabbits. The extract was administered twice daily before and after cataract induction. Results showed that the fennel seed extract eye drops significantly reduced lens opacity score($p<0.01$) and malondialdehyde (MDA) level in aqueous humor($p<0.01$) compared to the untreated cataract group. Scanning electron microscopy revealed preservation of normal lens fiber structure in the treated group. The study concluded that Foeniculum vulgare seed extract possesses anti-cataract activity, mainly through its antioxidant properties.

2.Nafees et al.2022,Reviewed the potential of various Indian traditional medicinal plants in the management of ophthalmic diseases. The mini-review highlighted plants such as Atropa belladonna, Abrus precatorius, Coptis teeta, Crocus sativus (saffron), Foeniculum vulgare (fennel), Ginkgo biloba, and Zingiber officinale. For Foeniculum vulgare, the authors cited its oculohypotensive activity in experimental glaucoma models and the protective/therapeutic effects of 0.5% aqueous seed extract eye drops against selenite-induced cataract in rabbits, attributing these benefits mainly to its antioxidant and anticholinesterase properties.

3.Wankhede et al. 2026,Conducted a comprehensive review on the role of Foeniculum vulgare seed extract in ocular vision enhancement and reduction of eye inflammation. The review collated evidence on its antioxidant, anti-inflammatory, and oculohypotensive properties, citing studies demonstrating significant reduction in lens opacity and oxidative stress in selenite-induced cataract models, along with IOP-lowering effects. The authors proposed fennel seed extract as a promising multi-targeted herbal approach for managing ocular inflammation, dryness, and oxidative damage, while recommending further clinical validation.

4.Dighade Narendra et al.2023,reviewed the medicinal potential of fennel (Foeniculum vulgare) seeds. The review summarized its botanical characteristics, nutritional composition, major phytochemical constituents (trans-anethole, fenchone, estragole, flavonoids, etc.), and a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects. The authors emphasized the therapeutic importance of fennel as a promising medicinal plant for developing new phytopharmaceuticals.

5.Downey and Leigh 1998,In their article "Eye movements: pathophysiology, examination and clinical importance" published in the Journal of Neuroscience Nursing, provided a comprehensive overview of the ocular motor system. The paper explains the key functions of eye movements — gaze-holding (fixation, vestibular, and optokinetic mechanisms) to keep images steady on the retina and gaze-shifting (saccades, smooth pursuit, and vergence) to bring objects of interest onto the fovea. It discusses the underlying pathophysiology of abnormal eye movements, bedside clinical examination techniques, and their diagnostic importance in identifying neurological disorders. The authors emphasize that systematic evaluation of eye movements can help localize lesions and guide treatment in various neurological conditions.

6.Chu et al.2024,In their mini-review titled "Inflammation mechanism and anti-inflammatory therapy of dry eye" published in Frontiers in Medicine, provided an updated overview of the role of inflammation in dry eye disease (DED). The paper describes the vicious cycle of dry eye (tear film instability → hyperosmolarity → inflammation → ocular surface damage → further tear instability), key inflammatory pathways (MAPK, NF-κB), immune cell involvement (macrophages, T cells), and major inflammatory mediators/cytokines. It also discusses various anti-inflammatory therapeutic strategies and their mechanisms for managing dry eye.

7.Srivastava and Ramana 2008,Reviewed the role of nuclear factor-kappaB (NF-κB) in ocular physiology and pathology. As a key transcription factor, NF-κB regulates the expression of genes involved in inflammation, immune responses, cell survival, and apoptosis. The authors highlighted its activation in multiple ocular disorders such as uveitis, AMD, cataract, and glaucoma, underscoring NF-κB as an important molecular target for therapeutic intervention in inflammatory eye diseases.

8. Miller and Hanumunthadu 2022,Reviewed inflammatory eye diseases, highlighting their varied clinical presentations ranging from benign conditions (e.g., episcleritis) to vision-threatening emergencies (e.g., uveitis, scleritis). The authors discussed diagnostic approaches and management principles, stressing the need for early intervention with corticosteroids, immunosuppressants, or biologics depending on severity and underlying systemic associations. This review underscores the importance of timely diagnosis and multidisciplinary management to preserve vision in patients with ocular inflammation.

9.Sharma et al.2020,Conducted a standardization study of Darvyadi Eye Ointment, a modified Ayurvedic formulation based on classical Darvyadi Raskriya described in Sharangdhar Samhita. The ointment was prepared using seven herbal drugs — Daruharidra (Berberis aristata), Patol (Trichosanthes dioica), Yashtimadhu (Glycyrrhiza glabra), Nimba (Azadirachta indica), Padmak (Prunus cerasoides), and Utpal/Prapaundarik (Nelumbo nucifera) — in equal proportions. The preparation involved making Ghana Satva (aqueous extract concentrate) from the decoction of the drugs, which was then incorporated into a base of Goghrita (cow ghee) and paraffin wax.

10.Patwegar et al. 2022,Developed a levofloxacin ophthalmic ointment using petroleum jelly as the base for the treatment of bacterial ocular infections. The optimized formulation (F4) exhibited acceptable pH (6.74), good spreadability and extrudability, high viscosity, 94% drug content, and 81% in-vitro drug release over 7 hours. The formulation passed sterility and stability tests as per ICH guidelines. This study demonstrates a conventional approach for formulating prolonged-release antibiotic eye ointments.

11.Sreya Rajan et al.2022,Formulated and optimized a Ketoprofen film-forming gel using HPMC and PVP polymeric blend for topical management of inflammatory conditions like rheumatoid arthritis and osteoarthritis. The optimized formulation (F3) showed desirable spreadability, rapid film formation, high drug content, and sustained drug release (97% in 6 hours) following zero-order kinetics. The prepared film-forming gel was non-irritant, stable, and provided an aesthetic, patient-compliant alternative to conventional topical formulations by forming a thin, flexible, transparent film on the skin upon application.

12.Mithuna Mohanan et al.2022,Investigated the in vitro anti-inflammatory potential of Cinnamomum tamala leaf extract and formulated it into a topical ointment. Phytochemical analysis revealed the presence of flavonoids, polyphenols, and other bioactive compounds. The ethanolic extract demonstrated significant anti-inflammatory activity through protein denaturation assay. The prepared ointment exhibited good physicochemical properties, antioxidant activity, and antimicrobial effects against Staphylococcus aureus and Escherichia coli. The study supports the traditional use of Cinnamomum tamala as a natural anti-inflammatory agent for topical applications.
13.The World Health Organization 2019, in its "World Report on Vision," reported that at least 2.2 billion people globally have vision impairment, with at least 1 billion cases being preventable or unaddressed. The report underscores the growing burden of eye conditions due to ageing populations and lifestyle changes, and advocates for integrated people-centred eye care (IPCEC) embedded in strong primary health care systems. It highlights the need to address both vision-threatening conditions (such as cataract, glaucoma, and diabetic retinopathy) and non-vision-threatening but highly prevalent conditions (such as dry eye and conjunctivitis). This global call for action supports the development of accessible, safe, and effective eye care interventions, including herbal and topical formulations, to reduce the burden of ocular inflammation and vision impairment.

Chapter3: Scope of Study

AIM:

FORMULATION AND EVALUATION OF OPTHALMIC OINTMENT USING FOENICULUM VULGARE MILLER SEEDS EXTRACT.

OBJECTIVES:

1. To authenticate and perform preliminary phytochemical screening of Foeniculum vulgare Miller seeds.
2. To prepare decoction extract of Foeniculum vulgare seeds and Tulsi leaves.
3. To formulate an ophthalmic ointment incorporating Foeniculum vulgare seed extract using suitable ointment base (Beeswax and Castor oil).
4. To evaluate the prepared ophthalmic ointment for various physicochemical parameters such as physical appearance, pH, spreadability, viscosity, drug content, and stability.
5. To assess the in vitro anti-inflammatory activity of the formulated ophthalmic ointment using protein denaturation method.
6.To study the stability of the optimized ophthalmic ointment formulation as per ICH guidelines.
7. To compare the prepared herbal ophthalmic ointment with standard parameters for ocular preparations and assess its suitability for ocular use.
8. To summarize the overall findings and suggest the potential of Foeniculum vulgare seed extract as a natural ingredient in ophthalmic formulations.

Chapter4: Plan of Work

Materials Used: Fennel seeds (Foeniculum vulgare), Tulsi leaves (Ocimum sanctum), honey, castor oil, and beeswax were procured from the local market. The fennel seeds and Tulsi leaves were cleaned, dried, and subjected to aqueous extraction by decoction method to obtain their extracts. Beeswax was used as the ointment base and was melted before formulation. Castor oil was incorporated as an emollient and vehicle, while honey was added for its therapeutic properties. All the materials used in the study were of suitable quality and were utilized for the preparation of the herbal ophthalmic ointment.

Authentication:

The plant material was authenticated by the Department of Botany,Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur and a voucher specimen was preserved. The plant material was authenticated by the Department of Botany,Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur and a voucher specimen was submitted.

Fig No:9 Authentication of Fennel Seeds

Extraction method

1: Decoction Extraction Method:

Decoction Method:

The fresh herb material (fennel seed) is used in an amount (15 gms) in dry form and immersed in four times its volume of water for overnight period (approximately 8 hours). Then on another day, seeds are crushed in mortar and pestle and decoction preparation is done until it reduces to 1/4th of the total volume. Similarly, Tulsi is crushed in mortar and pestle, and decoction is prepared till it is reduced to 1/4th of the total volume[18].

Fig No:10Fennel Seed Extract

Fig No:11Decoction of fennel seed extract

Fig No:12Decoction of Tulsi Extract

Formulation of Eye ointment:

1. Preparation of Oil Phase:

Firstly, 5 mL of castor oil was taken in a clean beaker and heated gently in a heating mantle. Later, 5 g of beeswax was dissolved in castor oil with constant stirring.

2.Addition of Aqueous Phase:

The aqueous phase consisting of 0.50 g of fennel seed extract (decoction) was then mixed with the oil phase with constant stirring to form a uniform ophthalmic ointment preparation.

3.Addition of Preservative:

The preservatives used in this preparation were honey and tulsi extract decoction (0.2 mL) were then added to the formulation with continuous stirring to obtain a uniform ophthalmic formulation.

4.Packaging and storage condition:

The prepared ophthalmic ointment was then packed in a sterile ophthalmic ointment container and properly sealed to avoid contamination. The formulation was stored in a cool and dry place away from direct sunlight to maintain its stability and effectiveness[19].

Table No:8 List of Ingredients:

Ingredients	Use/Application	F1 Batch	F2 Batch	F3 Batch	F4 Batch	F5 Batch	F6 Batch	F7 Batch
Fennel seed Exract(Decoction)	API/Drug	0.50ml	0.50ml	0.50ml	0.50ml	0.50ml	0.50ml	0.50ml
Bees Wax	Ointment Base	5gm	4gm	5gm	4gm	5gm	4gm	5gm
Castor oil	Lubricant,Emollient, moisturizing agent	5ml	4ml	6ml	6ml	5ml	4ml	6ml
Tulsi(Decoction)	Preservative	-	0.2ml	-	-	-	0.2ml	-
Honey	Preservative	-	-	0.2ml	-	-	-	0.2ml

Fig No:13Formulation of ointment

Evaluation of Opthamic Ointment:

1. Physical Appearance:

It was observed that the prepared ophthalmic ointments of formulations F1–F7 were slightly yellow in color without having any distinct smell. The prepared ointments had a smooth and uniform texture.

2. PH:

The determination of pH of ointments was carried out with the use of a calibrated digital pH meter. One gram of ointment from each formulation (F1–F7) was mixed with 100 ml of distilled water for 2 hours. The determination of pH was done in triplicate for each formulation and the mean values were calculated.

3. Spreadability:

Spreadability of the formulations (F1–F7) was assessed by measuring the spreading diameter of 0.5 g of ointment formulation between two horizontal smooth surface glass plates (20 cm × 20 cm). The initial diameter of the spreading of the ointment in centimeters that was formed by applying the ointment on the glass plate was recorded. Another glass plate of the same size was then put over the ointment formulation and left for 1 minute until there was no further spreading of the ointment. The upper glass plate was slowly removed, and the diameter of the circle formed was measured in centimeters.

4. Viscosity:

Viscosity of ophthalmic preparations (F1–F7) was determined by means of a Brookfield Viscometer. Rotation of ointments was carried out at speeds of 0.3, 0.6, and 1.5 revolutions per minute; the readings on the dial corresponding to each rotation were noted. Viscosity of ointments was calculated by multiplying the readings on the dial with the factor specified in the Brookfield Viscometer manual.

5. Stability Test:

Stability studies of the developed ointment formulations (F1–F7) were carried out according to ICH guidelines. The formulated ointments were filled in collapsible tubes and kept at different temperatures and humidity levels, i.e., 25°C ± 2°C/60% ± 5% RH, 30°C ± 2°C/65% ± 5% RH, and 40°C ± 2°C/75% ± 5% RH for 3 months. The parameters such as appearance, pH, viscosity, and spreadability were studied at predetermined intervals.

6. Drug Content:

An accurately weighed quantity of ointment from each formulation (F1–F7), weighing around 1 g, was dissolved in 50 ml of phosphate buffer solution having a pH value of 7.4 in a 100 ml volumetric flask, and its volume was adjusted up to the mark using phosphate buffer solution. The solution of ointment was sonicated for 15 minutes to ensure complete dissolution of the drug. After that, the solution was passed through a 0.45 µm pore size membrane filter. The absorbance of the prepared solution was determined at λmax of 376 nm against phosphate buffer (pH 7.4) as a blank using a UV-visible spectrophotometer.

7. Anti-Inflammatory Activity:

Anti-inflammatory activity of formulations F1–F7 was evaluated in vitro by protein denaturation technique as described by Mizushima and Kobayashi (1968). Reaction mixture (5 ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline (PBS, pH 6.4), and 2 ml of different concentrations of plant extract (10, 20, 50, and 100 µg/L). In order to have control, the same quantity of double distilled water was taken. The mixture was heated at 70°C for 5 minutes after keeping it in a BOD incubator at 37°C for 15 minutes. The absorbance of samples was recorded at 660 nm by taking vehicle as blank. Percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Inhibition (\%)} = [(Ac(660\ nm) - At(660\ nm)) / Ac(660\ nm)] \times 100$$

Where,

Ac = Absorbance of control solution

At = Absorbance of test sample.

% Inhibition Range:

According to the percentage inhibition scale, inhibition values of less than 20% indicate negligible anti-inflammatory activity, 20–40% indicate mild activity, 40–60% indicate moderate activity, 60–80% indicate good activity, and values greater than 80% indicate excellent anti-inflammatory activity. The formulated ophthalmic ointment exhibited 44% and 58% inhibition at 10 µg/mL and 20 µg/mL, respectively, indicating moderate anti-inflammatory activity. At higher concentrations of 50 µg/mL and 100 µg/mL, the formulation showed 70% and 75% inhibition, respectively, indicating good anti-inflammatory activity. The maximum inhibition observed was 75% at 100 µg/mL, demonstrating concentration-dependent anti-inflammatory activity of the developed herbal ophthalmic ointment.

Result

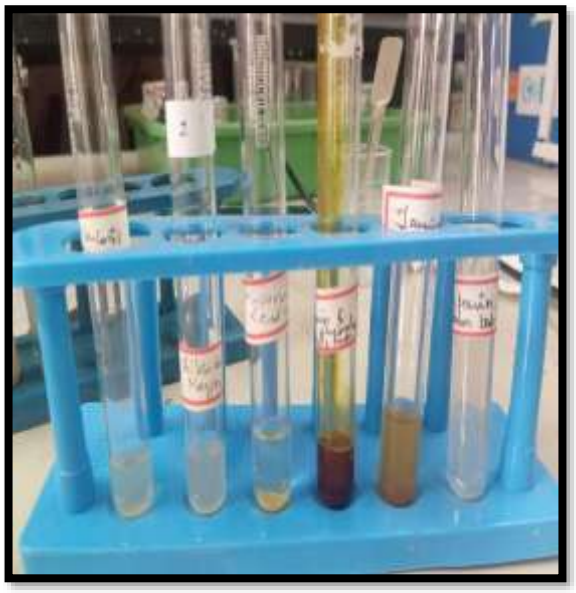
1.Authentication:

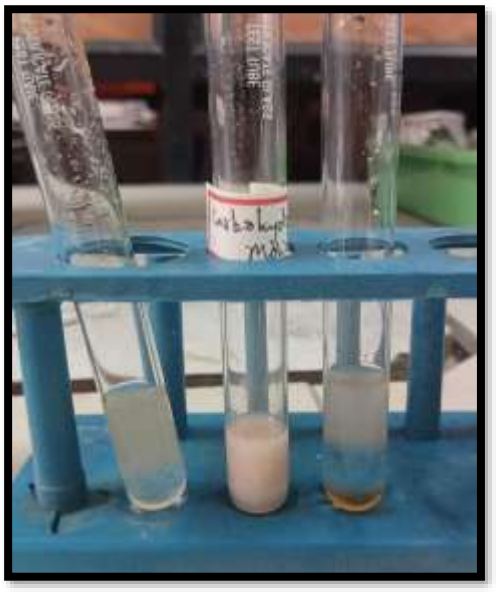
The plant material was authenticated by the Department of Botany,Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur and a voucher specimen was preserved. The plant material was authenticated by the Department of Botany,Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur and a voucher specimen was submitted.

Fig No:10 Authentication of Fennel Seeds

Table No: 2Phytochemical Screening of Fennel Seeds Extract:

	Phytoconstituent	Test Performed	Observation	Result
1	Alkaloids	Mayer's Test	White or creamy precipitate was observed.	Present (+)
2	Flavonoids	Lead Acetate Test	Yellow precipitate was observed.	Present (+)
3	Tannins	Ferric Chloride Test	Blue/green-black colour was observed.	Present (+)
4	Saponins	Foam Test	Persistent frothing was observed.	Present (+)
5	Carbohydrates	Molisch Test	Violet ring formation was observed.	Present (+)





Phytochemical Test of Fennel Seeds Extract

Table No.7: 3.Physical Appearance:

Sr. No.	Batch	Colour	Odour	Texture/Consistency
1	F1	Slightly yellow	Odourless	Smooth and uniform
2	F2	Slightly yellow	Odourless	Smooth and homogeneous
3	F3	Pale yellow	Odourless	Smooth and uniform
4	F4	Slightly yellow	Odourless	Smooth and free from grittiness
5	F5	Slightly yellow	Odourless	Smooth and consistent
6	F6	Slightly yellow	Odourless	Homogeneous and smooth
7	F7	Slightly yellow	Odourless	Smooth and uniform

The physical appearance of all formulated ophthalmic ointment batches (F1–F7) was evaluated for colour, odour and texture. All formulations were found to be slightly yellow in colour, odourless and possessed smooth, uniform and homogeneous texture without any grittiness or phase separation. The results indicated acceptable physical characteristics suitable for ophthalmic application.

Table No.8:4.PH:

Sr.No	Batches	PH
1.	F1	7.1
2.	F2	7.0
3.	F3	7.2
4.	F4	7.1
5.	F5	7.3
6.	F6	7.2

7.	F7	7.0

The pH of all formulated batches (F1–F7) of the herbal ophthalmic ointment was found to be in the range of 7.0–7.3. The obtained pH values were close to the normal pH of tear fluid, indicating good compatibility of the formulation with ocular tissues. Therefore, the prepared ophthalmic ointment formulations were considered suitable for ocular application with minimal chances of irritation or discomfort to the eyes.

Table No.9:4.Spreadability:

Sr.No	Batches	Spreadability(cm)
1.	F1	6.2
2.	F2	5.8
3.	F3	6.5
4.	F4	6.1
5.	F5	6.7
6.	F6	6.3
7.	F7	6.0

The spreadability of all formulated batches (F1–F7) of the herbal ophthalmic ointment was found to be in the range of 5.8–6.7 cm. The formulations exhibited good spreadability with smooth and uniform application characteristics. The obtained results indicated that the ointment could be easily applied over the ocular surface, ensuring better patient compliance and effective therapeutic action.

Table No10:5.Viscosity:

Sr.No	Batches	Viscosity(Cp)
1.	F1	2850
2.	F2	2600
3.	F3	3000
4.	F4	2750
5.	F5	3150
6.	F6	2900
7.	F7	2700

The viscosity of all formulated batches (F1–F7) was found to be satisfactory, indicating good consistency and suitability of the herbal ophthalmic ointment for ocular application.

6.Stability Studies:

The developed herbal ophthalmic ointment formulations were found to be stable during the stability study period. No significant changes were observed in appearance, pH, viscosity, and spreadability at different storage conditions. The formulations retained their physical stability and were found suitable for ophthalmic use throughout the study period.

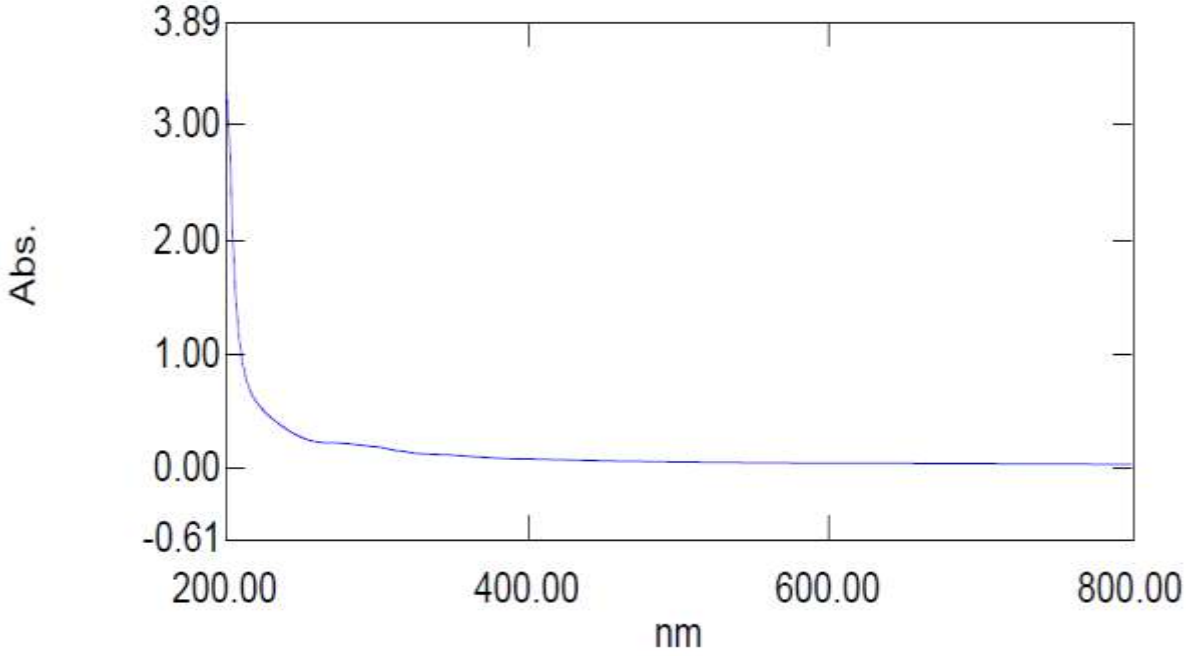
Table No.11:7.Drug content:

Sr. No.	Batches	Drug Content
1.	F_1	85.98 %
2.	F_2	90.02 %
3.	F_3	92.57 %
4.	F_4	94.38%
5.	F_5	95.20%
6.	F_6	95.10%

7	F_7	93.33%

The drug content of all batches (F_1–F_7) was found to be satisfactory and ranged from 85.98% to 95.20%. Among all formulations, batch F_5 showed the highest drug content (95.20%) and was considered the best batch due to its better drug uniformity and suitability for ocular application.

1.Calibration Curve of Drug Content:



2.Graph of Drug Content Fennel Seed Extract Ointment:

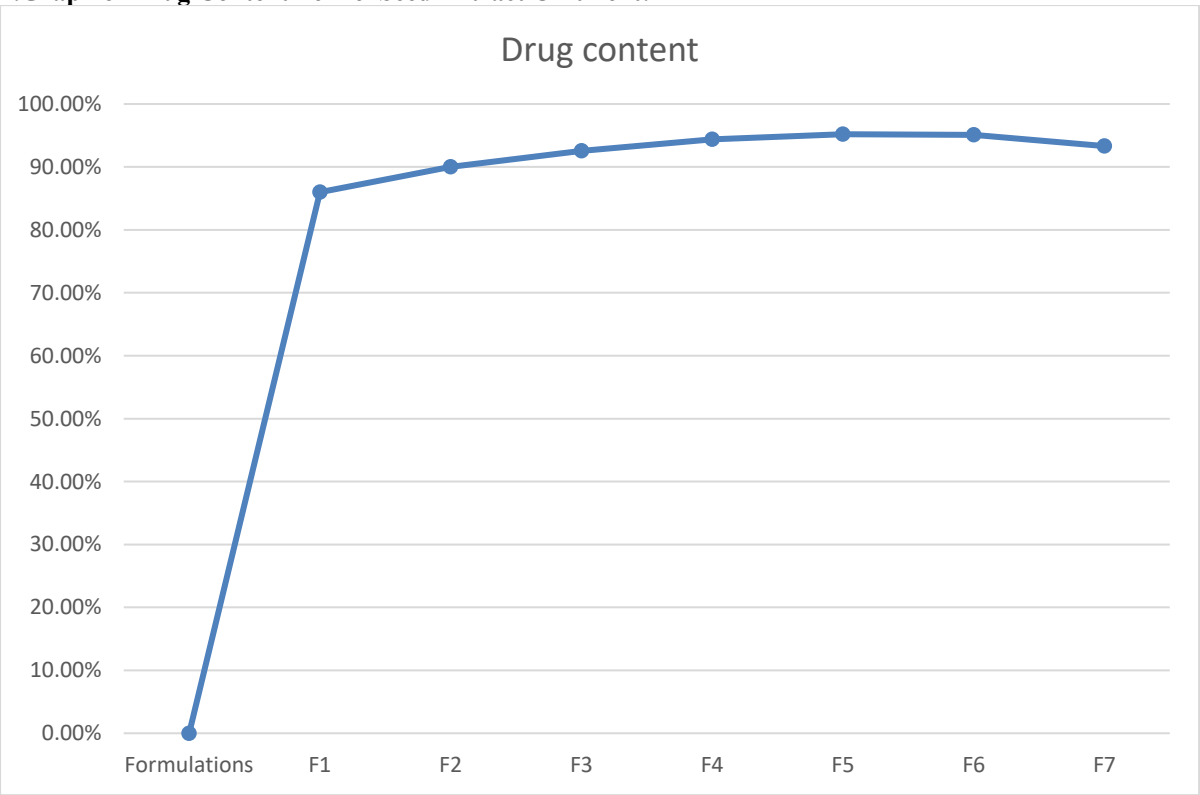


Table No12:8.Anti-Inflammatory Activity:

1.Anti-Inflammatory Activity of Formulation:

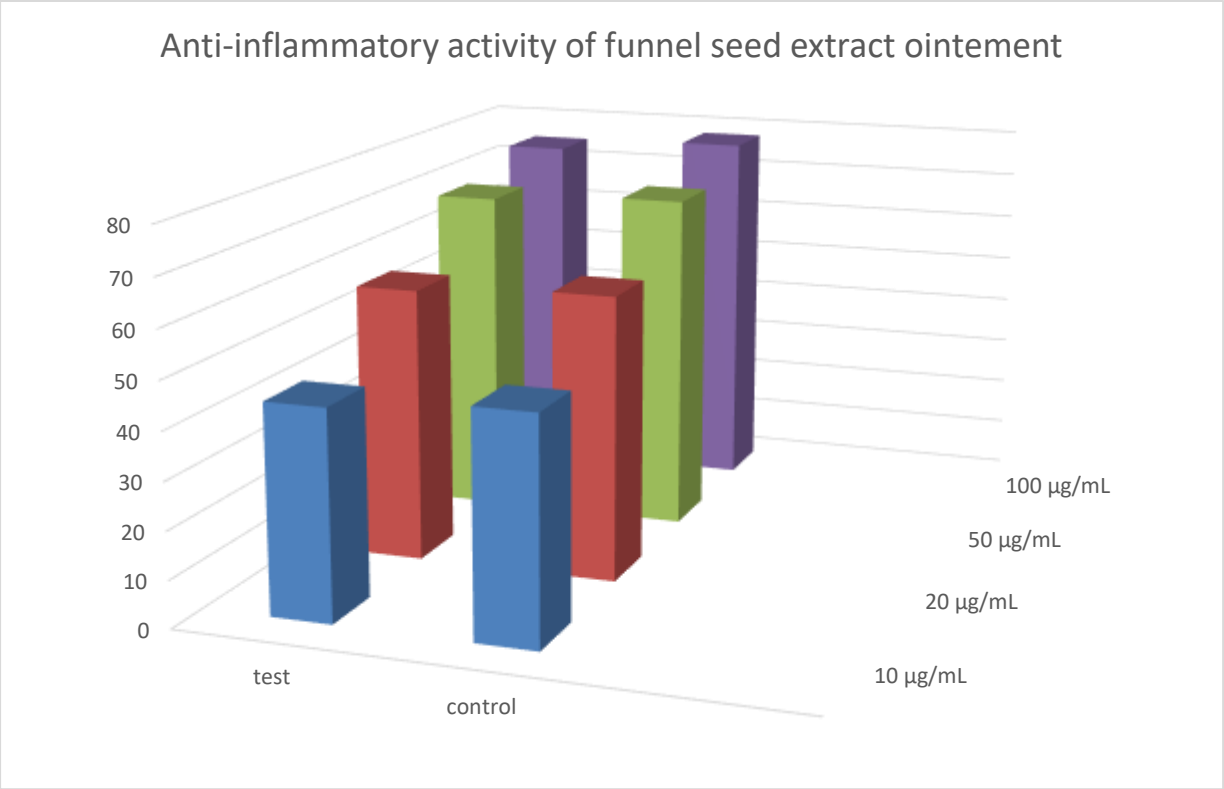
	10 µg/mL	**20 µg/mL**	**50 µg/mL**	**100 µg/mL**
Test	44	58	70	75
Control	47	60	72	78

2.Anti-Inflammatory Activity of Marketed Ointment:

	10 µg/mL	20 µg/mL	50 µg/mL	100 µg/mL
Marketed eye ointment	53	62	66	70
Standard	55	64	69	72

The anti-inflammatory activity of the formulated ophthalmic ointment was evaluated at different concentrations (10–100 µg/mL). The percentage inhibition increased with increasing concentration, indicating concentration-dependent anti-inflammatory activity. The formulation exhibited 44% inhibition at 10 µg/mL and 58% inhibition at 20 µg/mL**,** which indicates moderate anti-inflammatory activity. At higher concentrations, the formulation showed 70% inhibition at 50 µg/mL and 75% inhibition at 100 µg/mL**, i**ndicating**i** good anti-inflammatory activity**.** The highest anti-inflammatory activity was observed at 100 µg/mL (75%)**.** These results suggest that the developed herbal ophthalmic ointment possesses significant anti-inflammatory potential and is suitable for ophthalmic application.

3.Anti-Inflammatory Activity:



Chapter5:Summary and Conclusion

Summary:

The present study was undertaken with the aim to formulate and evaluate a herbal ophthalmic ointment incorporating aqueous decoction extract of Foeniculum vulgare Miller (fennel) seeds for the management of ocular inflammation. The plant material was authenticated and subjected to preliminary phytochemical screening, which confirmed the presence of flavonoids, phenols, carbohydrates, glycosides, and other bioactive constituents. The aqueous decoction extract of fennel seeds was successfully incorporated into an ointment base composed of beeswax and castor oil using the fusion method. Honey and Tulsi decoction were added as natural preservatives. Seven batches (F1 to F7) of the ophthalmic ointment were prepared by varying the concentration of the base components.

The prepared formulations were evaluated for various physicochemical parameters. All batches showed smooth, uniform texture with yellowish color and neutral pH (≈7.0), which is suitable for ocular application. The spreadability was found in the range of 5.8–6.7 cm, indicating good spreadability on the ocular surface. Viscosity values ranged from 22,650 to 64,320 cP, with Batch F3 showing optimum viscosity (38,720 cP). Drug content was found to be within acceptable limits. Stability studies as per ICH guidelines showed that the optimized formulation remained stable with no significant changes in appearance, pH, spreadability, and viscosity.

In vitro anti-inflammatory activity performed by the protein denaturation method demonstrated significant anti-inflammatory potential of the formulated ointment. The results indicated that the fennel seed extract retained its bioactivity even after incorporation into the ointment base.

Conclusion

The present investigation successfully demonstrated that a stable and effective herbal ophthalmic ointment can be formulated using Foeniculum vulgare Miller seed extract. The developed formulation exhibited desirable physicochemical properties such as smooth texture, neutral pH, good spreadability, and optimum viscosity, making it suitable for ocular application. The significant anti-inflammatory activity observed in the protein denaturation assay highlights the therapeutic potential of the fennel seed extract in managing ocular inflammation. This study establishes Foeniculum vulgare as a promising natural ingredient for the development of herbal ophthalmic preparations. The use of natural preservatives (honey and Tulsi) further enhances the safety and acceptability of the formulation. The prepared ointment may serve as a safer alternative to synthetic preparations with fewer side effects for the treatment of ocular inflammatory conditions.

However, further *in vivo* studies, ocular irritation tests, and clinical trials are recommended to confirm the safety, efficacy, and therapeutic effectiveness of the developed formulation before commercial use.

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