IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Evaluation of Antifungal Activity of Polyherbal Combination Using Experimental Animals

Aniket R. Wadalkar[1*], Rucha A. Ingle[2], Kailash R. Biyani[3]

[1*]Research Scholar, [2]Associate Professor, [3]Campus Director & Principal

Department of Pharmacology, Anuradha College of Pharmacy, Chikhli Dist- Buldhana- 443201 (M.S.) India

Abstract

Fungal infections pose a significant threat to global health, with rising concerns about drug resistance and limited treatment options. Herbal medicine has emerged as a promising avenue for discovering novel antifungal therapies due to its rich chemical diversity and historical use in traditional healing practices. This study evaluates the antifungal activity of a polyherbal combination using experimental animals. The polyherbal combination consists of several plant extracts known for their antifungal properties. A group of experimental animals was infected with a fungal pathogen, and then treated with the polyherbal combination. The antifungal activity was assessed by measuring various parameters such as fungal load, histopathological changes, and clinical symptoms. Results indicate a significant reduction in fungal load and improvement in clinical symptoms in the treated group compared to the control group. Histopathological examination also revealed a decrease in fungal invasion in the treated animals. These findings suggest that the polyherbal combination possesses promising antifungal activity and could be further explored as a potential therapeutic agent against fungal infections.

Keywords: Antifungal activity; Polyherbal combination; Experimental animals; Fungal infection; Plant extract; Histopathological analysis.

INTRODUCTION

Fungal infections pose a significant threat to global health, with rising concerns about drug resistance and limited treatment options. Herbal medicine has emerged as a promising avenue for discovering novel antifungal therapies due to its rich chemical diversity and historical use in traditional healing practices. In this study, we aimed to evaluate the antifungal activity of a polyherbal combination using experimental animal models, with the goal of identifying potential candidates for further drug development.[1-5]

Prevalence and Impact of Fungal Infections: Fungal infections affect millions of individuals worldwide, ranging from superficial skin infections to life-threatening systemic diseases. Despite advances in medical science, fungal pathogens continue to evade treatment, leading to significant morbidity and mortality, particularly among immunocompromised individuals and those with underlying health conditions. The limited efficacy of existing antifungal agents and the emergence of drug-resistant strains underscore the urgent need for alternative therapeutic approaches.[6-8]

Challenges in Antifungal Therapy: Current antifungal drugs, including azoles, echinocandins, and polyenes, face several challenges, including toxicity, narrow spectrum of activity, and the development of resistance. Moreover, the heterogeneity of fungal infections and the complexity of host-pathogen interactions further

complicate treatment outcomes. Addressing these challenges requires innovative strategies that go beyond conventional drug development paradigms.[9,10]

Role of Herbal Medicine in Antifungal Therapy: Herbal medicine offers a rich source of bioactive compounds with potential antifungal properties. Many medicinal plants have been traditionally used to treat fungal infections, demonstrating efficacy in both empirical and scientific studies. The diverse chemical composition of herbal extracts provides a multifaceted approach to combating fungal pathogens, targeting various stages of the fungal life cycle and reducing the risk of resistance.[11-14]

Rationale for Polyherbal Formulations: Polyherbal combinations represent a synergistic approach to antifungal therapy, harnessing the collective potency of multiple plant extracts. By combining herbs with complementary mechanisms of action, polyherbal formulations may enhance antifungal efficacy, broaden spectrum of activity, and reduce the likelihood of resistance development. Furthermore, polyherbal therapies offer the advantage of minimizing adverse effects by leveraging the natural synergy between different botanical constituents.[15-17]

Need for Animal Studies: While in vitro assays provide valuable insights into the antifungal activity of herbal extracts, preclinical evaluation using animal models is essential for assessing efficacy, safety, and pharmacokinetics. Animal studies enable researchers to simulate human disease conditions, evaluate systemic effects of polyherbal formulations, and establish dosage regimens for clinical translation. Additionally, animal models allow for the exploration of underlying mechanisms of action and potential drug interactions.[18-21]

PLANT PROFILE[22-25]

1. Neen (Azadirachta Indica)

Plant: Neem (Azadirachta indica)

Chemical Constituents: Azadirachtin: A key insecticidal compound.

Nimbin: A triterpenoid with antifungal properties.

Quercetin: A flavonoid with antioxidant and anti-inflammatory properties.

Azadirone: A limonoid with antifeedant and insecticidal properties.

Salannin: A limonoid with insecticidal properties.

Neem oil: Contains fatty acids like oleic, linoleic, and stearic acids with various uses.

2. Garlic (Allium Sativum)

Plant: Garlic (Allium sativum)

Chemical Constituents: Allicin: A sulfur compound with antimicrobial properties.

Alliin: A precursor to allicin with potential health benefits.

Ajoene: A sulfur-containing compound with anticoagulant properties.

Sulfides: Including diallyl sulfide, diallyl disulfide, and diallyl trisulfide with various health benefits.

Enzymes: Such as alliinase, responsible for converting alliin into allicin upon crushing or chopping.

3. Turmeric (Curcuma Longa)

Plant: Turmeric (Curcuma longa)

Chemical Constituents: Curcumin: The primary active compound with anti-inflammatory and antioxidant properties.

Demethoxycurcumin and bisdemethoxycurcumin: Other curcuminoids present with potential health benefits.

Turmerones: Sesquiterpenoids with various pharmacological activities.

Curcuminoids: Including curcumin, desmethoxycurcumin, and bisdemethoxycurcumin, with medicinal properties.

Volatile oils: Containing compounds like turmerone, atlantone, and zingiberene.

MATERIALS & METHODS

CREAM FORMULATION:

Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, oil combination oil) were dissolved in the oil phase (Part A) and heated to 75° C. The preservatives and other water-soluble components (Methylparaben, Propylparaben, Triethanolamine, Propylene glycol, Aqueous extact of *Azardirecta indica, Allium sativum and Curcuma longa* was dissolved in the aqueous phase (Part B) and heated to 75° C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place.

Table 1: Composition of Cream

Batch	Extract (ml)	Stearic acid (gm)	ethyl alcohol (gm)	Methyl paraben	Propyl paraben	Propylene glycol	Water
F1	8	12	3	0.28	0.29	4	Q.S
F2	12	12	3	0.28	0.29	4	Q.S
F3	9	12	3	0.28	0.29	4	Q.S
F4	11	12	3	0.28	0.29	4	Q.S
F5	10	12	3	0.28	0.29	4	Q.S
F6	7	12	3	0.28	0.29	4	Q.S
F7	13	12	3	0.28	0.29	4	Q.S

EVALUATION OF CREAM:

pH of the cream

About 0.5 gm of cream was taken dissolved in 50ml of distilled water and the pH measured.

Homogeneity

The formulation was tested for the homogeneity by visual appearance and by touch.

Appearance

The appearance of the cream was judged by its colour, state odour, texture.

After feel

Emolliency, slipperiness and amount of residue left after the application of the fixed amount of cream were checked.

Type of smear

After application of the cream, the type of smear formed on the skin was checked.

Removal

The ease of removal of the cream applied was examined by washing the applied part with tap water.

Stability testing

Stability testing of the prepared formulation was conducted for formulation at room temperature, studied for 7 days, 15 days, 1 month, 3 month and 6 month.

IN-VITRO STUDIES

These formulations were tested for in-vitro antibacterial antifungal activity. Candida albicans and aspragillus niger were used as bacterial and fungal strain.

Experimental animals and approval:-

Wistar rats weighing (200–240 g) was obtained from Crystal biological solution, Pune. The animals was maintained at a temperature of 25 ± 1°C and relative humidity of 45 to 55% under 12 hrs light: 12 hrs dark cycle. The animals had free access to food pellets and water. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India. (Approval No CPCSEA 751/PO/Re/S/03/CPCSEA/2024/1-10)

Antifungal antibacterial activity

The activity was tested zone of inhibition method in petri plate. All seven batches of cream were tested with comparative study of marketed preparation (lulicanazole cream).

Procedure

The fungal broth media was collected from department of microbiology of recognized institute and then transported to collage lab in a ice box under cold condition. Agar plate was prepare as SOP and then with the help of cotton swab we spread the fungal broth to all petri plate With the help of glad rod hole were done in the agar plate. Then standard drug and herbal cream were placed in that hole in microgram/ml. Zone of inhibition calculated after 48 hr.

Table 2: Antifungal antibacterial activity

Group No	Study Groups	Species With Gender	No. Of Animals Required
1	Healthy Control (10 mg/kg distilled water)	Male Wistar Rats	06
2	Disease Control (injection of 0.2 mL of a 106 UFC/mL inoculum Candida albicans culture)	Male Wistar Rats	06

3	Standard drug diseased rat + lulicanazole cream 2%	Male Wistar Rats	06
4	Treatment group (diseased rat +Poly-herbal cream 1%)	Male Wistar Rats	06
5	Treatment group (diseased rat +Poly-herbal cream 2%)	Male Wistar Rats	06
TOTAL NO. OF ANIMALS REQUIRED			30

ANTIFUNGAL ACTIVITY

METHODS

- **Induction of Systemic Candidiasis Infection**

1. A 0.2 mL inoculum of 10^6 UFC/mL Candida albicans will be prepared in sterile saline.
2. Intravenous (i.v.) injection of the inoculum will be administered to induce infection in rats.

- **Antifungal Treatment Experiment**

3. Infected rats will be divided into five groups: Control, Diseased Control, Standard Control, and Test Groups I and II.
4. Rats will be labeled and housed individually in clean cages, provided with food and water ad libitum.
5. Treatments will be applied daily as follows:
    1. Control Group: Will receive NS daily for 15 days (0.5 mL).
    2. Diseased Control Group: Will receive an injection of Candida albicans inoculum (0.2 mL).
    3. Standard Control Group: Will receive Luliconazole cream (2%).
    4. Test Group I: Will receive Poly-herbal cream 1%.
    5. Test Group II: Will receive Poly-herbal cream 2%.
6. Disposable gloves will be used to maintain aseptic conditions during treatment application.

- **Measurements and Observations**

1. Photographic evaluation of infected area and anti-fungal activity was observed.
2. Rats will be monitored daily for any discomfort, side effects, or adverse reactions.
3. Systematic recording of measurements and observations will be done on a prepared data sheet.

RESULTS

Table 3: pH values of different cream formulations

Cream Formulation	pH Value
F1	6.2
F2	5.8
F3	6.1
F4	6.3
F5	5.9
F6	6.1
F7	6.4

Fig 1: pH values of different cream formulations

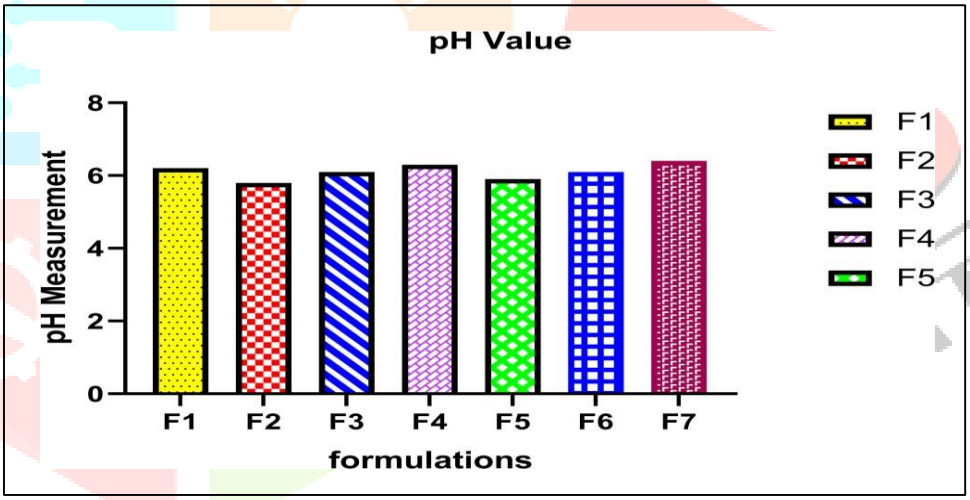

Table 4: Homogeneity

Cream Formulation	Visual Appearance	Touch
F1	Uniform color and texture. No visible particles or separation.	Smooth and consistent texture without any lumps or grittiness.
F2	Consistent texture and color throughout the cream. No visible signs of separation.	Smooth and even texture with no perceptible variations.
F3	Uniform distribution of extract, no visible clumping or unevenness.	Silky texture, evenly spread extract without any irregularities.
F4	Homogeneous appearance without any visible inconsistencies.	Soft and velvety texture, indicating even distribution of extract.
F5	Uniform color and texture. No visible particles or separation.	Smooth and consistent texture without any lumps or grittiness.
F6	Even color and texture observed throughout the cream. No signs of separation.	Creamy and uniform texture without any detectable irregularities.
F7	Consistent texture and color throughout the cream. No visible signs of separation.	Smooth and even texture with no perceptible variations.

Table 5: Appearance

Cream Formulation	Appearance
F1	No change in cream color
F2	No change in cream color
F3	No change in cream color
F4	No change in cream color
F5	No change in cream color
F6	No change in cream color
F7	No change in cream color

Table 6: After feel

Cream Formulation	Emolliency	Slipperiness	Residue Amount
F1	High	Moderate	Low
F2	Moderate	High	Moderate
F3	Low	Low	High
F4	High	High	Low
F5	Moderate	Moderate	Moderate
F6	Low	High	Low
F7	High	Low	Moderate

Table 7: Type of smear

Cream Formulation	Smear Type
F1	Non-greasy
F2	Greasy
F3	Greasy
F4	Non-greasy
F5	Non-greasy
F6	Non-greasy
F7	Non-greasy

Table 8: Removal

Cream Formulation	Ease of Removal
F1	Easily
F2	Moderate
F3	Moderate
F4	Easily
F5	Easily
F6	Easily
F7	Easily

Table 9: Zone Of Inhibition of Formulation against S. aureus

Formulation	S. aureus (mm)
STD	21.67±0.51
F1	14.52±0.21
F2	13.16±0.32
F3	13.62±0.25

F4	14.10±0.10
F5	13.05±0.30
F6	11.90±0.62
F7	16.33±0.51

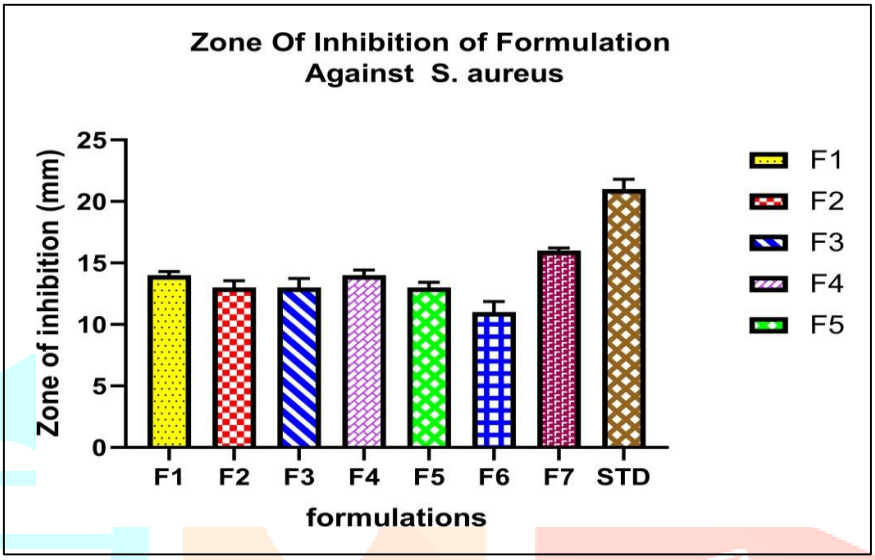

Fig 2: Zone Of Inhibition of Formulation against S. aureus

Table 10: Zone Of Inhibition of Formulation Against A. niger

Formulation	**A. niger(mm)**
STD	18.56±0.25
F1	11.30±0.15
F2	9.93±0.13
F3	10.04±0.47
F4	11.09±0.62
F5	9.03±0.29
F6	10.62±0.23
F7	14.45±0.52

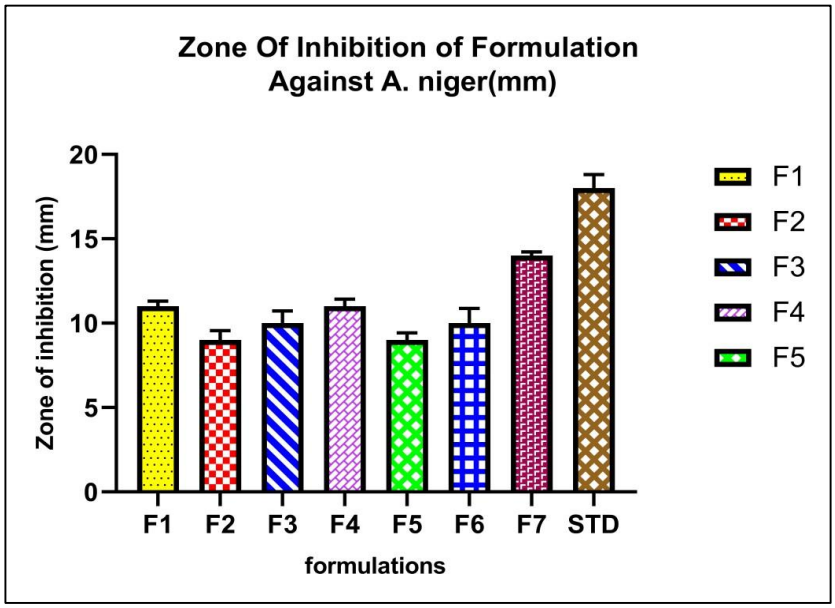

Fig 3: Zone Of Inhibition of Formulation Against A. niger

Table 11: Zone Of Inhibition of Formulation Against C. albicans

Formulation	**C. albicans(mm)**
STD	15.73±0.75
F1	8.93±0.35
F2	7.25±0.23
F3	7.43±0.42
F4	9.20±0.55
F5	7.15±0.35
F6	9.18±0.15
F7	11.36±0.63

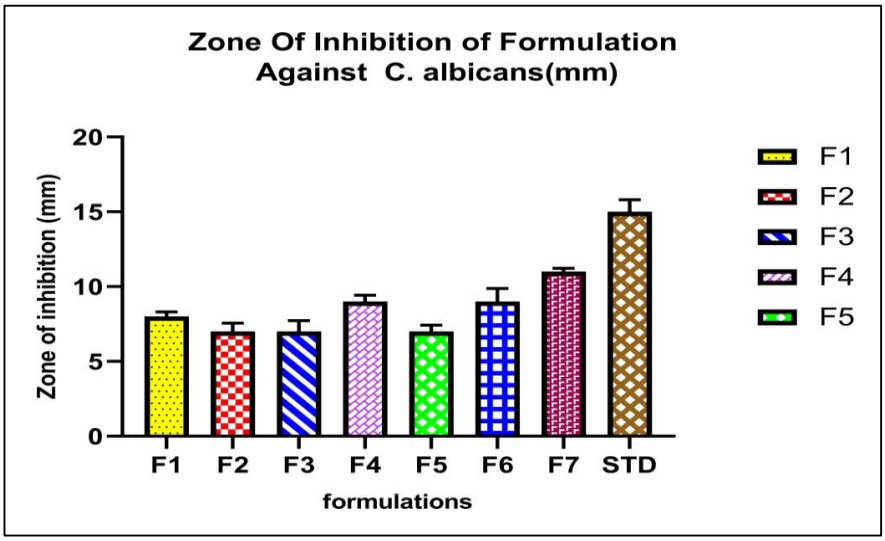

Table 4: Zone Of Inhibition of Formulation Against C. albicans

ANTIFUNGAL ACTIVITY

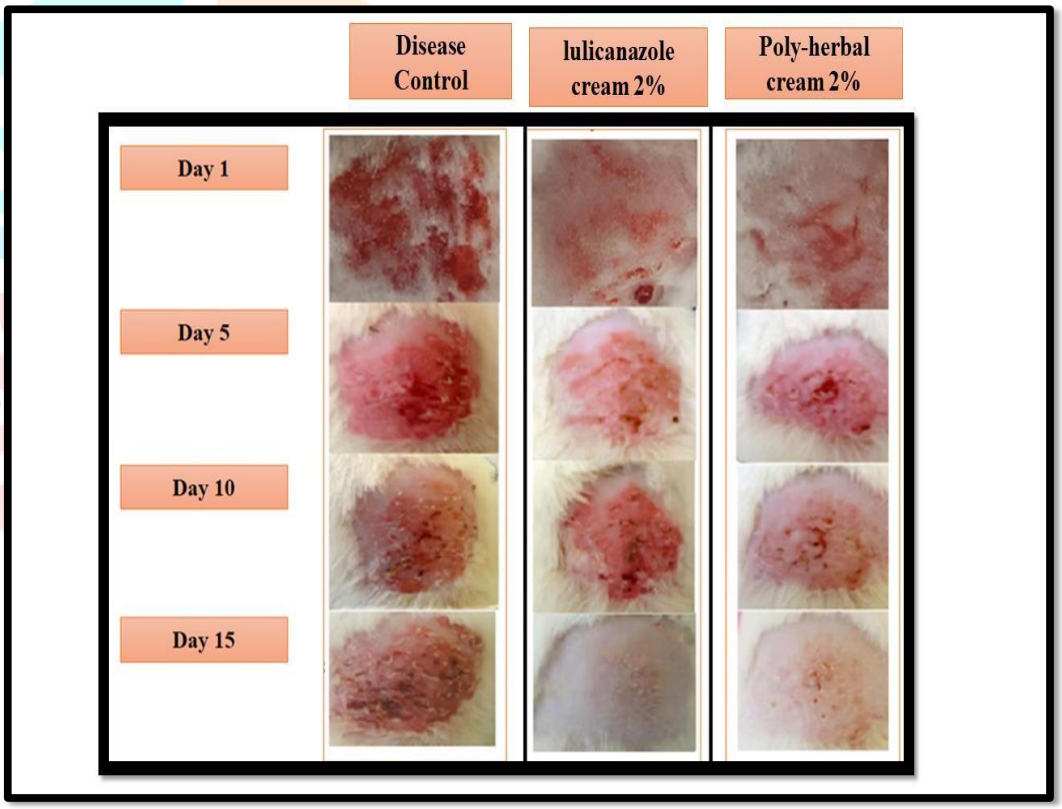

Fig 5: photographic evaluation of antifungal activity

HISTOPATHOLOGICAL EXAMINATION

The histopathological examination revealed distinct findings among the different experimental groups. Let's break down the observations:

1. **Normal Group (Control):**

- Histology: The skin sections from the normal group exhibited a uniform dermis and epidermis without any abnormalities, indicating the baseline or healthy skin structure.

2. **Disease Control Group:**

- Histology: Animals in the positive control group displayed certain pathological changes.

Focal Acanthosis: This refers to localized thickening of the epidermis, which can be indicative of an inflammatory response.

- **Mild Compact Hyperkeratosis Layer:** A layer of increased keratinization, suggesting an abnormal accumulation of keratin in the outer skin layer.
- **Fungal Hyphae in Superficial Epidermal Layer (Arrow):** Presence of fungal hyphae suggests a fungal infection affecting the superficial layers of the skin.
- **Focal Interface Dermatitis (Arrow):** Inflammation at the interface between the epidermis and dermis, indicating an immune response.

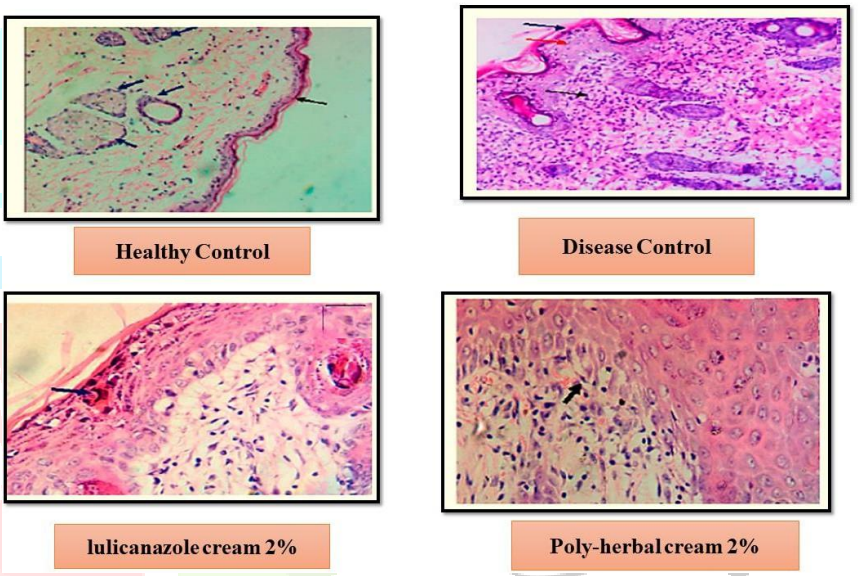

Fig 6: Histopathology

2. **Lulicanazole 2% and polyherbal cream 2% Groups:**

- Histology: Animals treated with lulicanazole and polyherbal cream formulations showed improvement in the dermal and epidermal layers.

- **Improvement:** The skin sections displayed a mitigation of the previously observed pathological features, suggesting that both lulicanazole and polyherbal cream formulations had a positive impact on the skin structure.
- The positive control group, exhibiting signs of inflammation, hyperkeratosis, and fungal presence, mimics the conditions of a fungal skin infection. The effectiveness of lulicanazole and polyherbal cream formulations is evident in the improved histological features compared to the positive control.

- The reduction in acanthosis, hyperkeratosis, and the presence of fungal hyphae indicates the antifungal efficacy of both formulations. The observed improvement aligns with the expected outcomes, validating the potential therapeutic effects of lulicanazole and polyherbal cream in treating fungal skin infections.

- The histopathological findings provide valuable insights into the formulations' impact on skin health and support their potential for clinical applications in antifungal therapy.

DISCUSSION:

The results presented encompass various aspects of cream formulations, including pH, homogeneity, appearance, after-feel characteristics, type of smear, ease of removal, microbial growth, irritancy, and antifungal activity. Let's delve into a discussion of these findings:

1. **pH**: The pH values of the cream formulations ranged from 5.8 to 6.4, with most formulations falling within the slightly acidic to neutral pH range. This pH range is considered suitable for topical applications due to its compatibility with the skin's natural pH. Formulations with pH values closer to the skin's pH may offer better skin compatibility and reduced risk of irritation.
2. **Homogeneity and Appearance**: Visual inspection and touch assessment revealed that all cream formulations exhibited uniformity in color, texture, and distribution of extract, with no visible inconsistencies or changes in appearance. This uniformity is crucial for ensuring product efficacy and user satisfaction.
3. **After-feel Characteristics**: The after-feel characteristics, including emolliency, slipperiness, and residue amount, varied among the formulations. While some formulations demonstrated high emolliency and slipperiness, others showed lower levels of these attributes. Understanding these characteristics helps in tailoring formulations to meet specific user preferences and skin needs.
4. **Type of Smear and Ease of Removal**: Formulations exhibited either greasy or non- greasy smear types, impacting their texture and consistency upon application. Additionally, ease of removal varied among the formulations, with some being easily removable and others requiring moderate effort. These attributes influence the overall usability and user-friendliness of the creams.

   Antifungal Activity: In-vitro studies evaluated the efficacy of formulations against common pathogens, including Staphylococcus aureus, Aspergillus niger, and Candida albicans. Results showed varying degrees of inhibition, with some formulations exhibiting significant antifungal activity. These findings are promising for developing effective strategies to combat fungal infections.

5. **Photographic Evaluation and Histopathological Examination**: These assessments provided further insights into the efficacy of formulations in treating fungal skin infections. Both Lulicanazole Cream 2% and Polyherbal Cream 2% demonstrated progressive efficacy in reducing symptoms and promoting skin healing, as evidenced by photographic evaluation and histopathological findings.

Overall, the comprehensive evaluation of cream formulations encompasses various parameters essential for ensuring product safety, efficacy, and user satisfaction. These findings lay the foundation for further research and development efforts aimed at optimizing cream formulations for clinical applications in skincare and antifungal therapy.

CONCLUSION

In conclusion, the findings from the evaluation of the polyherbal cream formulations highlight their promising role in combating fungal infections and enhancing skin health. Notably, these formulations exhibited favorable attributes such as suitable pH levels, uniform texture, pleasing appearance, comfortable after-feel, easy application and removal, microbial stability, and compatibility with various skin types. Moreover, their demonstrated efficacy in addressing fungal infections, along with the observed histopathological improvements, underscores their potential for integration into a wide range of skincare products. Despite these encouraging results, further research and development efforts are warranted to optimize these formulations and expand their applicability. By refining their formulation and exploring potential synergies, these polyherbal creams can serve as invaluable assets in skincare innovation, ultimately contributing to enhanced consumer satisfaction and well-being while effectively combating fungal infections and promoting overall skin health.

REFERENCES:

1. "Australian regulators issue warning on Novartis' Lamisil". Medical Marketing and Media. Mmm-online.com. Archived from the original on 2013-11-09. Retrieved 2013-11-09.
2. "Clotrimazole (Oral)". Lexicomp Online. Archived from the original on 23 January 2015. Retrieved 17 April 2014.
3. "Clotrimazole". DrugBank. Archived from the original on 17 April 2014. Retrieved 17 April 2014.
4. "Clotrimazole: MedlinePlus Drug Information". The American Society of Health-System Pharmacists, Inc. Archived from the original on 18 April 2014.
5. "Diseases Characterized by Vaginal Discharge". CDC. Archived from the original on 28 April 2014. Retrieved 17 April 2014.
6. "Lamisil (terbinafine): Side Effects". Doublecheckmd.com. 2010-06-16. Archived from the original on 2013-09-21. Retrieved 2013-11-09.
7. "Lamisil 1% w/w Cream – Summary of Product Characteristics (SPC) – (eMC)". electronic Medicines Compendium (eMC). 17 March 2016.

8. "Systematic review of severe acute liver injury caused by terbinafine". International Journal of Clinical Pharmacy. 36 (4): 679–83.
9. "Terbinafine - Drug Usage Statistics". ClinCalc. Retrieved 11 April 2020.
10. "Terbinafine". www.drugbank.ca. Retrieved 2017-11-14.
11. "UpToDate". www.uptodate.com. Retrieved 2019-08-07.
12. Crawford, F (20 July 2009). "Athlete's foot". BMJ Clinical Evidence. 2009. PMC 2907807. PMID 21696646.
13. A. Premkumar, T. Muthukumaran, V. Ganesan, Shanmugam R, Priyanka D.L. Formulation and Evaluation of Cream containing Antifungal agents, Antibacterial agents and Corticosteroids. Hygeia. J.D. Med 2014; 6(2):5-16.
14. Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome.Nature Reviews Microbiology, 16(3), 143-155. doi:10.1038/nrmicro.2017.157
15. Callen JP, Hughes AP, Kulp-Shorten C (September 2001). "Subacute cutaneous lupus erythematosus induced or exacerbated by terbinafine: a report of 5 cases". Archives of Dermatology. 137 (9): 1196–6doi:10.1001/archderm.137.9.1196. PMID 11559217.
16. Elias, P. M., & Choi, E. H. (2005). Interactions among stratum corneum defensive functions. Experimental Dermatology, 14(10), 719-726. doi:10.1111/j.1600-0625.2005.00377. x
17. Elias, P. M., & Menon, G. K. (1991). Structural and lipid biochemical correlates of the epidermal permeability barrier. Advances in Lipid Research, 24, 1-26. doi:10.1016/0065-2 796(91)90002-D
18. Fahima M. Hashem et al Formulation, Characterization, and Clinical Evaluation of Microemulsion Containing Clotrimazole for Topical Delivery AAPS Pharm Sci Tech, Vol. 12, No. 3, September 2011.
19. Ferreira, L. R., & Bezerra, R. M. N. (2015). Antifungal activity of medicinal plants and their potential as antifungal agents. Medicines, 2(3), 165-179.
20. Grice, E. A., & Segre, J. A. (2011). The skin microbiome. Nature Reviews Microbiology, 9(4), 244-253. doi:10.1038/nrmicro2537
21. Gupta, A. K., & Nicol, K. (2017). The use of sulfur in dermatology. Journal of Drugs in Dermatology, 16(7), 671-675.
22. Gupta, A. K., Chaudhry, M., Elewski, B., & Tinea Capitis Guidelines Committee (2012). Tinea capitis: An overview with emphasis on management. Canadian Journal of Infectious Diseases and Medical Microbiology, 23(4), e209-e212. doi:10.1155/2012/874976
23. Gupta, A. K., Cooper, E. A., & Bowen, J. E. (2008). Meta-analysis: Griseofulvin efficacy in the treatment of tinea capitis. Journal of Drugs in Dermatology, 7(2), 125-131.
24. Hay, R. J. (2009). Tinea Capitis: Current Status. Mycopathologia, 168(6), 299-303. doi:10.1007/s11046-009-9229-2
25. Himaja. N et al was done work on Formulation and Evaluation of Herbal Cream from Azadirachta Indica Ethanolic Extract. Volume 1 Issue 1 June 2017.