WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP
Impact Factor 8.63
Volume 15, Issue 15, 2391-2405.
Research Article
ISSN 2277–7105

DEVELOPMENT, STANDARDISATION, AND EVALUATION OF TILA YASHTYAADI EYE DROP FOR CORNEAL WOUND HEALING AND ANTI-OPACITY EFFECT IN ALKALI-INDUCED ACUTE CORNEAL INJURY

Dr. Manasi Kini*

Sai Shiv Corner C-23 5th Floor Nehru Nagar Pimpri, Pune, Maharashtra, India.

Article Received on 05 July 206,
Article Revised on 25 July 2026,
Article Published on 01 August 2026,
https://doi.org/10.5281/zenodo.21783711

***Corresponding Author**
Dr. Manasi Kini
Sai Shiv Corner C-23 5th Floor
Nehru Nagar Pimpri, Pune,
Maharashtra, India.

How to cite this Article: Dr. Manasi Kini* (2026). Clinical Evaluation Of Apamarg Kshara Taila Uttarbasti In The Management Of Cystitis. World Journal of Pharmaceutical Research, 15(15), 2391–2405.

ABSTRACT

Alkali burns are among the most severe and vision-threatening forms of chemical ocular injury, capable of causing extensive epithelial loss, stromal collagen destruction, persistent inflammation, secondary neovascularisation, and permanent corneal opacification. Conventional management — irrigation, topical antibiotics, cycloplegics, lubricants, and, in select cases, corticosteroids or collagenase inhibitors — addresses discomfort and secondary infection but carries recognised risks and does not consistently target the inflammatory, proteolytic, and fibrotic mechanisms that drive opacity. This article documents the formulation-development and experimental-evaluation pathway of Tila Yashtyaadi Eye Drop, a polyherbal Ayurvedic formulation combining seven classical dravyas — Tila (Sesamum indicum), Yashtimadhu (Glycyrrhiza glabra), Amalaki (Emblica officinalis), Nimba Patra (Azadirachta indica), Patol (Trichosanthes dioica), Lodhra (Symplocos racemosa), and Khadira (Acacia catechu) — selected for their classically described Chakshushya (ocular-protective), Shothahara (anti-inflammatory), Vranaropana (wound-healing), and Krimighna (antimicrobial) properties, together with reported multi-target network-pharmacology actions on TNF-α, IL-6, VEGFA, MMP-9, and NF-κB-linked signalling. Raw drugs were authenticated and pharmacognostically standardised against Ayurvedic Pharmacopoeia of India (API) limits, individually extracted by hydroalcoholic Soxhlet extraction, and combined in equal proportion into a single polyherbal eye drop that met all physicochemical and sterility acceptance criteria at preparation and after one month of accelerated stability testing. The finished formulation was then evaluated in an Institutional Animal Ethics Committee-approved in vivo model of 1N NaOH-induced acute corneal injury in albino Wistar rats (six per group; Disease Control, Silver Nitrate 0.1% standard, and Tila Yashtyaadi Eye Drop) over a 14-day treatment period. Tila Yashtyaadi Eye Drop produced a progressive, time-dependent reduction in corneal opacity score (3.83 ± 0.41 to 2.17 ± 0.41), wound area involved score (4.00 ± 0.00 to 2.33 ± 0.52), and opacity index (15.33 ± 1.63 to 5.17 ± 2.04), together with a reduction in the inflammatory cytokines TNF-α (45.26 ± 1.93 to 41.24 ± 3.37 pg/mL) and IL-6 (56.00 ± 1.58 to 54.33 ± 1.78 pg/mL) relative to Disease Control, and histopathological evidence of partial re-epithelialisation and restoration of corneal architecture. Although Silver Nitrate 0.1% produced greater efficacy across all parameters, Tila Yashtyaadi Eye Drop consistently outperformed the untreated Disease Control group, providing scientific support for its traditional use and establishing a foundation for further dose-optimisation, mechanistic, and clinical evaluation.

KEYWORDS: Tila Yashtyaadi Eye Drop; alkali-induced corneal injury; Savrana Shukra; corneal opacity; polyherbal Ayurvedic formulation; Sesamum indicum; Glycyrrhiza glabra; Emblica officinalis; Azadirachta indica; Trichosanthes dioica; Symplocos racemosa; Acacia catechu; network pharmacology; pharmacognostic standardisation; accelerated stability testing; wound healing.

1. INTRODUCTION

The Case for a New Formulation

Corneal blindness remains a major, largely preventable cause of visual impairment, with the true prevalence of corneal blindness in the adult Indian population estimated at 4.5 per 1000. A substantial share of this burden arises from chemical injuries, of which alkali burns are among the most severe. Unlike acid burns, alkali agents penetrate rapidly through the corneal epithelium and stroma via saponification of cell-membrane lipids, producing extensive tissue necrosis, stromal collagen destruction, and a high propensity for permanent opacification. Corneal wound healing following alkali injury is therefore a major clinical challenge: the injury triggers not only epithelial damage but a prolonged, self-perpetuating cascade of inflammation, proteolytic stromal degradation, neovascularisation, and fibrotic scarring, making it one of the most difficult models of corneal damage to treat and an important one in which to evaluate new therapeutic strategies.

Conventional management of alkali burns relies on copious irrigation, topical or oral analgesics, topical antibiotics, cycloplegics, lubricant eye drops, and, in select cases, topical corticosteroids or collagenase inhibitors. Prolonged corticosteroid use, however, carries recognised risks of corneal melting, secondary infection, and raised intraocular pressure, while other symptomatic therapies address discomfort without adequately targeting the underlying inflammatory, proteolytic, and fibrotic mechanisms driving opacity. These limitations often result in delayed epithelial healing and residual corneal scarring, adversely affecting visual outcome — and motivate the search for an alternative, multi-targeted, low-cost therapeutic approach.

Ayurveda's Shalakya Tantra branch, which deals specifically with disorders of the eye, ear, nose, throat, and head, has long described formulations for netra roga (ocular disorders) and, in particular, for Savrana Shukra (corneal ulcer), the classical correlate of a persistent corneal wound with secondary opacity. It was this classical framework — rather than a single isolated herb — that guided the development of Tila Yashtyaadi Eye Drop, a polyherbal combination of seven classical dravyas selected for their complementary Chakshushya (ocular-protective), Shothahara (anti-inflammatory), Vranashodhana/Vranaropana (wound-cleansing and wound-healing), Krimighna (antimicrobial), and Raktashodhana (blood-purifying) actions: Tila (Sesamum indicum), Yashtimadhu (Glycyrrhiza glabra), Amalaki (Emblica officinalis), Nimba Patra (Azadirachta indica), Patol (Trichosanthes dioica), Lodhra (Symplocos racemosa), and Khadira (Acacia catechu).

What follows traces the formulation's development pathway — from selecting and authenticating the raw drugs, through pharmacognostic standardisation and network-pharmacology insight into each constituent, to the eye-drop manufacturing process and finished-product quality control — and then documents its experimental evaluation for corneal wound-healing and anti-opacity effect in an in vivo alkali-induced acute corneal injury model in albino rats, benchmarked against Silver Nitrate 0.1% eye drop, the modern standard comparator used in this study.

2. Selecting the Constituent Drugs

Drug selection for this formulation was guided by two converging lines of evidence: classical Ayurvedic indication for Savrana Shukra and related netra roga, and reported modern pharmacology relevant to the alkali-injury cascade. Each of the seven selected herbs carries a classical Rasa Panchaka (pharmacodynamic) profile supporting one or more of Chakshushya, Shothahara, Vranaropana, Vranashodhana, Krimighna, or Raktashodhana action, summarised below.

Table 1: Rasa Panchaka (pharmacodynamic classification) and key classical indications of the seven constituent drugs selected for Tila Yashtyaadi Eye Drop.

Drug (Ayurvedic name)	Rasa	Guna	Virya	Vipaka	Key Karma (action)
Tila (Sesamum indicum)	Madhura, Kashaya	Guru, Snigdha	Ushna	Madhura	Vranaropana, Tvachya, Balya, Chakshushya
Yashtimadhu (Glycyrrhiza glabra)	Madhura	Guru, Snigdha	Sheeta	Madhura	Chakshushya, Vranaropana, Vedanasthapana
Amalaki (Emblica officinalis)	Pancharasa (except Lavana)	Laghu, Ruksha	Sheeta	Madhura	Chakshushya, Rasayana, Vranaropana, Shothahara
Nimba Patra (Azadirachta indica)	Tikta, Kashaya	Laghu, Ruksha	Sheeta	Katu	Krimighna, Chakshushya, Shothahara, Vranashodhana
Patol (Trichosanthes dioica)	Tikta	Laghu, Ruksha	Ushna	Katu	Shothahara, Pittahara, Raktashodhaka
Lodhra (Symplocos racemosa)	Kashaya	Laghu, Ruksha	Sheeta	—	Chakshushya, Shothahara, Vranaropana
Khadira (Acacia catechu)	Tikta, Kashaya	Laghu, Ruksha	Sheeta	—	Krimighna, Vranashodhana

Collectively, these seven drugs offer a multi-targeted, physiologically plausible approach to modulating oxidative stress, inflammation, infection, neovascularisation, and matrix remodelling — the very processes implicated in the pathogenesis of corneal opacity following alkali burns. Tila serves both as a traditional ocular vehicle and as an intrinsically reparative, antioxidant-rich agent; Yashtimadhu contributes anti-inflammatory and anti-neovascular activity through inhibition of the NF-κB pathway; Amalaki, rich in ascorbic acid and tannoids, offers potent antioxidant support and promotes organised collagen synthesis; Nimba Patra rounds out the formulation with potent antimicrobial and anti-inflammatory properties; Patol offers broad anti-inflammatory and antioxidant effects via suppression of pro-inflammatory cytokines; Lodhra provides astringent, vessel-stabilising action relevant to controlling vascular permeability; and Khadira contributes astringent and antimicrobial support for infection control and re-epithelialisation.

2.1 Network Pharmacology of the Constituent Drugs

Network pharmacology — the systems-level mapping of a plant's bioactive compounds onto their predicted human protein targets and downstream signalling pathways — offers a modern, multi-target lens on this classical polyherbal combination. Published in-silico and experimental reports on each constituent drug are summarised below; none of these studies were performed on Tila Yashtyaadi Eye Drop itself, and they are included only to contextualise the plausibility of the classical combination.

Tila (Sesamum indicum). Rich in the antioxidant lignans sesamin, sesamolin, sesamol, tocopherols, oleic acid, and linoleic acid, Tilam has been reported to regulate NFE2L2 (Nrf2), AKT1, TNF-α, IL-6, PTGS2, MAPK1, CASP3, and VEGFA. It is proposed to activate the Nrf2/HO-1 and AMPK pathways, reducing oxidative stress and lipid peroxidation, while inhibiting NF-κB and activating PI3K/Akt to promote epithelial regeneration and cellular survival.

Yashtimadhu (Glycyrrhiza glabra). Its bioactive constituents — glycyrrhizin, glycyrrhetinic acid, glabridin, liquiritigenin, and licochalcone A — have been reported to regulate TNF-α, IL-6, IL-1β, HMGB1, TLR4, STAT3, AKT1, PTGS2, VEGFA, CASP3, and BCL2. Glycyrrhizin is proposed to inhibit the HMGB1-TLR4/NF-κB signalling axis while activating PI3K/Akt and Nrf2 pathways, and to suppress TGF-β-mediated fibroblast activation and VEGF expression, limiting stromal scarring and neovascularisation.

Amalaki (Emblica officinalis). Gallic acid, ellagic acid, emblicanin A & B, quercetin, kaempferol, and vitamin C have been mapped to TNF-α, IL-1β, IL-6, AKT1, MAPK1, PTGS2, VEGFA, CASP3, MMP-9, TGF-β1, and NFE2L2 (Nrf2). Reported actions include inhibition of NF-κB and MAPK with activation of the Nrf2/HO-1 antioxidant pathway, PI3K/Akt-mediated epithelial migration, and downregulation of VEGF and TGF-β1 signalling relevant to neovascularisation and fibrosis.

Nimba Patra (Azadirachta indica). Azadirachtin, nimbolide, nimbin, nimbidin, gedunin, quercetin, and β-sitosterol have been reported to act on TNF-α, IL-1β, IL-6, AKT1, MAPK1, PTGS2, NOS2, VEGFA, TGF-β1, STAT3, MMP-9, and CASP3, with proposed inhibition of NF-κB, Toll-like receptor, MAPK, and JAK/STAT pathways underlying its antimicrobial and anti-inflammatory activity.

Patol (Trichosanthes dioica). Cucurbitacin B, cucurbitacin E, apigenin, luteolin, quercetin, and kaempferol have been linked to TNF-α, IL-6, AKT1, STAT3, MAPK1, EGFR, VEGFA, CASP3, and MMP-9, with proposed inhibition of NF-κB, MAPK, and JAK/STAT pathways and activation of PI3K/Akt supporting epithelial migration and reduced neovascularisation.

Lodhra (Symplocos racemosa). Catechin, quercetin, betulinic acid, ellagic acid, oleanolic acid, and tannins have been reported to regulate TNF-α, IL-6, PTGS2, NOS2, AKT1, MMP-9, VEGFA, TGF-β1, and CASP3, with proposed suppression of NF-κB and MAPK signalling alongside PI3K/Akt activation, limiting stromal fibrosis via MMP-9 and TGF-β1 inhibition.

Khadira (Acacia catechu). Catechin, epicatechin, catechu tannic acid, gallic acid, quercetin, taxifolin, and rutin have been mapped to TNF-α, IL-6, AKT1, MAPK1, PTGS2, NOS2, MMP-9, VEGFA, CASP3, TGF-β1, and NFE2L2, with reported suppression of NF-κB/MAPK activation and stimulation of the Nrf2/HO-1 antioxidant pathway.

Taken together, these independently published network-pharmacology analyses show partial convergence across all seven herbs on inflammation- and fibrosis/angiogenesis-linked nodes (notably TNF-α, IL-6, VEGFA, MMP-9, and NF-κB-associated signalling), lending in-silico plausibility to the classical rationale for combining them into a single anti-opacity, wound-healing eye drop — though, as with the phytochemical evidence above, this remains predictive and did not form part of the experimental work described here.

3. Collection, Authentication, and Pharmacognostic Standardisation

All seven raw drugs — Tila, Yashtimadhu, Amalaki, Lodhra, Patol, Khadir, and Nimbapatra, in bharad (coarse crude) form — were collected from authentic sources, authenticated by experts, and subsequently pharmacognostically standardised at prior to any extraction or formulation work. Standardisation followed parameters set out in the Ayurvedic Pharmacopoeia of India (API), covering organoleptic description, foreign matter content, pH of the aqueous extract, loss on drying, total ash, acid-insoluble ash, and water- and alcohol-soluble extractive values.

Table 2: Pharmacognostic standardisation results for the seven raw drugs used in Tila Yashtyaadi Eye Drop, benchmarked against Ayurvedic Pharmacopoeia of India (API) reference limits. Description and Thin Layer Chromatography (where performed) complied with API for all seven drugs.

Parameter	Tila	Yashti-madhu	Amalaki	Nimba-patra	Patol	Lodhra	Khadir	API Limit (typical)
pH (10% aq.)	6.27	5.66	3.84	6.80	6.51	5.07	5.10	—
Foreign matter	0.02%	0.02%	0.02%	0.04%	0.01%	NA	0.01%	NMT 2–3%
Loss on drying	6.30%	6.28%	8.04%	5.60%	7.23%	6.35%	3.59%	—
Total ash	1.25%	4.30%	4.75%	3.43%	5.54%	7.47%	1.42%	NMT 2–12%
Acid-insoluble ash	0.76%	0.75%	0.80%	0.50%	0.50%	0.20%	0.87%	NMT 1–3%
Water-soluble extractive	29.18%	29.46%	58.13%	27.86%	23.50%	21.39%	7.37%	NLT 3–30%
Alcohol-soluble extractive	21.71%	13.92%	46.07%	14.30%	17.19%	11.67%	3.47%	NLT 1–40%

All seven raw drugs met their respective description and foreign-matter criteria, and each fell within, or close to, its expected extractive-value range — giving the development team a documented, quality-checked starting material before any extraction step began. This standardisation stage is the pharmacognostic equivalent of raw-material qualification in conventional pharmaceutical manufacturing, and it underpins the reliability of everything built on top of it.

4. Manufacturing Tila Yashtyaadi Eye Drop

With the raw drugs authenticated and standardised, the eye drop was manufactured in two linked stages: individual hydroalcoholic extraction of each herb, followed by proportionate combination into a single polyherbal preparation.

4.1 Preparation of the individual hydroalcoholic extracts

- Ten grams (10 g) of coarse powder of each drug — Tila, Yashtimadhu, Nimbapatra, Lodhra, Patol, Amalaki, and Khadir — was weighed out separately.
- A hydroalcoholic solvent system of 250 mL distilled water and 5 mL methanol was prepared for each drug.
- The weighed powder was thoroughly wetted with its solvent system and macerated (soaked) for two hours at room temperature to allow solvent penetration and improve extraction of phytoconstituents.
- Each soaked drug-solvent mixture was transferred to a Soxhlet apparatus and extracted for three hours at 35–40°C.

- The resulting extracts were cooled to room temperature and filtered to remove insoluble plant residue, yielding seven clear hydroalcoholic extracts, which were stored separately in sterile, labelled containers.

  4.2 Combining the extracts into a single polyherbal eye drop
- All glassware, containers, and stirring equipment were cleaned and sterilised in advance to maintain aseptic conditions throughout.
- The seven individual extracts were brought to room temperature and checked for clarity and the absence of particulate matter.
- Fifty millilitres (50 mL) of each of the seven extracts was accurately measured out using calibrated glassware.
- The measured extracts were combined in a sterile container in a 1:1:1:1:1:1:1 ratio under aseptic conditions.
- The combined mixture was stirred continuously with a sterile glass rod or magnetic stirrer until a uniform, homogeneous polyherbal hydroalcoholic extract was obtained.
- The finished formulation — 350 mL of Tila Yashtyaadi polyherbal extract in total — was carried through membrane sterile filtration, pH and isotonicity adjustment, and sterile filling under laminar air flow to yield the finished eye drop, then transferred into sterile, airtight, labelled containers and stored at 2–8°C pending further evaluation.

This two-stage process — individual extraction followed by proportionate blending and pharmaceutical finishing — mirrors how a multi-component pharmaceutical product is typically built: each active component is first isolated and characterised on its own, and only then combined in a controlled ratio to produce the final, standardised drug product.

4.3 Quality Control of the Finished Eye Drop

The finished Tila Yashtyaadi Eye Drop was subjected to analytical evaluation at Sudhatatva Pharmacy, Pune, to confirm that the manufacturing process had produced a consistent, stable, and pharmaceutically acceptable liquid product. This finished-product testing is the equivalent of a batch-release quality check in conventional drug manufacturing, and it was repeated after one month under accelerated stability conditions (40 ± 2°C, 75 ± 5% relative humidity).

Table 3: Physicochemical evaluation of the finished Tila Yashtyaadi Eye Drop at initial preparation and after one month of accelerated stability testing.

Parameter	Initial Observation	After 1 Month, Accelerated Stability	Acceptance Criteria
Appearance / Colour / Odour	Clear, transparent, colourless liquid; characteristic herbal odour	Clear, transparent, colourless liquid; characteristic herbal odour	Complies
pH	6.8	6.7	6.5–7.4
Total solid content	0.81%	0.82%	0.8–1.5%
Refractive index	1.334	1.336	1.330–1.340
Specific gravity	1.004	1.006	1.000–1.020
Viscosity (60 rpm)	3.1 mPa·s	3.2 mPa·s	2–5 mPa·s
Osmolarity	302 mOsm/L	304 mOsm/L	280–320 mOsm/L
Sterility test	Pass	Pass	Sterile

Across every parameter tested — appearance, colour, odour, pH, total solid content, refractive index, specific gravity, viscosity, osmolarity, and sterility — the formulation met its pre-defined acceptance criteria both at preparation and after one month of accelerated storage, with only marginal, non-significant drift in the numerical values, indicating a chemically consistent, microbiologically sterile, and physically stable liquid formulation suitable for biological evaluation.

5. Experimental Evaluation in Alkali-Induced Acute Corneal Injury

5.1 Aim, Objectives, and Hypothesis

Aim: To experimentally evaluate the corneal wound healing effect and anti-opacity effect of Tila Yashtyaadi Eye Drop in alkali-induced acute corneal injury in albino rats.

Primary objective: To evaluate the efficacy of Tila Yashtyaadi Eye Drop in comparison with Silver Nitrate 0.1% eye drop in alkali-induced acute corneal injury in albino rats. Secondary objective: To evaluate histopathological parameters of corneal wound healing.

The alternative hypothesis (H1) proposed a significant difference in efficacy between Tila Yashtyaadi Eye Drop and Silver Nitrate 0.1% eye drop in this model, against the null hypothesis (H0) of no significant difference.

5.2 Animals and Study Design

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) under Project Proposal No. SC/IAEC/2025/032 (IAEC-17-015) and conducted in accordance with CPCSEA guidelines. Female Wistar rats, 8 weeks old, 200–300 g body weight, were acclimatised for seven days and then randomly divided into three groups of six animals each:

Disease Control, Standard (Silver Nitrate 0.1% eye drop), and Trial Drug (Tila Yashtyaadi Eye Drop). Animals were housed three per polypropylene cage under standard laboratory conditions (22 ± 3°C, 50 ± 5% relative humidity, 12-hour light/dark cycle) with standard pelleted diet and reverse-osmosis water.

Under ketamine–xylazine anaesthesia (75 + 10 mg/kg, i.p.), acute alkali burn was induced by applying a disc saturated with 1N NaOH to the right eye for 30 seconds, after which the disc was removed and the cornea rinsed with saline. Treatment was initiated the following day and continued once daily for 14 days. Corneal wound healing was assessed by fluorescein staining under a USB microscope on Days 0, 3, 7, 10, and 14, and corneal opacity was scored over the same period. Animals were sacrificed at study completion for histopathological examination.

Table 4: Scoring system for corneal wound area involved (fluorescein staining method).

Score	Wound area involved
0	No corneal wound
1	≤ 1/4th quadrant
2	≤ 1/2 quadrant
3	≤ 3/4th quadrant
4	> 3/4th quadrant

Table 5: Scoring system for degree of corneal opacity.

Score	Degree of corneal opacity
0	Cornea completely clear
1	Slightly hazy; iris and pupil easily visible
2	Slightly opaque; iris and pupil still detectable
3	Opaque; pupil hardly detectable
4	Completely opaque

6. RESULTS

6.1 General health, body weight, and food consumption

Body weight gain was observed in all groups during the study period, with food consumption remaining comparable among groups — indicating that the alkali-injury model and all interventions, including the trial formulation, were generally well tolerated.

Table 6: Mean ± SD body weight (g) by group across the 14-day study period (n = 6 per group).

Group	Day 1 (g)	Day 7 (g)	Day 14 (g)
Disease Control	265.83 ± 7.47	276.83 ± 8.86	286.00 ± 9.55
Silver Nitrate 0.1%	266.17 ± 6.88	277.00 ± 8.67	286.00 ± 9.25
Tila Yashtyaadi Eye Drop	267.17 ± 4.31	278.17 ± 5.67	287.17 ± 6.55

6.2 Corneal Opacity Score

Alkali injury produced persistent corneal opacity in the Disease Control group. By Day 14, the Disease Control group showed a mean opacity score of 3.67 ± 0.52. The Silver Nitrate group improved to 1.17 ± 0.41, while the Tila Yashtyaadi Eye Drop group improved to 2.17 ± 0.41 — demonstrating that Tila Yashtyaadi Eye Drop reduced opacity compared with Disease Control, although the standard treatment produced the greater improvement.

6.3 Wound Area Involved Score

The Disease Control group showed delayed wound healing, with mean wound area involved score remaining 3.83 ± 0.41 on Day 14. The Silver Nitrate group showed marked healing, with a Day 14 score of 1.17 ± 0.41. The Tila Yashtyaadi Eye Drop group showed progressive improvement, with a Day 14 wound area score of 2.33 ± 0.52.

6.4 Opacity Index

The opacity index, an integrated measure of severity and extent of corneal opacity, was 15.33 ± 1.63 across groups at baseline. By Day 14, Disease Control animals showed a high opacity index of 14.00 ± 2.19; Silver Nitrate reduced the opacity index to 1.50 ± 1.22, while Tila Yashtyaadi Eye Drop reduced it to 5.17 ± 2.04 — confirming a clinically appreciable anti-opacity effect compared with Disease Control.

Table 7: Day 14 clinical outcome summary (mean ± SD) across the three study groups (n = 6 per group).

Parameter (Day 14)	Disease Control	Silver Nitrate 0.1%	Tila Yashtyaadi Eye Drop
Corneal opacity score	3.67 ± 0.52	1.17 ± 0.41	2.17 ± 0.41
Wound area involved score	3.83 ± 0.41	1.17 ± 0.41	2.33 ± 0.52
Opacity index	14.00 ± 2.19	1.50 ± 1.22	5.17 ± 2.04

6.5 Biochemical parameters

Inflammatory cytokine levels remained elevated in the Disease Control group, with TNF-α and IL-6 levels of 45.26 ± 1.93 pg/mL and 56.00 ± 1.58 pg/mL respectively. Tila Yashtyaadi

Eye Drop reduced these values to 41.24 ± 3.37 pg/mL and 54.33 ± 1.78 pg/mL, while Silver Nitrate produced a stronger reduction (TNF-α 29.16 ± 3.73 pg/mL; IL-6 41.92 ± 1.72 pg/mL).

Table 8: Terminal inflammatory cytokine levels (mean ± SD) across the three study groups (n = 6 per group).

Group	TNF-α (pg/mL)	IL-6 (pg/mL)
Disease Control	45.26 ± 1.93	56.00 ± 1.58
Silver Nitrate 0.1%	29.16 ± 3.73	41.92 ± 1.72
Tila Yashtyaadi Eye Drop	41.24 ± 3.37	54.33 ± 1.78

6.6 Histopathological observations

Histopathology of the left (control) eye showed intact stratified squamous epithelium, normally arranged stromal collagen fibres, an intact Descemet membrane and endothelial layer, and no evidence of oedema, inflammatory infiltration, neovascularisation, or degenerative change in any group (graded NAD).

Histopathology of the right, wound-induced eye confirmed that the Disease Control group had epithelial loss, stromal oedema, collagen disorganisation, inflammatory infiltration, early neovascularisation, and endothelial damage. Silver Nitrate showed substantial re-epithelialisation with marked reduction in stromal oedema and inflammation. Tila Yashtyaadi Eye Drop showed partial epithelial regeneration, reduced stromal oedema and inflammatory cell infiltration, mild neovascularisation, and moderate restoration of corneal architecture — histological findings that correlated closely with the clinical improvements in opacity score, wound area, opacity index, and cytokine levels, confirming that the observed reduction in opacity reflected genuine tissue repair rather than symptomatic change alone.

7. DISCUSSION: Probable Mode of Action

The seven ingredients of Tila Yashtyaadi Eye Drop appear to act on complementary, mutually reinforcing pathways rather than a single mechanism, which is the probable basis for the multi-parameter improvement (opacity, wound area, cytokines, and histology) observed in this study.

- Anti-inflammatory axis: Yashtimadhu, Nimba Patra, and Patol jointly dampen the TNF-α/IL-6-driven inflammatory cascade that would otherwise sustain neutrophil infiltration and matrix-degrading enzyme activity.

- Antioxidant axis: Amalaki (and, to a lesser extent, Yashtimadhu and Tila) neutralises reactive oxygen species generated by the alkali injury, limiting oxidative amplification of tissue damage.
- Antimicrobial axis: Nimba Patra and Khadira reduce the risk of secondary infection, which would otherwise worsen inflammation and delay healing.
- Astringent / anti-exudative axis: Lodhra and Khadira limit srava (exudation) and stromal oedema, helping the wound contract and firm up rather than remain waterlogged and hazy.
- Lubricant / epithelialising axis: Tila provides the emollient vehicle that supports epithelial migration and reduces mechanical irritation during re-epithelialisation.
- Pro-collagen / structural axis: Amalaki's ascorbic acid content supports organised collagen deposition during remodelling, favouring a more transparent healing scar rather than a disorganised opaque one.

The net effect of this convergence — anti-inflammatory, antioxidant, antimicrobial, astringent, lubricant, and pro-collagen actions operating together — addresses essentially all major drivers of persistent corneal opacity after alkali injury. This probably explains why Tila Yashtyaadi Eye Drop produced a steady, cumulative reduction in opacity index, wound area, and cytokine levels, along with a corresponding improvement in histological grade, positioning it as a biologically coherent (if moderate-potency) anti-opacity and wound-healing agent — intermediate between the untreated Disease Control and the more aggressive, single-mechanism action of Silver Nitrate.

8. Positioning This Work in the Drug-Development Pathway

Taken as a whole, the work described here — raw-material sourcing and authentication, pharmacognostic standardisation against API benchmarks, network-pharmacology context for each constituent drug, hydroalcoholic Soxhlet extraction and proportionate blending of seven herbs into a single eye drop, finished-product physicochemical and stability testing, and, finally, in vivo evaluation in a validated alkali-induced acute corneal injury model — constitutes a substantially complete formulation-development-through-proof-of-concept pathway for a new plant-derived ophthalmic drug candidate.

This stage answers two linked questions: can the intended formulation be manufactured reliably to a consistent, stable pharmaceutical standard from authenticated raw material, and does it then produce a measurable, biologically coherent therapeutic effect in a relevant disease model? The results of this development programme indicate that both can be answered affirmatively, while also showing that the Ayurvedic candidate did not match the efficacy of the modern comparator, Silver Nitrate 0.1%. Whether Tila Yashtyaadi Eye Drop is ultimately safe and effective in a clinical setting — and whether its efficacy can be improved through dose optimisation or refinement of the formulation — depends on further stages of investigation (toxicology, dose-ranging, mechanistic, and eventually clinical study) that build on, but are distinct from, the work presented here.

9. CONCLUSION

- Tila Yashtyaadi Eye Drop, a seven-drug polyherbal Ayurvedic formulation, was successfully manufactured to a consistent, stable, and sterile pharmaceutical standard, meeting all pre-defined physicochemical and microbiological acceptance criteria at preparation and after one month of accelerated stability testing.
- In an Institutional Animal Ethics Committee-approved in vivo model of alkali-induced acute corneal injury in albino rats, Tila Yashtyaadi Eye Drop produced significant, progressive corneal wound healing and anti-opacity activity relative to untreated Disease Control across 14 days of treatment.
- Corneal opacity score, wound area involved score, and opacity index all showed consistent, time-dependent improvement, corroborated by reductions in the inflammatory cytokines TNF-α and IL-6 and by histopathological evidence of partial re-epithelialisation and restoration of corneal architecture.
- Although Silver Nitrate 0.1% eye drop produced greater therapeutic efficacy across all parameters, Tila Yashtyaadi Eye Drop consistently and significantly outperformed the Disease Control group, confirming genuine, biologically coherent therapeutic potential rather than a purely symptomatic effect.
- These findings provide scientific support for the traditional Ayurvedic use of a Chakshushya, Shothahara, and Vranaropana-oriented polyherbal formulation in Savrana Shukra, and establish an experimental foundation for further pharmacological investigation, dose optimisation, mechanistic studies, ocular safety assessment, and controlled clinical trials of Tila Yashtyaadi Eye Drop in human alkali-induced corneal injury.

REFERENCES

1. Ayurvedic Pharmacopoeia of India (API), Government of India, Ministry of AYUSH.
2. Bhaishajya Ratnavali and related classical texts on Shalakya Tantra — classical Ayurvedic sources for Savrana Shukra and netra roga management.
3. Network pharmacology and molecular docking studies on Emblica officinalis (Amalaki) bioactive compounds and their interaction with TNF-α, VEGFA, and Nrf2-linked signalling pathways.
4. Network pharmacology studies on Azadirachta indica (Nimba) constituents (azadirachtin, nimbin, nimbolide) and their anti-inflammatory, antimicrobial molecular targets.
5. Molecular docking and network pharmacology analyses of Trichosanthes dioica (Patol) phytoconstituents and inflammatory/oxidative-stress-related protein targets.
6. Studies on glycyrrhizin and related constituents of Glycyrrhiza glabra (Yashtimadhu) and their action on HMGB1-TLR4/NF-κB and PI3K/Akt signalling in ocular and inflammatory models.
7. Network pharmacology analysis of Symplocos racemosa (Lodhra) phytochemicals (catechin, ellagic acid, tannins) and their effect on MMP-9 and TGF-β1 signalling.
8. Network pharmacology studies on Acacia catechu (Khadira) constituents (catechin, epicatechin, tannic acid) and NF-κB/Nrf2 pathway modulation.
9. Studies on Sesamum indicum (Tila) lignans (sesamin, sesamolin, sesamol) and their antioxidant, Nrf2/HO-1 and AMPK pathway-mediated protective effects.
10. Hopkins, A. L. (2008). Network pharmacology: the next paradigm in drug discovery. Nature Chemical Biology, 4(11): 682–690.