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Evaluation of Polyherbal Film- Forming Gel for Treatment of Fungal Infection

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Abstract

Aim of study was the evaluation of a topical film-forming gel containing polyherbal extracts for treatment of fungal infection. The evaluation of anti-fungal potential was done using Wistar rats. Four hours before to the start of the study, the dorsal side of the skin was shaved with an electric clipper, and any leftover hair was removed by applying Veet cream for five minutes. The region was then washed with alcohol and wiped with moist cotton. Total 18 healthy female Wistar albino rats weighing between 200g-250g were infected and split into six groups: group 1 (normal control), group 2 (disease control), group 3 (test drug), group 4 (standard drug), group 5 (optimal formulation), and group 6 (blank). Following confirmation of the fungal growth, the drug's application began. Rats with the infection received treatment for 14 days. In results, the anti-fungal potential was observed and found significant at day 1, day 3, day 7, day 10 and day 14 when compared with the blank/placebo. It concluded that polyherbal film forming gel is effective as anti-fungal which might destroy the growth of fungus stains on Wistar rats. It suggests, to determine the mode of action of anti-fungal potential of film forming gel.

Keywords: Film forming gel, *A. indica, O. sanctum*, anti-fungal, Wistar rats

Introduction

Tissue glues, such as films or gels, have been used to seal surgical wounds (Bajaj et al. 2016). It can be utilized for non-medical applications, such the delivery of active ingredients in cosmetics, like the silicone film-forming technology used to create ointments and creams (Klykken et al. 2009). Film forming technologies offer a number of benefits over more conventional formulation types. Because of their rapid drying and absorbing qualities, they can limit product transference losses onto clothing or other individuals, improve medicine administration, apply to wide application areas with ease, and administer a unit dose (Frederiksen K., et.al. 2015). The neem belongs to family meliaceae. It is highly cultivated in India, Pakistan, Bangladesh, and Nepal. It is a rapidly tree that can reach 20-23 m in height and has a straight trunk with a width of 4-5ft. The leaf is complex, imparipinnate and have 5-15 (leaflets) each. Its fruits are greenish drupes that changes in golden yellow on ripe (Alzohairy, 2016).

Taxonomy

Kingdom	:	Plantae
Order	:	Rutales
Family	:	Meliaceae

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Genus : *Azadirachta*

Species : *indica*

Figure 1: Leaves of *Azadirachta indica* (Neem)

Many plants have been utilized all around the world to cure a variety of illnesses or achieve other medical objectives. Different varieties of Ocimum tenuiflorum were favored and suitable as medicinal formulations in the Indian medical system. Ocimum tenuiflorum is referred to as the "Mother medicine of nature," The incomparable one, "Queen of Herbs," "Elixir of Life," and other names in Ayurveda. O. tenuiflorum is often referred to as "Holy Basil" in English and "Tulsi" in Hindi. It is a key component in the conventional "Ayurvedic" and "Unani" systems. One species or variant of the Lamiaceae family of plants is Ocimum tenuiflorum L. It has contributed to science from ancient times to the present day in current practices because of its many restorative potentials (Cohen, 2014).

Taxonomy

Kingdom: Plantae

Division: Magnoliophyta

Order: Lamiales

Figure 2: Leaves of *Ocimum tenuiflorum*

Family: Lamiaceae

Genus: *Ocimum*

Species: *tenuiflorum*

Tulsi is a significant symbol of Hindu intellectual traditions. Tulsi is one of the most important plants in Ayurveda. Tulsi is the finest Ayurvedic knowledge tonic for the body and mind, and it may treat a variety of contemporary disorders (Cohen, 2014).

Materials and Methods

Materials

Polyherbal based Film forming gel, Miconazole (std. drug), *Candida albicans* fungus, Female Wistar albino rats, methanol, distilled water.

Animal preparation

The animal house at Glocal University Pharmacy College, Mirzapur Pole Saharanpur UP, provided the 18 healthy female Wistar albino rats (200–250g). The rodents were kept at room temperature with a 12-hour light and dark cycle. In addition to providing frequent rodent food and unrestricted access to water, the relative humidity was maintained between 44 and 56 percent (Bhajoni et al. 2016).

Group design

Rats were divided into 6 groups as follows (n=5):

Group 1: Control

Group 2: Disease control

Group 3: Test drug

Group 4: Standard drug

Group 5: Optimized formulation

Group 6: Blank

Evaluation of anti-fungal potential

Prior to starting the study, electric clipper utilized for hair removal on the dorsal side, any remaining hair was removed with Veet cream for five minutes, and the skin was cleaned with wet cotton and alcohol four hours later. The inoculation was administered under general anesthesia. Each inspected rat's back was marked after a careful 3 cm2 skin cut was made with an electric clipper. The washed and shaven skin was carefully cleaned by gently rubbing it with a suspension of Candida albicans (107 CFU/ml) using a sterile switch with a cotton tip. To stop any fluid from being applied, they were using a sterile, cotton-tipped swap to gently imprint their shaved skin until no more fluid was visible. To prevent skin licking, the animals were kept apart in cages after each mixture was administered. Rats were monitored for infections and fed and watered on a regular basis. The drug was given after the

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fungal growth was confirmed. For 14 days, the sick rats were treated. Additionally, the skin's papules, scaling, redness, and dryness were microbiological and clinical characteristics. Microscopic observations of papules, papulovesicular, scales, and crusts were among the microbiological findings (Abdullah and Patil, 2021).

Score are calculated as follows:

None erythema = 0

Minor erythema = 1

Reasonable erythema = 2

Modest-Severe erythema = 3

Severe erythema = 4

Results and Discussion

Evaluation of anti-fungal potential *(in-vivo)*

In the evaluation, the mean score of erythema, edema, papule, flakiness and dryness were observed at day 1, day 3, day 7, day 10 & day 14. Mean score was evaluated for normal control, disease control, test drug, std. drug, optimized film-forming gel formulation, and blank, separately. In normal control, on day 1 & day 3, mean score was found zero. On day 7, erythema edema and dryness were observed. On day 10 & day 14, flakiness was found as zero.

Table 1: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the normal control

Parameter	Normal Control				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	0	0	2	1	1
Edema	0	0	1	1	1
Papule	0	0	0	1	1
Flakiness	0	0	0	0	0
Dryness	0	0	3	2	1

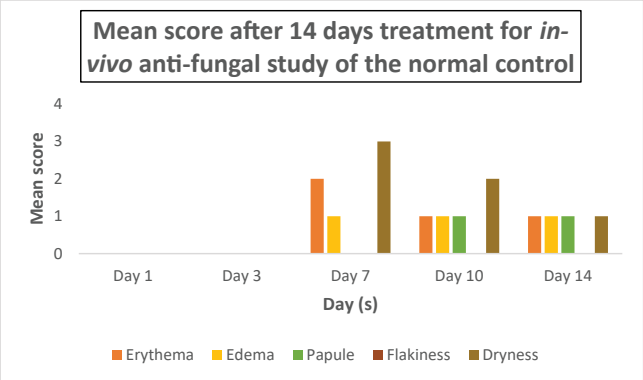

Figure 3: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the normal control

Highest mean scores were observed in disease control due to prevalence of fungal infections. On each experimental day, erythema, edema, papule, flakiness and dryness were observed except day 1 when papule was absent.

Table 2: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the disease control

Parameter	Disease Control				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	1	2	3	3	4
Edema	1	1	1	2	3
Papule	0	3	2	3	2
Flakiness	1	2	2	1	1
Dryness	0	1	3	2	3

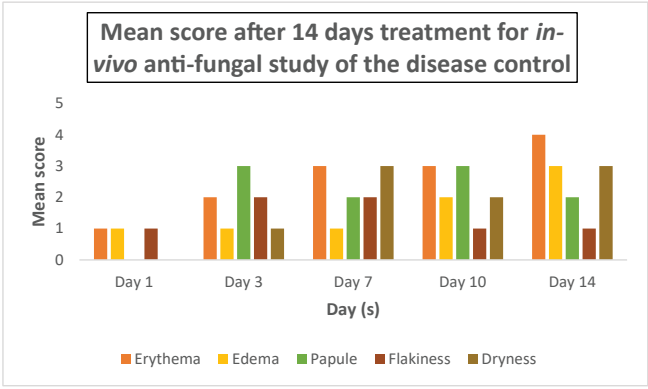

Figure 4: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the disease control

In test drug observation, Neem and Tulsi extracts exhibited anti-fungal potential when observed. It exhibited zero score on day 1 & day 3. Moreover, on day 7, day 10 & day 14, it decreased the mean scores while papule and flakiness were absent on day 14.

Miconazole was used as standard drug. It diminished the fungal infection completely on day 14, however minimal scores were observed on day 7 & day 10. In this treatment, dryness was observed chiefly.

Table 3: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the test drug

Parameter	Test drug				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	0	0	1	1	1
Edema	0	0	2	2	1
Papule	0	0	1	0	0
Flakiness	0	0	0	1	0
Dryness	0	0	1	2	1

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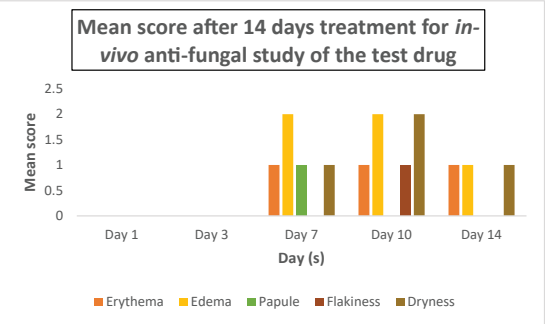

Figure 5: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the test drug

Table 4: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the standard drug

Parameter	Std. drug				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	0	0	0	1	0
Edema	0	0	1	0	0
Papule	0	0	0	0	0
Flakiness	0	0	0	0	0
Dryness	0	0	1	1	0

The optimized polyherbal based film forming gel was utilized in the test. On day 1 & day 3, it showed anti-fungal potential. On day 7, erythema edema and dryness were observed with mean score= 1.

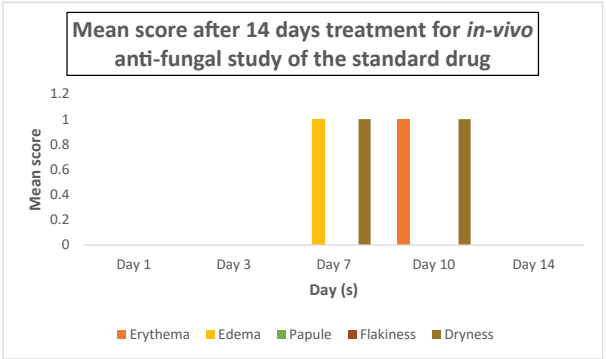

Figure 6: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the standard drug

Table 5: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the optimized formulation

Parameter	Optimized formulation				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	0	0	1	1	0
Edema	0	0	1	0	0
Papule	0	0	0	1	0
Flakiness	0	0	0	0	0
Dryness	0	0	1	1	0

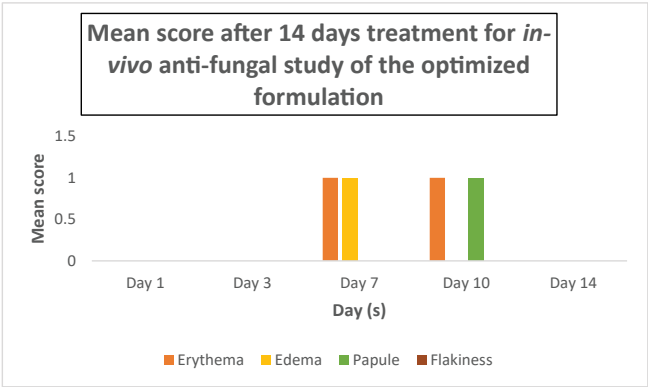

Figure 7: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the optimized formulation

Blank group also showed zero score on day 1 & day 3. Day 7, 10 & 14 mean scores were observed significantly as 3, 2, 1. However, flakiness was found absent throughout the observations.

Nevertheless, a hydrophilic polymer matrix was created by the successful encapsulation of MF into NE. Furthermore, NE-loaded films could have become more hydrophilic when 12 weight percent of a hydrophilic surfactant was added, which would have impacted their ability to absorb water. As seen in Figure 13, the drug loading efficiency of NE-loaded films was higher (94.1±6.53) than that of the physical mixture film (72.8±6.11). It appears that MF was able to be encapsulated inside NE and successfully incorporate the medication into films due to the existence of nanosized droplets. NE tends to offer high stability due to their nanoscale droplet size, which drastically reduces the probability of droplets approaching and inhibits coalescence, which might lead to phase separation. The mometasone furoate nanoemulsion-based film's content uniformity was 95.14±8.9, whereas the control film's was 94.0±4.6, indicating that the drug was uniformly distributed throughout the films (Singh V K., et al. 2024).

The formulation was developed to accomplish the following objectives, according to a study: maximum antifungal activity, drug content, film-forming qualities, optimal drying time, and desired aesthetic appearance. It contains PVP K30 (10%), Eudragit RS 100 (1%), and

Table 6: Mean score after 14 days treatment for *in-vivo* anti-fungal study of the blank

Parameter	Blank				
	Day 1	Day 3	Day 7	Day 10	Day 14
Erythema	0	0	2	3	2
Edema	0	0	1	1	1
Papule	0	0	1	2	1
Flakiness	0	0	0	0	0
Dryness	0	0	3	2	1

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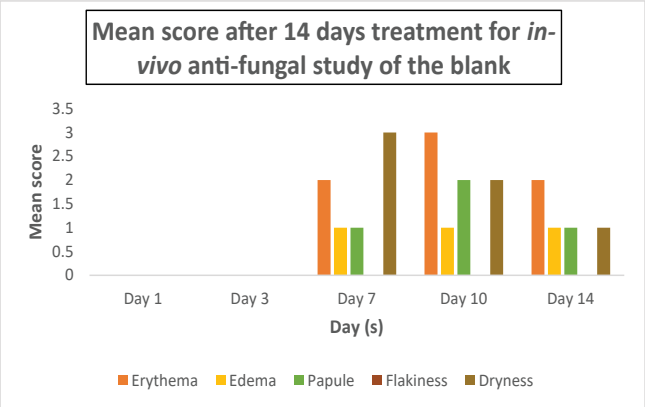

Figure 8: Graphical data of mean score after 14 days treatment for *in-vivo* anti-fungal study of the blank

propylene glycol (20%) as a plasticizer in a solvent composed of a unique ethanol:acetone (8:2) ratio (Shaikh A G., et al., 2023).

In results, the anti-fungal potential was observed and found significant at day 1, day 3, day 7, day 10 and day 14 when compared with the blank/placebo.

Conclusion

This polyherbal formulation created a film that stuck to the skin well and gradually released herbal extracts, which could improve therapeutic efficacy and patient compliance. Furthermore, the fact that the transdermal route of administration produces controlled release of the drug, which lowers systemic side effects, occasionally improves efficacy over other dosage forms, and boosts patient compliance, shows that it is recognized as one of the possible routes for the local and systemic delivery of medications. It concluded that polyherbal film forming gel is effective as anti-fungal which might destroy the growth of fungus stains on Wistar rats. It suggests, to determine the mode of action of anti-fungal potential of film forming gel.

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