International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 11 (1), pp. 001-003, January, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Short Communication

Dano: A herbal solution for dandruff

J R Krishnamoorthy[1], S. Ranganathan[2]*, S. Gokul Shankar[2] and M. S. Ranjith[2]

[1]CCRAS, Govt. of India, No.6, Perumal Kovil Street, Kuntrathur, Chennaiv, India.
[2]Department of Microbiology, The New College, Chennai, India.

Accepted 10 October, 2016

Dano, a poly-herbal hair oil was studied for anti -dandruff activity using microbiological and clinical tests. There was a clear symptomatic relief from dandruff in all the volunteers after 10 days of use. Further, the isolation of *Pityrosporum ovale*, the causative organism of the dandruff in culture was not possible after use of the Dano oil. The plant extracts is from *Wrightia tinctoria* (Indrajev), *Cassia alata* (Dadmari) and bitter fraction of *Azadirachta indica* (Neem or Nimba). Methylene blue reductase test was employed to study the anti-dandruff efficacy of the oil.

Key words: *Pityrosporum ovale*, dandruff, methylene blue reductase test.

INTRODUCTION

Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries. The problem manifests as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching. Hair fall is also not uncommon in dandruff sufferers (Paul, 1999). *Pityrosporum ovale*, a yeast-like lipophilic basidiomyce-teous fungus, is considered to be the chief cause of the problem (Roberts, 1969). Besides this, *Candida* sp. is also suspected in the disease process of dandruff. These organisms are widely considered to be the commensal flora of the scalp and skin region. Why and when this commensal organisms turns to be pathogens is not clearly understood (Rippon, 1998) . It is believed that, *P. ovale* converts the sebum lipid into fatty acids and triglycerides. These fatty acids may presumably accelerate hyper proliferation of keratinocytes (Nazzaro-Porro and Passi, 1976). Management of dandruff must essentially contain the chief causative agent, *P. ovale* and other yeast fungi that exist in the scalp as commensal flora as well as the hyper proliferation of keratinocytes. Dano, a poly-herbal hair oil, is known to have activity against *P. ovale in vitro* (Krishnamoorthy and Ranganathan, 2000). The oil contains the extracts of *Wrightia tinctoria* (Indrajev), *Cassia alata* (Dadmari) and bitter fraction of *Azadirachta indica* (Neem or Nimba). Sufficient support is available in the ancient Indian literature regarding the antidandruff activity of the above plant materials (Kirtikar and Basu, 1995). In the light of the existing knowledge on the antidandruff activity of plants that are present in Dano, a detailed clinical and microbiological study of Dano on severe dandruff sufferers in Chennai was planned. The findings of the study are presented in the paper.

MATERIALS AND METHODS

Three clinical isolates of *P. ovale* and one standard isolate procured from Institute of Microbial Technology, Chandigarh, India (strain No. MTCC 1374) were used for the *in vitro* study. Similarly one clinical isolate of *C. albicans* isolated in our earlier study was also used. All the isolates were maintained in Sabouraud's agar medium until use.

MIC (Minimum Inhibitory Concentration) determination

MIC was determined by incorporating different concentrations (by weight) of Dano in 10 ml of Sabouraud's medium (Ranganathan et al., 1996). The medium with the Dano was emulsified thoroughly with the help of Tween 20 and was allowed to solidify at room temperature. Later the organism at 10^3 cfu was inoculated on each plate with different concentrations of Dano. The plates were incubated at 37°C for 5 days. Organism in plain medium was maintained as control. The MIC was determined as the lowest concentration of Dano that inhibited the growth of the organism

*Corresponding author. E mail: subushika@yahoo.com.

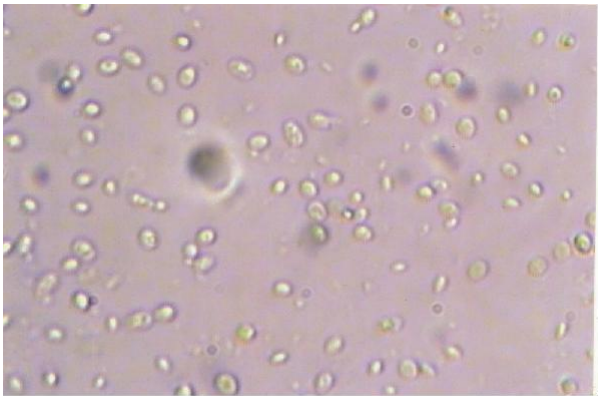

Figure 1. Live cells of *Candida albicans* (untreated, control).

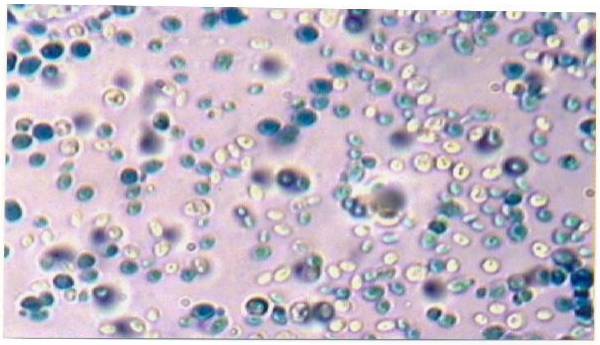

Figure 2. Dead cells of *Candida albicans* stained by methylene blue after treatment with sub lethal level of Dano.

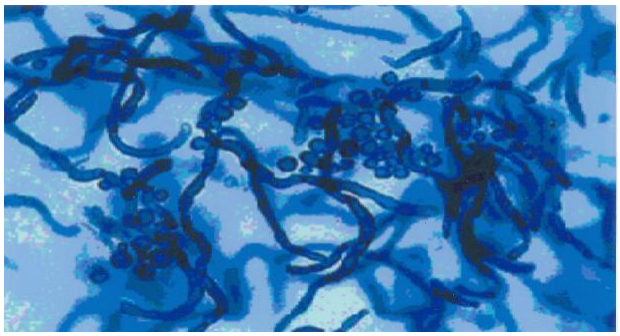

Figure 3. Microscopic examination of scalp scrapings showing hyphal and yeast cells of *P. ovale* before Dano treatment.

when compared to control.

Zone of inhibition study

Diffusion dependent activity of the Dano was studied by zone of inhibition study. The organism was uniformly inoculated on the surface of the Sabouraud's medium. A well of 10 mm diameter was cut in the center of the medium and 50 mg of the oil + Tween 20 was loaded in the well. The plate was incubated at 37°C for 5 days. The zone of inhibition was measured.

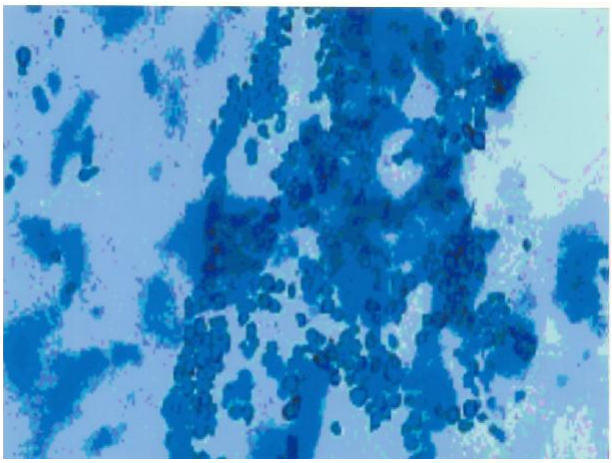

Figure 4. Microscopic examination of scalp scrapings after Dano treatment.

Methylene blue reductase test

This test was done to establish the effect of Dano on *Ca. albicans* at sub MIC level. Like the MIC test, the Dano was incorporated into 10 ml of the medium at its sub-MIC level, and the organism was inoculated and incubated for 24 h. After the incubation, the organism was scooped from the surface of medium and was stained with Methylene blue stain and was examined under microscope (Rippon, 1998). The dead cell takes up the stain and as a result cells appear blue in color, while the live cells appear colorless as these organisms has an enzyme methylene blue reductase which reduces the dye. In a microscopic field, the total number of stained vs unstained cells would reflect the killing effect of Dano at sub-MIC level on *C. albicans*. The percentage death was calculated by the above method.

***In vivo* study**

10 volunteers with severe dandruff were included in the trial. All the volunteers were in the age group of 18-22 years and were studying in a city college. Each of the volunteer was supplied with 100 ml of Dano and requested to use the same every day for their hair oil. During Dano usage, all the volunteers were requested to abstain from the use of other antidandruff shampoo/hair oil/hair cream or any antifungal medicament. All the volunteers were reviewed every alternative day for 21 days. The severity of the scaling was determined before Dano use and the reduction of the scaling subsequent to Dano use was scored as 'Severe' 'Moderate', 'Mild' and 'Traces to Nil'.

Scalp scrapings were collected from the volunteers on every alternative day after oil application as well as prior to oil use. The scrapings were divided into two parts and used for direct examination and culture separately. The scraped material was digested with 10% KOH and was observed under microscope. Randomly, 10 fields were selected and the number of spores was counted in the each of the 10 fields and totaled. Total spore burden on the scalp before and after Dano use was assessed.

The scraped materials were inoculated onto Sabouraud's medium and incubated at 37°C for 7 days. The rate of isolation of *P. ovale* from the scraped materials before and after Dano use at different time intervals was studied.

Table 1. Effect of Dano on *P. ovale* burden in the scalp.

No of volunteers	Cfu of *P. ovale*/2 cm^2 scalp (mean of 10 volunteers)					
	Pre-use	Day 2	Day 4	Day 6	Day 8	Day 10
10	TNTC	2000	800	540	218	12

TNTC: Too numerous to be counted.

Table 2. Culture study of *P. ovale* in the scalp of volunteers after Dano use.

No. of volunteers	Isolation of *P. ovale* (%)					
	Pre use	Day 2	Day 4	Day 6	Day 8	Day 10
10	100	80	50	20	-	-

RESULTS AND DISCUSSION

Dano inhibited the growth of *P. ovale* at 30 mg/ml concentration. However the MIC of Dano for *C. albicans* was 50 mg/ml. The zone of inhibition of Dano was 10 mm diameter for *P. ovale* and 6 mm for *C. albicans*. 90% death of *C. albicans* cells was observed at sub-MIC level (25 mg/ml) (Figure 2). In the case of control, death of *C. albicans* cells could not be seen (Figure 1).

Out of 10 volunteers, 9 had severe scaling while 1 had moderate scaling before Dano use. 8 days of Dano use had reduced the scaling from severe to 'Mild' to 'Traces to Nil' scale in all the 10 volunteers. Complete elimination of scaling was observed in all the volunteers in 10 days of Dano use see Figures 3 and 4). In 4 day use of Dano, the reduction *P. ovale* burden was 2000 cells/2 cm^2. The mean reduction of *P. ovale* cells were in the order of 800, 540, 218, 12 cells/2 cm^2 for days 6, 8, 10, 12 after Dano use (Table 1). After 8 days of Dano use, the isolation of *P. ovale* in culture was not possible in all the volunteers (Table 2).

The present study reveals that Dano is very effective in the management of dandruff. Dano is very effective against *P. ovale in vitro* (Krishnamoorthy and Ranganathan, 2000). In our earlier study, we have reported that the oil extracts of *W. tinctoria* possess antifungal activity especially against *P. ovale*. Similarly, the bitter fraction of neem is also known to possess antifungal activity. The extracts of *W. tinctoria* are also known to have an effect on keratinocyte proliferation (cell line study, unpublished data). The antifungal activity coupled with keratinocyte proliferation inhibition activity of *W. tinctoria* in Dano makes the oil very effective in the management of dandruff. Further, the localized immune elicitation activity of bitter fraction of neem also offers better protection against relapse of dandruff for prolonged period of time.

In this study, both *P. ovale* and *C. albicans* were found to be very sensitive to Dano. The anti-fungal activity observed against *P. ovale* and *C. albicans* can be extrapolated to other members of the same genus whose involvement in dandruff may not be ruled out. The *in vivo* experiments suggest that Dano may be very effectively in killing the cells of *P. ovale*. At present we are evaluating the effect of Dano on dandruff of unknown etiology in order to establish its effect on hyper proliferating keratinocytes.

REFERENCES

Paul KB (1999). ABC of Dermatology. BMJ Publishing Group, London.

Rippon JW (1988) Superficial infections, In Rippon, JW. (ed). Medical Mycology – Pathogenic fungi and pathogenic actinomycetes, 2 nd ed. WB Saunders, Philadelphia, pp 154-159.

Nazzaro-Porro M, Passi S (1976). Growth requirements and lipid metabolism of *Pityrosporum obiculare*. J. Invest. Dermatol. 66: 178-182.

Krishnamoorthy J, Ranganathan S (2000). Anti – Pityrosporum ovale activity of a herbal drug combination of *Wrightia tinctoria* and *Hibiscus rosasinensis*. *Indian J. Dermatol* 45 (3): 21-28.

Kirtikar KR, Basu BD (1995). Indian Medicinal Plants, two periodical experts, Delhi, India.

Ranganathan S, Menon T, Balajee SAM, Mahendraraja S (1996) Antidermatophytic activity of *Azadirachta indica* : an *in vitro* and *in vivo* study. Indian J. Dermatol. 41: 113-115.