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Preclinical Evaluation on Rabbit of *Lakshadi Anjana* Drops: Physico-Chemical, Histopathological, and Anti-Inflammatory Insights for Ocular Therapeutic Applications

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Abstract

Introduction: Lakshadi Anjana Drops, a traditional Ayurvedic ocular formulation, is designed to alleviate eye-related conditions. Composed of herbal ingredients with therapeutic properties, this study aimed to evaluate the formulation's quality, safety, and efficacy through physico-chemical, microbiological, and preclinical analysis.

Methods: Formulation was analyzed for physico-chemical parameters such as pH, refractive index, peroxide value, saponification value, and iodine value. Preclinical testing was conducted using New Zealand white rabbits, where the Draize test was used to evaluate ocular safety and carrageenan-induced ocular inflammation model was employed to assess anti-inflammatory efficacy. Histopathological analysis of ocular tissues was performed to assess potential tissue damage.

Results: pH of Lakshadi Anjana Drops was found to be 7.82, confirming its suitability for ocular use. Physico-chemical parameters, including refractive index (1.4542), peroxide value (2.18 meq/kg), saponification value (210), and iodine value (38.49), were within acceptable limits, ensuring stability. Preclinical results indicated minimal ocular irritation and significant anti-inflammatory activity, comparable to a marketed formulation. Histopathological analysis showed minimal epithelial thickening, with no significant tissue damage.

Conclusion: Lakshadi Anjana Drops exhibited strong physico-chemical stability, and biocompatibility, supporting its therapeutic potential for ocular applications. The combination of traditional Ayurvedic ingredients with modern scientific validation highlights its promise for clinical use in ocular therapeutics.

Keywords Lakshadi Anjana Drops; Ayurvedic Formulation; Ocular Safety; Anti-Inflammatory Efficacy; Physico-Chemical Analysis; Preclinical Study

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1. Introduction

Lakshadi Anjana Drops is a traditional Ayurvedic formulation used to treat various eye-related conditions. The formulation combines a unique blend of herbal ingredients, each selected for its therapeutic properties in Ayurvedic medicine[1]. Ayurvedic treatments have long been recognized for their holistic approach to healing, emphasizing the balance of the body's inherent energies or doshas (Vata, Pitta, and Kapha). Lakshadi Anjana Drops are believed to have beneficial effects in relieving ocular discomfort, inflammation, and irritation, while promoting overall eye health[2]. Despite the extensive historical use of this formulation, scientific validation through modern research has been limited. In recent years, there has been a growing interest in the integration of traditional herbal medicine with contemporary scientific approaches to demonstrate the safety, efficacy, and consistency of these treatments. Ayurvedic formulations often face challenges in gaining acceptance in mainstream medicine due to the lack of empirical evidence supporting their therapeutic claims[3]. Therefore, it is essential to conduct rigorous scientific studies to validate the claims associated with these herbal remedies[4]. This study aims to provide comprehensive scientific data on the quality, safety, and efficacy of Lakshadi Anjana Drops through detailed physico-chemical analysis, microbiological testing, and preclinical animal studies[5]. By combining traditional knowledge with modern scientific methods, this research seeks to bridge the gap between Ayurvedic practices and contemporary ocular therapeutics, opening avenues for the broader application of Ayurvedic formulations in global healthcare[6].

2. Materials and Methods

2.1. Materials

Lakshadi Anjana Drops formulation utilized high-quality, natural ingredients sourced from reputable suppliers. The primary components included Laksha (*Laccifer lacca*) resin, Nirgundi (*Vitex negundo*) fresh juice, Bhringraj (*Eclipta prostrata*) fresh juice, and Darvi (*Berberis aristata*) root extract (figure 1), which are known for their therapeutic properties in Ayurvedic medicine, especially in treating ocular disorders. Cow ghee (clarified butter) was used for the preparation process, sourced from an organic dairy farm to ensure purity and authenticity. All ingredients were processed according to traditional Ayurvedic methods, ensuring the preservation of

Figure 1. Laksha (*Laccifer Lacch*), Nirgundi (*Vitex negundo*), Bhringraj (*Eclipta prostrata*), Darvi (*Berberis aristata*)

their active constituents. The final formulation was stored in sterilized, aseptic glass containers to avoid contamination and ensure the formulation's stability throughout the study period.

2.2. Preparation of Lakshadi Anjana Drops

For the preparation of Lakshadi Anjana Drops adhered to a traditional Ayurvedic recipe, emphasizing the use of natural, therapeutic ingredients. The formulation process began by soaking cotton pads in the combined fresh juices of Nirgundi and Bhringraj, along with the Laksha resin and Darvi extract. These soaked cotton pads were air-dried under controlled conditions for seven days to concentrate the medicinal properties. The dried cotton was then soaked in cow ghee, a critical step in the formulation, which was heated to collect the resultant ash[7]. This ash was carefully processed to minimize contamination, and diluted with ghee at a ratio of 100:1 to create the final product. The formulation was then bottled under sterile conditions to maintain its integrity and ensure it was free from microbial contamination. This preparation process follows Ayurvedic standards that guarantee the therapeutic efficacy of the product[8].

2.3. Ethical Approval

Preclinical study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: CPCSEA/ISF/065/2024) and was conducted in compliance with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for animal research. The study adhered to the ARRIVE 2.0 (Animal Research: Reporting In Vivo Experiments) guidelines, which emphasize ethical considerations, transparency, and the reduction of animal suffering. Ethical approval was obtained prior to the commencement of any experimental procedures, ensuring the study complied with all relevant regulatory requirements for the use of animals in research[9].

2.4. Animal Model

New Zealand white rabbits were selected for the study due to their well-established use in ocular research, given their similar eye anatomy to humans and their responsiveness to ocular formulations. Healthy adult rabbits, weighing between 2.0 and 3.0 kg, were used in the study. The animals were housed in individual cages under standard laboratory conditions (temperature: 22–25°C, humidity: 50–60%, and a 12-hour light/dark cycle). They were provided with a standard pellet diet and clean water ad libitum throughout the study. The selection of rabbits as the animal model was based on their ability to simulate human ocular conditions and provide reliable data regarding the formulation's safety and therapeutic potential[10].

2.5. Experimental Design

Study included three groups of rabbits (n = 6 per group) to evaluate the efficacy and safety of Lakshadi Anjana Drops: Control Group: Rabbits in this group received sterile normal saline, serving as the baseline comparison, Test Group: This group was treated with Lakshadi Anjana Drops and Positive Control Group: This group received a commercially available ophthalmic formulation, used as a standard for comparison. Each rabbit received 50 µL of the respective formulation instilled into the conjunctival sac of one eye twice daily for 14 days. The contralateral eye of each rabbit served as an untreated intra-animal control. The experimental design followed a randomized, parallel-group format, ensuring unbiased treatment allocation and reliable comparison of the effects of the test and control formulations[11].

2.6. Ocular Safety Assessment (Draize Test)

Ocular safety of Lakshadi Anjana Drops was assessed using the Draize test, a widely accepted method for evaluating the irritation potential of ophthalmic formulations. Following the instillation of 50 µL of the formulation into the conjunctival sac, the animals were observed for signs of ocular irritation at 1, 24, 48, and 72 hours post-administration. The parameters evaluated included redness, swelling, discharge, and corneal opacity, which were scored on a scale of 0 (no irritation) to 4 (severe irritation). This method allowed for the systematic quantification of potential irritation caused by the formulation and enabled a comparison between the test formulation and the control group[12].

2.7. Anti-Inflammatory Efficacy

To evaluate the anti-inflammatory efficacy of Lakshadi Anjana Drops, a carrageenan-induced ocular inflammation model was employed. Carrageenan is commonly used to induce localized ocular inflammation in rabbits, mimicking the inflammatory response that occurs in ocular diseases. A 50 µL injection of 1% carrageenan solution was instilled into the conjunctival sac of each rabbit to induce inflammation. The test and control formulations were administered immediately after carrageenan induction and every 6 hours for 24 hours. Edema thickness was measured using a digital vernier caliper. The

percentage inhibition of inflammation was calculated by comparing the edema thickness in the test groups with that in the control group. This approach allowed for the assessment of the anti-inflammatory effects of the formulation in an ocular setting[12].

2.8. Histopathological Analysis

After the completion of the treatment period, animals were euthanized humanely under anesthesia, and ocular tissues, including the cornea, conjunctiva, and stroma, were excised for histopathological examination. The tissues were fixed in 10% neutral-buffered formalin, processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Histological examination was performed using a light microscope to assess any structural damage, cellular infiltration, or inflammatory responses induced by the formulations. This analysis provided critical insights into the safety and long-term tissue effects of the test formulation[13].

2.9. Physico-Chemical Analysis

The pH of Lakshadi Anjana Drops was measured using a pH meter (EUTech pH Tutor instrument), calibrated with pH 4 and pH 7 buffer standards. Other physicochemical parameters, including peroxide value, saponification value, refractive index, and iodine value, were measured using standard laboratory methods as outlined in pharmacopeial guidelines. These parameters are essential for determining the stability and quality of the formulation over time[13].

3. Results and Discussion

3.1. Ocular Safety (Draize Test)

Draize test scores for ocular irritation were recorded for the control, test, and positive control groups. Observations were made at 1, 24, 48, and 72 hours post-administration. The test group (Lakshadi Anjana Drops) exhibited minimal irritation, with a mean Draize score significantly lower than the control group, indicating good ocular tolerance Shown in Table 1 and Figure 2. The scores were comparable to the positive control group, confirming the mild irritant profile of the formulation, suggesting that natural ingredients may have a lower irritant profile compared to synthetic ophthalmic drugs.

3.2. Anti-Inflammatory Efficacy

In the carrageenan-induced ocular inflammation model, the test group showed significant anti-inflammatory effects, which were comparable to the positive control group. The test group showed a significant reduction in edema thickness compared

Table 1. Draize Test Scores for Ocular Safety of Lakshadi Anjana Drops

Parameter	Control (Normal Saline)	Test (Lakshadi Anjana)	Positive Control (Marketed Formulation)
Redness	3.5 ± 0.3	0.5 ± 0.2	0.6 ± 0.2
Swelling	3.0 ± 0.4	0.3 ± 0.1	0.5 ± 0.1
Discharge	2.0 ± 0.3	0.4 ± 0.2	0.5 ± 0.2
Corneal Opacity	2.5 ± 0.3	0.3 ± 0.2	0.4 ± 0.1

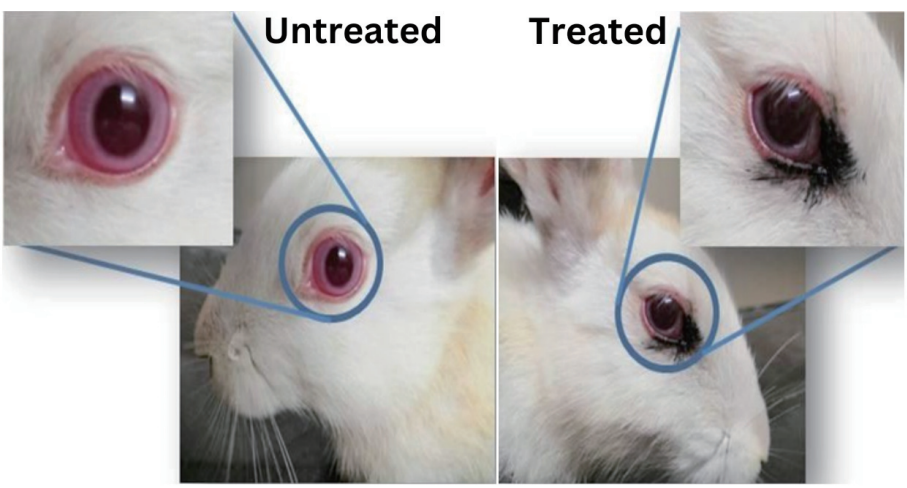

Figure 2. Ocular Administration of Lakshadi Anjana Drops in Rabbit Eye

to the control group at both 24 and 48 hours. The percentage inhibition of inflammation was 53.1%, closely matching the 56.3% inhibition observed in the positive control group in Table 2, suggesting effective anti-inflammatory action. These effects were comparable to the positive control group, supporting its therapeutic potential in managing ocular inflammation.

3.3. Histopathological Findings

Test group showed Mild Epithelial Thickening and no significant histological damage. The ocular tissues remained mostly intact, similar to the Positive Control Group, suggesting that Lakshadi Anjana Drops do not cause significant tissue damage or adverse inflammatory responses. This finding supports the ocular safety and biocompatibility of the formulation, which is crucial for long-term use in treating eye-related conditions, Result are shown in Table 3.

3.4. Physico-Chemical Analysis

pH, peroxide value, saponification value, and refractive index were assessed to determine the chemical stability of the formulation in Table 4. The pH of Lakshadi Anjana Drops was found to be in the optimal range for ocular applications. The peroxide value (2.18 meq/kg) and saponification value (210) confirmed that the formulation was chemically stable and within the acceptable limits for long-term storage.

4. Conclusion

This study presents a thorough scientific evaluation of Lakshadi Anjana Drops, a traditional Ayurvedic formulation, through rigorous physico-chemical, microbiological, and preclinical animal testing. The formulation demonstrated optimal stability with physico-chemical parameters, including pH, peroxide value, and saponification value, all falling within acceptable pharmacopeial ranges. Ocular safety (Draize test) and anti-inflammatory efficacy results revealed that the formulation exhibits minimal irritation and effective anti-inflammatory properties, comparable to commercially available formulations. Histopathological analysis of ocular tissues indicated biocompatibility and absence of significant tissue damage. These findings provide strong evidence supporting the therapeutic potential of Lakshadi Anjana Drops in ocular health. By merging traditional Ayurvedic knowledge with modern scientific methodologies, this

Table 2. Edema Thickness and Percentage Inhibition of Inflammation in Carrageenan-Induced Ocular Inflammation Model

Group	Edema Thickness at 24 hrs (mm)	Edema Thickness at 48 hrs (mm)	% Inhibition of Inflammation
Control (Saline)	3.2 ± 0.4	3.5 ± 0.5	—
Test (Lakshadi Anjana)	1.5 ± 0.3	1.7 ± 0.4	53.1%
Positive Control	1.4 ± 0.2	1.6 ± 0.3	56.3%

Table 3. Histopathological Examination of Ocular Tissues in Different Treatment Groups

Tissue	Control (Saline)	Test (Lakshadi Anjana)	Positive Control
Cornea	Significant edema and cellular infiltration	Mild epithelial thickening, no inflammation	Mild epithelial thickening, no inflammation
Conjunctiva	Inflammatory cell infiltration, edema	Minimal inflammatory response	Minimal inflammatory response
Stroma	Severe edema, damaged tissue	Minimal changes, intact structure	Minimal changes, intact structure

Table 4. Physico-Chemical Parameters of Lakshadi Anjana Drops

Parameter	Test (Lakshadi Anjana)	Acceptable Range
pH	7.82 ± 0.1	7.0–8.5
Peroxide Value (meq/kg)	2.18 ± 0.3	< 10.0
Saponification Value	210 ± 5	190–210
Refractive Index	1.4542 ± 0.02	1.45 ± 0.05

study opens avenues for further clinical research and integration of Ayurvedic formulations into mainstream healthcare practices, promoting the wider acceptance and utilization of traditional medicines in the global medical community.

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Conflict of interest

The writers attest that there is not a conflict between their interests in the article's content.

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