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Original Research Article

In Vivo Biodistribution and Mechanistic Insights of Herbal Lipiodol Eye Drop in Rat Corneal Tissue Following Ocular Administration

Article History:

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Abstract: ***Background***: Ayurveda, the ancient Indian medical system, views health as a balance of body, mind, and spirit. Within its eight specialized branches, Shalakya Tantra addresses diseases of the head and sensory organs, particularly the eyes. Eye health is emphasized in classical texts, with vision described as the most vital sense. Modern ocular drug delivery, however, faces challenges due to the eye's protective anatomy. Topical eye drops are commonly used, yet their bioavailability is low—typically under 5%—owing to tear turnover, nasolacrimal drainage, and corneal barriers. Ayurveda offers many traditional herbal lipid-based formulations out of which we prepared herbal lipiodol Phyllanthus Emblica linn eye drop, which are claimed to support ocular health, but lack pharmacokinetic validation. ***Objective***: To evaluate the corneal biodistribution of lipiodol Phyllanthus Emblica linn formulation following topical ocular administration in an animal model, and to provide scientific evidence supporting its Ayurvedic claims regarding ocular penetration and retention. ***Methods***: An experimental study was conducted using Albino Wistar rats, selected for their anatomical similarity to human eyes and suitability for ophthalmic pharmacokinetic research. Lipiodol Phyllanthus Emblica linn was prepared according to classical Ayurvedic methods. Topical administration was performed, followed by corneal tissue extraction at predefined intervals. High-Performance Liquid Chromatography (HPLC) was employed for the quantitative analysis of ellagic acid, selected as a marker compound for its chemical stability and reliable detectability. Other potential markers like ascorbic acid and gallic acid were excluded due to analytical interference with corneal proteins. ***Results***: Preliminary analysis confirmed the presence of ellagic acid in corneal tissues following topical application of lipiodol Phyllanthus Emblica linn, indicating successful tissue penetration. Quantifiable levels were detected across multiple time points, suggesting sustained retention in the corneal layers. These findings offer initial pharmacokinetic support for the traditional use of lipid-based preparations in localized ocular therapy. ***Conclusion***: The study demonstrates that lipiodol Phyllanthus Emblica linn exhibits measurable corneal penetration and tissue retention after topical application, thereby validating its traditional use in Ayurvedic ophthalmology. The lipid-rich herbal formulation base likely enhances transcorneal absorption, aligning with modern drug delivery principles. These findings support the rational integration of Ayurvedic herbal formulations into evidence-based ophthalmic care and highlight the need for further pharmacodynamic and clinical studies to expand on this foundational work.

Keywords: Ayurvedic Ophthalmology, Albino Rats, Corneal Biodistribution, Drug Delivery, HPLC, Phyllanthus Emblica linn.

INTRODUCTION

Ophthalmic drug delivery remains a complex challenge due to the corneal epithelium's selectivity and tear dynamics that limit drop bioavailability to less than 5% . The search for superior carriers has found echoes in traditional medicine, where lipid-based (ghee) formulas have been applied as bioenhancers for centuries. Herbal lipiodol

formulation , prepared from Phyllanthus Emblica linn and clarified cow ghee, has caught scientific interest for its reputed rejuvenative activities and potent phytochemical profile . Yet, empirical studies exploring its pharmacokinetics in vivo are limited. This work addresses that gap using a validated animal model and quantitative methods to investigate whether classical formulations live up to their claims in modern settings. The aim of study is to evaluate the bio-distribution of lipiodol Phyllanthus Emblica linn in the cornea of Albino rats following ocular instillation. The objective of this study is to evaluate the bio-distribution of lipiodol Phyllanthus Emblica linn in the cornea of Albino rats following ocular instillation. Specifically, the study aims to assess the distribution of lipiodol Phyllanthus Emblica linn in corneal tissue, determine the maximum concentration (Cmax) of its active constituents absorbed in the cornea, and estimate the time required to reach this maximum concentration (Tmax). Additionally, the study seeks to generate evidence-based support for the corneal bio-distribution of lipiodol Phyllanthus Emblica linn, thereby contributing to a deeper understanding of the ocular pharmacokinetics of Ayurvedic formulations.

MATERIALS AND METHODOLOGY

Ethical Clearance

The design and scope of the study is based on consideration of the study objectives. The experimental procedures were performed based on protocol set forth and approved by IAEC Committee under the Project Proposal No.: SC/IAEC/2024/056.

Preparation of Amalaki Ghrita

The raw material Phyllanthus emblica L. used for the preparation of lipiodol Phyllanthus Emblica linn was procured from the local market of Pune. Authentication of the plant material was carried out at the Agharkar Research Institute (ARI), Maharashtra Association for the Cultivation of Science, Pune (An autonomous body under the Department of Science and Technology, Govt. of India).

Fresh fruits of Phyllanthus emblica L Approx. 200 g were authenticated botanically, then processed into lipoidol formulation. The lipiodol Phyllanthus Emblica linn was prepared in the department of Rasa-Shastra of Dr. D. Y. Patil College of Ayurved & Research Centre, Pimpri, Pune-18. following ancient Ayurvedic protocols . Quality control included physicochemical assays and reference to national pharmacopoeia standards.

Experimental Design

Female Albino Wistar rats weighing 150–200 grams were used for the study. They were maintained under standard laboratory conditions with food and water provided ad libitum, Ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC).

Study Design

This was an in-vivo experimental study designed to evaluate the biodistribution of lipiodol Phyllanthus Emblica linn in the cornea of Albino rats after ocular instillation. The study was conducted at Sciore Research Private Limited, Pune, following approval from the Institutional Animal Ethics Committee (IAEC) (Approval No.: SC/IAEC/2024/056). All procedures were conducted in accordance with the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), as outlined in The Gazette of India dated December 15, 1998.

A single topical ocular instillation of lipiodol Phyllanthus Emblica linn was administered to the right eye of each rat. Corneal samples were collected at 0, 0.5, 1, 2, 4, 6, 8, and 12 hours post-administration. Rats received carefully dosed instillations of lipiodol Phyllanthus Emblica linn. Corneal samples were harvested at intervals from baseline up to 12 hours, with all procedures strictly aligned to ethical guidelines 4.

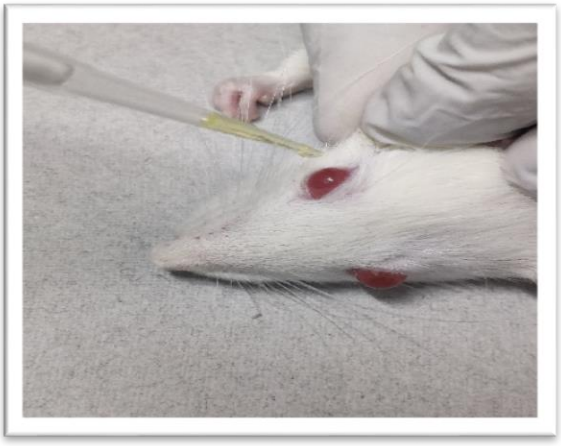


Figure 1: Topical ocular instillation of lipiodol Phyllanthus Emblica linn in Albino rat

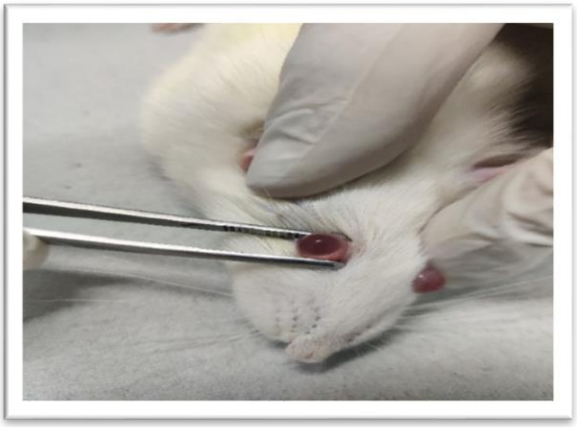


Figure 2: Enucleation of eye of Albino rat

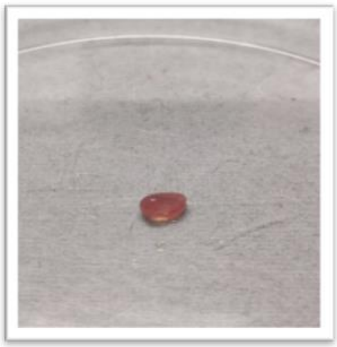


Figure 3: Separation of cornea

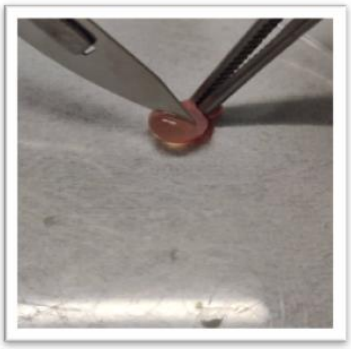


Figure 4: Cornea

Analytical Approach

Tissue collection was followed by protein precipitation and homogenization. Ellagic acid was chosen for its stability as a biomarker and quantified using HPLC with PDA detection 4. Pharmacokinetic parameters such as C-max, T-max, and AUC were calculated using PK Solver software 1.

HPLC Analysis:

Ellagic acid was used as the marker compound for quantification. High-Performance Liquid Chromatography (HPLC) was performed under standardized conditions using a C18 column and a UV detector.

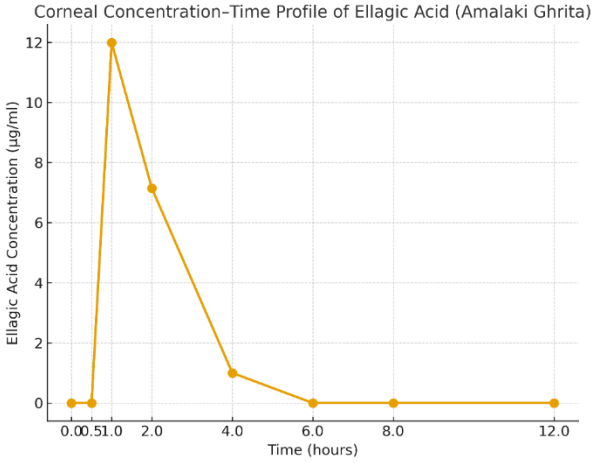
Statistical Analysis:

The concentration-time data were analyzed to calculate pharmacokinetic parameters such as Cmax (maximum

concentration) and Tmax (time required to reach this maximum concentration).

RESULTS

Ellagic acid was detected reliably in rat corneal tissues across all studied time points. Peak concentrations occurred around 1–2 hours post-administration, with measurable levels sustained for up to 12 hours. The distribution curve suggested rapid uptake, sustained retention, and gradual clearance-mirroring the theoretical advantage of lipid carriers, which adhere better to the tear surface and resist precorneal elimination 1.



Summary Table – Ellagic acid absorption in cornea

Sr. no	Parameter Name	Parameter Indication	Unit	Ellagic Acid				
				Animal 1	Animal 2	Animal 3	Mean	SD
1	Elimination rate constant	Lambda_z	1/h	0.86672	0.82779	0.84947	0.848	0.020
2	t1/2	t1/2	h	0.79974	0.83735	0.81598	0.818	0.019
3	**Tmax**	Tmax	h	**1**	**1**	**1**	**1.000**	0.000
4	**Cmax**	Cmax	µg/ml	**10.5252**	**11.8072**	**13.6633**	**11.999**	1.578
5	Tlag	Tlag	h	0	0	0	0.000	0.000
6	Clast_obs/Cmax	Clast_obs/Cmax		0.08246	0.08937	0.08035	0.084	0.005
7	AUC	AUC 0-t	µg/ml*h	22.5939	23.7602	24.7924	23.716	1.100

Cmax – Maximum concentration in corneal tissue
Tmax – Time to reach maximum concentration
t½ – Elimination half-life

AUC (0–t) and AUC (0–∞) – Area under concentration-time curve
The use of PK Solver ensured precise and validated pharmacokinetic modeling of ellagic acid distribution in corneal tissues

DISCUSSION

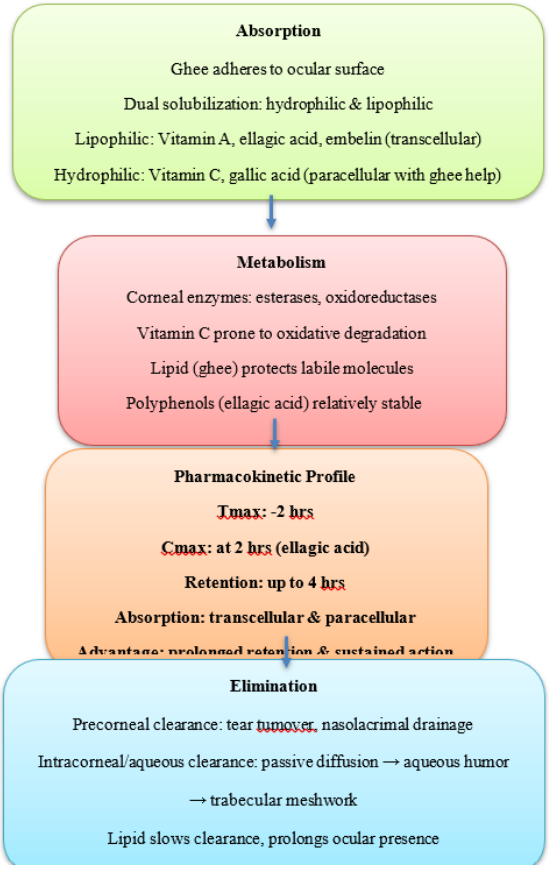
Mechanistic Insights into Corneal Action

- Lipiodol Phyllanthus Emblica linn effects in corneal tissue can be attributed to synergistic activities of its constituents and delivery vehicle:
- Lipid-mediated Penetration: The ghee base is naturally lipophilic, promoting the transcellular transport of active compounds across the corneal epithelium, typically the biggest hurdle in topical drug delivery 1,2.
- Antioxidant Defense: High levels of vitamin C, ellagic acid, and gallic acid confer strong antioxidant potential, scavenging ROS and preserving stromal collagen and epithelial integrity, which is critical in protection against UV-induced and inflammatory damage 4.
- Anti-inflammatory Effects: Key polyphenols and embelin suppress cytokine cascades, reducing tissue irritation and facilitating the healing of microtraumas 4.
- Barrier Restoration: Vitamin A and associated phytochemicals contribute to epithelial repair, activation of

keratocytes, mucin secretion, and stabilization of the tear film 5.

- Retention and Release: The viscous nature of ghrita prolongs residency on the ocular surface, allowing for a slow and sustained release of both hydrophilic and lipophilic molecules, unlike fast-draining aqueous drops 2.
- Lipophilic moieties preferentially utilize the transcellular route, while hydrophilic antioxidants benefit from ghrita's encapsulation properties for deeper stromal access 1. By overcoming both major corneal barriers, lipiodol Phyllanthus Emblica linn epitomizes a dual-mode delivery system combining ancient design and modern nanocarrier principles 2.

Probable Pharmacokinetics of Lipiodol Phyllanthus Emblica linn in the Cornea of Albino Rats



Study Limitations and Future Scope

As with many preclinical studies, the extrapolation from rodents to the human ocular milieu carries inherent limitations. Only ellagic acid was quantified due to analytical stability; future investigations should target additional bioactives and employ imaging modalities for complete profile mapping.

CONCLUSION

The study conclude that lipiodol Phyllanthus Emblica linn, a traditional Ayurvedic formulation, effectively penetrates and distributes within the corneal tissue of albino rats following ocular administration. High-performance liquid chromatography (HPLC) analysis revealed a maximum corneal concentration (Cmax) of 11.99 ± 1.57 µg/ml, observed one hour post-instillation (Tmax), with detectable levels retained for up to four hours. These findings highlight lipiodol Phyllanthus Emblica linn's potential as a lipid-based enhancer of ocular absorption. This research provides the first scientific evidence supporting the traditional Ayurvedic use of lipiodol Phyllanthus Emblica linn in topical ocular therapeutics, bridging ancient wisdom with modern ocular drug delivery approaches.

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