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Research Article

Efficacy, Evidence and Elegance of JRK's AF Antifungal Cream

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Abstract

The anti-fungal efficacy of JRK's AF antifungal cream was evaluated both by *in vitro* and by clinical trial. The *in vitro* studies have shown that JRK's AF antifungal cream is extremely effective against wide range of fungal pathogens viz., dermatophytes, species of Candida and *Pityrosporum ovale*. The clinical findings also shown that in two weeks usage of JRK's AF cream has completely relieved all the patients from severe fungal infections such as Tinea cruris, Tinea corporis, Candidiasis and Pityriosis vercicolor. The above findings clearly establish the validity, efficacy and the scientific sanctity of Siddha system of medicine and the two-important herbal antifungal drugs used in the cream such as *Cassia alataand Azadirachta indica*.

Introduction

Cutaneous mycosis is an emerging problem and is largely considered as major public health problem [1,2]. The cutaneous mycosis always limits to the superficial layer of the skin by limiting itself to stratum corneum, nail and hair[2,3]. Except *Pityriasis versicolor*, Candidiasis and dermatophytosis are symptomatic and cause severe itching due to their metabolites[4]. Treating cutaneous fungal infections is although easy but the patients with underlying conditions often show susceptibility to chronic infections which seldom respond to treatment. Variety of antifungal drugs are available for the treatment of cutaneous mycosis but the emerging anti-fungal drug resistance pose serious challenge to medical fraternity[5]. Therefore, safe, effective and non-specific broad spectrum anti-fungal agents are essential.

Siddha system of medicine is gifted to mankind by people with supernal wisdom and supreme spiritual attainment. Siddha system of medicine has several references of anti-fungal drugs and among them are the preparations with *Cassia alata*and neem[6-8]. Neem is considered to be a magical herb where its root to the terminal parts is medicinal and the medicinal value of neem cannot be described so easily[9].

JRK's AF antifungal cream is a proprietary siddha drug formulated with two credible anti-fungal siddha herbs such as *Cassia alata*and *Azadirachta indica.* Our earlier study using corneosurfametry has clearly shown that AF cream blocks the fungal adhesion. Blocking the fungal adhesion is quite novel and it can treat the cutaneous mycosis and simultaneously can avoid the possible drug resistance by the organism[10]. The present study is aimed at evaluating the antifungal activity of AF cream against different species of fungal pathogens viz., *Candida albicans*,*Pityrosporum ovale*and *Trichophyton mentagrophytes*. We have employed three different techniques such as MIC, determination of static effect and contact time Vs rate of kill. After establishing the antifungal effect of AF cream at laboratory level we have also subjected the siddha drug for rigorous clinical trial on patients with various cutaneous fungal infections over a period of 4 weeks. The findings are presented in the paper.

Materials and Methods

Determination of MIC

Saboraud's dextrose agar medium was used for MIC evaluation. Tween supplementation was used for studying *P.ovale*. *P.ovale*suspension adjusted to achieve 41 colonies/ml was used as inoculum. In case of *C.albicans*, the culture suspension was adjusted to 109 colonies/ml. In the case of *T.mentagrophytes*the spore suspension was prepared in such a way to achieve confluent colony covering the entire petri dish. The AF cream was incorporated with the medium separately at 1mg/ml, 0.5 mg/ml and 0.1mg/ml concentration and the above adjusted cultures were inoculated and the number of colony forming units were measured at either on day 2 and day 5 whichever is applicable.

Determination of Static Effect of AF Cream

5 mm well was made in saboraud's dextrose agar medium containing JRK's AF antifungal cream at 1mg/ml concentration. 5 mm plug prepared out of the respective fungi that were grown separately were placed in the well and the plates were allowed to incubate at 24°C for 2-5 days whichever is applicable.

The growth extension of the respective fungi that grown in the media with and without AF cream were compared and the static effect was arrived.

Contact Time Vs Rate of Kill

Into the spore/cell suspension of the 3 fungi 1mg/ml concentration of AF cream or C+ or A+ complex separately and incubated for 10 min. After 10 minutes, the spore suspension was plated on to plain Saboraud's dextrose agar medium and incubated for 2 -5 days whichever is applicable and the number of colonies grown were counted and compared with control to arrive the percentage kill Vs time.

Clinical Trials on JRK's AF Antifungal Cream

Clinical trial was done at quest life sciences as per complete bio ethical norms applicable for clinical trials.

- **Type of Study:** Prospective interventional clinical end point study (Pilot study).
- Study Design: A randomised open label single arm clinical study.
- Study Population: Patients suffering from Cutaneous Candidiasis or Dermatophytosis (Tinea/ Ring worm) or Pityriasis versicolor (Chromic/ Achromic).
- Sample Size: 20 patients diagnosed with either of the skin conditions like Cutaneous Candidiasis or Dermatophytosis (Tinea/ Ring worm) or Pityriasis Versicolor (Chromic/ Achromic)

Selection Criteria

- **Inclusion Criteria:** The following criteria's will be considered for subject enrollment in the study. Subjects in the age group between 18 to 75 years, inclusive of both male and female subjects. Availability of subject for the entire study period and willing to adhere to protocol requirements as evidenced by the written ICF duly signed by the volunteer. Subject who have no evidence of life-threatening disease during screening, medical history and whose physical examination is performed within 28 days prior to commencement of the study. Subjects suffering from either Cutaneous Candidiasis or Dermatophytosis (Tinea/ Ring worm) or Pityriasis Versicolor (Chromic/ Achromic). Atopic patients, dry skin and diabetes mellitus – definitely patients with either of the above problem and willing not to use any other anti-fungal drugs other than the investigational product during the study period. Two week wash out period may be required prior to the inclusion of patients to the study. Volunteers, who are willing to avoid sun bathing, swimming, prolonged sun exposure or artificial ultra-violet rays during the course of the study. Patients with localized epidermal dermatophytosis confirmed by presence of fungal hyphae in KOH (potassium hydroxide) preparation of skin scrapings from over the lesion at baseline visit.
- **Exclusion Criteria:** Volunteers with allergies to cosmetics, moisturizers, and whitening/bleaching agents. Volunteer who has sunburned, chapped, or irritated skin or open wounds on test sites. Volunteer who are not willing to discontinue any other personal care products containing whitening/bleaching properties during study. History of skin cancer or treatment for any type of cancer within the last 2 years. Volunteer who are with underlying immunosuppression. Volunteer who applied any other topical agent over the lesions prior to baseline visit.
- **Restrictions:** All the patients will be advised to restrict the use of antibiotics other than specified in the department protocol of the hospital. Patients will be instructed not to bathe, shower, wash or swim for at least 4 hours after the application of the study medication.

Intervention

The patients who fulfil the inclusion and exclusion criteria will be selected for the study. The start of therapy is considered as Visit 1 (Day 1). This is followed by Visit 2 (Day 7±1) and Visit 3 (Day 14 ±2).

Visit 1 (Day 1): Those patients who satisfy the inclusion and exclusion criteria will be recruited for the study. Evaluation of the response will be done by an Investigator/study team member (Dermatologist). The patient will be assessed as mentioned below for efficacy parameters and will be documented. Any concomitant medications taken by patients will be recorded. Test product will be applied topically on the affected area twice daily in morning and evening at the interval of every 12 hours (for example, 08.00 AM and 08.00 PM). Each patient will receive study drug until their next visit. They will also be instructed to come to the next visit as per schedule and report to the doctor for any unscheduled visit in case of adverse events. Patients will also be advised to update their patient compliance card/patient diary on daily basis until study completion. Patients will be told to return the empty medication tubes during the next visit.

Visit 2 (Day 7±1): Patients will be asked for any adverse events. Patients will be observed for any local or systemic adverse events due to study drug. All adverse events will be recorded in the CRF. All the patients will be assessed as mentioned below for efficacy parameters by the investigator and will be documented in the respective CRF. Number of days since the previous visit during

which the patient missed the drug due to unacceptable adverse events or missed doses will be asked for and recorded. Medications will be dispensed as mentioned above and the patients will be told to return the empty medication tubes on the next visit. They will also be instructed to come to the next visit as per the schedule and report to the doctor for any unscheduled visit in case of adverse events.

Visit 3 (Day 14**)**: Patients will be asked for any adverse events. Patients will be observed for any local or systemic adverse events due to study drug. All adverse events will be recorded in the CRF. All the patients will be assessed as mentioned below for efficacy parameters by the investigator and will be documented in the respective CRF. Number of days since the previous visit during which the patient missed the drug due to unacceptable adverse events or missed doses will be asked for and recorded. Empty medication tubes as well as remaining medication tubes will be collected for accountability.

Efficacy Assessment: An assessment will be made based on the presence or absence of fungal elements in KOH preparation and mycological cure. Additionally, during each visit, subject will be enquired regarding the symptomatic change from the initial stage and reduction in itching and scaling.

Visit 4 (Day 21):Patients will be asked for any adverse events. Patients will be observed for any local or systemic adverse events due to study drug. All adverse events will be recorded in the CRF. All the patients will be assessed as mentioned below for efficacy parameters by the investigator and will be documented in the respective CRF. Number of days since the previous visit during which the patient missed the drug due to unacceptable adverse events or missed doses will be asked for and recorded. Empty medication tubes as well as remaining medication tubes will be collected for accountability.

Post Treatment Enquiry: Patients were contacted telephonically on day 28 to check the recurrence of disease if any or its symptoms like itching or scaling. All the patients reported of no recurrence of the disease as they are completely cured of by JRK's AF cream.

Results

Determination of MIC

At 0.1 mg/ml concentration of AF cream, the percentage reduction of *P.ovale* was 26.8, Candida at 8.2 and *T.mentagraphytes* 10%. At 0.5 mg/ml concentration, AF cream exhibited the activity in reducing the number of colony forming units to 21.9 %, 74.31% and 60% respectively for *P.ovale*, *C.albicans* and *T.mentagraphytes*. At 1 mg/ml AF cream exhibited total inhibition of all the organisms.

Organisms	% Reduction of the organisms			Control (No. of colonies CFU/ml)
	0.1mg/ml	0.5mg/ml	1 mg/ml	
P.ovale	26.8	21.9	100	41
C.albicans	8.2	74.31	100	109
T.mentagrophytes	10	60	100	Abundant

Table 1: Growth of all the organisms were inhibited totally by AF cream when compared to control.

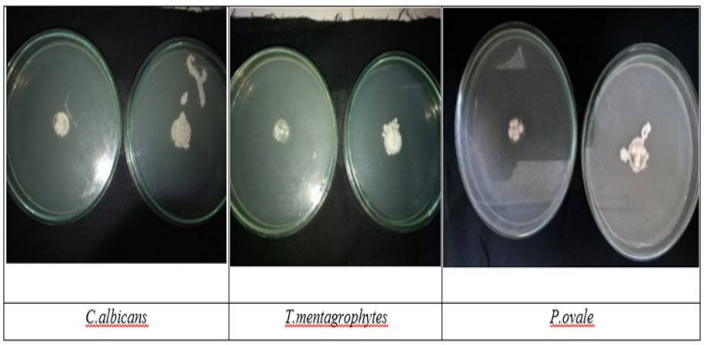


Figure 1: Determination of static effect of AF cream.

Organism	Extent of growth	
	Test	Control
P.ovale	No growth	Growth
C.albicans	No growth	Growth
T.mentagrophytes	No growth	Growth

Table 2: Determination of static effect of AF cream.

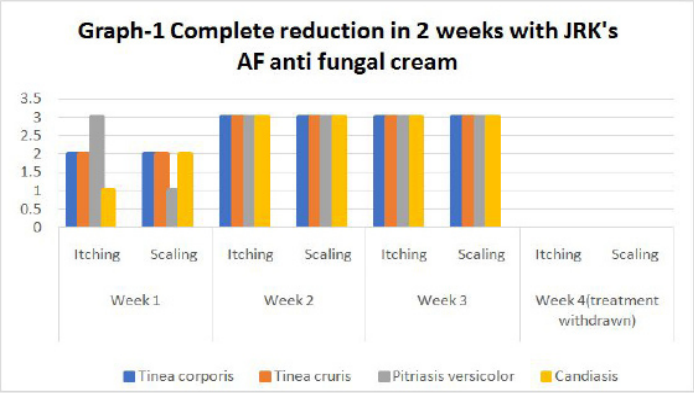
Organism	Percentage reduction of organisms			
	Control	AF cream	C+	A+
P.ovale	54	100	94.4	98.1
C.albicans	313	99.05	95.5	75.3
T.mentagrophytes	Abundant (100 app)	98	57	77

Table 3: Contact time Vs rate of kill within 10 min.

AF cream at 10 min contact had killed about 90% of the cells of all the organisms whereas C + or A+ complex did not show such effect individually.

Diagnosis	No. of patients	Reduction in signs and symptoms vis-à-vis duration of treatment/No. of patients							
		Week 1		Week 2		Week 3		Week 4(treatment with-drawn)	
		Itching	Scaling	Itching	Scaling	Itching	Scaling	Itching	Scaling
Tinea corporis	6	++/4	++/5	+++/5	+++/5	+++/6	+++/6	No recurrence	
Tinea cruris	6	++/5	++/4	+++/5	+++/6	+++/6	+++/6	No recurrence	
Pitriasis versicolor	4	+++/4	+/1	+++/4	+++/3	+++/4	+++/4	No recurrence	
Candiasis	4	+/1	++/4	+++/4	+++/4	+++/4	+++/4	No recurrence	
+ → Mild reduction ++ → Moderate reduction +++ → Complete reduction									

Table 4: Clinical Trial Findings.



Graph 1: Complete reduction in 2 weeks with JRK's AF anti-fungal cream.1→Mild reduction; 2→ Moderate reduction; 3→ Complete reduction;0→ No recurrence.

Discussion

The findings have shown that JRK's Af anti-fungal cream is extremely effective for the treatment of various cutaneous fungal diseases. Present laboratory studies have undoubtedly established that JRK's Af anti-fungal cream possesses strong anti-fungal activity against various species of fungi such as *P.ovale*, *C.albicans*and *T.mentagrophytes*.The laboratory findings clearly show that AF cream possesses broad spectrum antifungal activity.

The combination of CA+ complex is indeed superior in its efficacy over either of the individual constituents crediting the strong synergistic effect for the above. AF cream not only blocks the fungal adhesion but also kill the fungi. Further the extent of kill is quite quick and rapid. The broad spectrum anti-fungal effect of JRK's Af anti-fungal cream indeed makes it superior in the context of the AF cream having fungal adhesion blockage. The clinical trial finding was in full agreement with laboratory findings. In two weeks, remarkable reduction in itching, scaling and other signs and symptoms of fungal infections were observed. The Tinea corporisand T. crurishas responded very well in patients who were diabetic and some of the patients were in the menopause stage.

These findings superimpose that anti-fungal efficacy of JRK's Af anti-fungal cream and its therapeutic efficacy even in patients with strong predisposition for fungal diseases. To the best of our understanding JRK's Af anti-fungal cream is the only product that has the following benefits

1. Blocks the fungal adhesion.
2. Possess antifungal activity.
3. Restrict the fungal recolonization.
4. Kill the fungi in 10 min contact time.

All the above benefits are extremely inevitable for the treatment of active lesions as well as chronic lesions of cutaneous mycosis irrespective of the underlying conditions as AF cream is effective in both blocking the fungal adhesion as well as evicting the fungi. These findings testify the fundamental principle of siddha system of healing where the siddha preparations correct the Tridosha (eliminate the root cause) as well as etiological agent. Similarly, the JRK's Af anti-fungal cream block the adhesion which is parallel to correcting the tridosha and eliminate the causative agent which metaphorically equivalent to addressing the etiology.

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