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Dano Active AD Oil Flummox Drug Resistance Instinct in Certain Strains of Pityrosporum Ovale

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ABSTRACT

The present study describes the new rulebook in dealing dandruff where targeting the resistant isolates of Pityrosporum ovale and also preventing / delaying the process of the strains acquiring such defense mechanism. We have established a total herbal formulation, a Siddha cosmetic oil preparation having the dual effect in neutralizing the drug resistant strains causing dandruff as well as hindering the process of drug resistance or such adaptation in the strain. The entry of an array of antidandruff preparations just for the commercial endpoint only had made the lazy, commensal, lipophilic and highly fastidious yeast like fungi – Pityrosporum ovale to become resistant or adapt to deal various non-drug antidandruff agents like Zinc pyrithione, Climbazole, Octopirox etc. We have established how the herbal armamentarium in Dano active AD oil could achieve the above benefit through a well-designed scientific experiment and details are presented in the article.

Keywords: Dandruff, AD oil, Dano, P.ovale, drug resistance, anti-fungal

INTRODUCTION

Pityrosporum ovale and other species of the same genus with a few exceptions are always remembered in the medical realm as the etiological agents of the disease dandruff and Pityriasis versicolor.[1, 2] Both the diseases are superficial skin infections, often pose challenge to treatment due to drug resistance. The yeast like fungus do exhibit dimorphism which is seen in association with certain skin conditions such as chromic/achromic pityriasis; whether the dimorphic difference of the fungus or the host related factor contribute vice versa to the above chromic and achromic pityriasis versicolor is not clear.[3, 4, 5, 6]

Due to extravagant commercial exploitation of dandruff both by drug and personal care industries across the world, the fungus appears to have learned every trick in the book to escape the treatment either by developing drug resistance or by becoming part of normal microbial biome of the scalp and skin. The supra-intelligence of the fungus is posing a big problem in offering successful treatment with most medicaments that are available today.

A successful drug combination must flummox both the scope of the fungus developing resistance as well as the evolutionary instinct of the fungus where the possibility of the fungus learning/ acquiring the trait of drug resistance does not happen so easily especially when the drug is quite target specific.

The drug resistance can be hindered only through a strategy where the fungus is made confused and muddled than be clear, vigilant and intelligent in sensing the attack. Only through a 'treatment pandemonium', we can address and deal the above challenge, is our scientific instinct.

In our three decades of research expertise in medical mycology screening and studying antifungal sensitivity, we have learned that several herbs and herbal conglomerates do

possesses strong antifungal effect but that cannot be translated into antifungal drug that can be used for the treatment. However, such agents can be co-administered along with proven antifungals where the combat is made multi-level; the fungus has the least chance to develop resistance than fight simply for existence than get exit from the scalp habitat.[7, 8]

Due to abundant phyto-pharmacological agents present in the herbal conglomerate and many of them having different levels of antifungal activity targeting possibly different sites of the fungus like cell wall biosynthesis, cellular inclusion modification, nucleic acid synthesis, morphological alteration etc., the fungus to focus on unidirectional adaptation mode is not possible and hence the proven drug of choice in the presence of herbal conglomerate could do its best to arrest the fungus and offer best treatment delight to the patient.

In the present study we report the resistogram pattern of certain clinical and lab stored isolates of Pityrosporum ovale against standard antifungal agents, significant decline of MIC value when herbal conglomerate was used along and how such approach flummoxes the resistance pattern of the isolates after two generation growth in herb supplemented medium. Dano active dandruff oil, a proven antidandruff Siddha drug was used in the present study. Details are presented in the paper.

MATERIALS AND METHODS

Source and number of isolates used

Twenty freshly obtained clinical isolates of Pityrosporum ovale and 15 laboratories maintained (3-year-old) isolates of Pityrosporum ovale were used in the present study.

All the strains were grown in Sabouraud's dextrose agar medium supplemented with olive oil.

Details of Dano active AD oil

The Siddha formulation is composed of the following medicinal herbs in Mineral oil-Coconut oil base

Phyla nodiflora		:2.5
Trigonella foenum-graecum	:	2.5
Zingiber officinale	:	2.5
Hibiscus rosa-sinensis		:2.5
Wrightia tinctoria	:	2.5
Cassia alata	:	2.5
Azadirachta indica	:	2.5
Allium cepa	:	2.5

Preparation of herbal extract

All herbs were extracted individually using diethyl ether as solvent and after evaporation of the solvent, the extract was dissolved in 1% DMSO where the proportion of the herbal constituent in 1% DMSO was adjusted to 0.5% and the same was used for testing.

Antifungals used

The following antifungal such as Zinc pyrithione (Zpto), Octopirox (Octo), Climbazole (Climb), Selenium disulphide (Sel. Disul), Flucytosine (Flucyt), Fluconazole (Fluco) and Ketoconazole (Ketaco) were used. The antifungal agents were dissolved in DMSO for testing.

Antifungal susceptibility testing

IC_{50} value based on MIC testing was done for all antifungal agents. In brief, a stock of all antifungals was individually prepared in 0.5% DMSO and the concertation of the antifungals used for the test ranging from 10, 20, 30, 40, 50, 80, 100 and 150 µg/ml and was then incorporated in Sabouraud's dextrose agar medium supplemented with olive oil. Freshly prepared inoculum adjusted to the absorbance value of 0.6 at 450nm in physiological saline was used where 100 µl of such inoculum of each isolate was used for testing.

Antifungal-herbal conglomerate testing

The herbal conglomerate (equal proportion of all herbs) and individual herbal extracts at 40 µg/ml was incorporated into the

medium along with the standard antifungals and then the susceptibility assay was run to study any decline of IC_{50} of the respective isolates with reference to medium devoid of herbal extract.

Two generation exposure to herbal assay

All strains of the fungus were grown individually in Sabouraud's dextrose agar medium pre-nourished with either individual herbal extract or the conglomerate of Dano active AD oil at 100 µg/ml. Once the confluent growth of the organism was attained and the colony has covered entire petri dish, the isolates were re-inoculated into fresh agar plate devoid of herbal conglomerate. The fully grown fungus was again re-grown in herbal supplemented medium and after which the resistogram of the fungus was tested against standard antifungal agents. The decline in MIC concentration was attributed to inbuilt vulnerability of the fungus possibly caused by the herbal mix.

RESULTS

All clinical isolates show high sensitivity to all antifungals tested when compared to laboratory maintained isolates. The IC_{50} value of all antifungals did not exceed 80 µg/ml for the clinical isolates whereas the IC_{50} value of the antifungals to all laboratory maintained isolates ranged between 100 – 150 µg/ml, Table 1

Table 1

Isolates	Number	IC_{50} value in µg/ml/ antifungals						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	50	80	80	50	50	80	80
Lab stored	15	100	100	150	150	150	100	100

Table 2

Significant reduction in IC_{50} of all antifungals was observed against both the clinical and laboratory maintained strains when the herbal extract of *Phyla nodiflora* was incorporated in the system,

Table 2

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 1 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	50	50	20	30	30	50
Lab stored	15	80	80	100	100	100	80	80

Table 3

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory stored isolated was observed when herbal extract of *Trigonella foenum-graecum* was incorporated in the system,

Table 3

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 2 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	50	20	30	30	30
Lab stored	15	50	80	80	80	80	80	80

Table 4

Significant reduction in IC_{50} of all antifungals was observed against both clinical and lab maintained strains was observed when herbal extract of *Zingiber officinale* was incorporated in the system,

Table 4

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 3 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	50	80	80	80	50	50	80

Table 5

Significant reduction in IC_{50} of all antifungals was observed against both clinical and lab stored strains was observed when herbal extract of *Hibiscus rosa-sinensis* was incorporated in the system,

Table 5

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 4 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	30	80	50	80	50	50	80

Table 6

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory strains was observed when herbal extract of *Wrightia tinctoria* was incorporated in the system,

Table 6

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 5 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	30	80	50	80	50	50	50

Table 7

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory strains was observed when herbal extract of *Cassia alata* was incorporated in the system,

Table 7

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 6 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	30	80	50	80	50	50	50

Table 8

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory stored strains was observed when herbal extract of *Azadirachta indica* was incorporated in the system,

Table 8

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 7 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	30	80	80	80	50	80	50

Table 9

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory stored strains was observed when herbal extract of *Allium cepa* was incorporated in the system,

Table 9

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herb 8 (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	30	30	30
Lab stored	15	30	80	80	80	50	80	50

Table 10

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory strains was observed when herbal conglomerate was incorporated in the system,

Table 10

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herbal conglomerate (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	30	30	30	20	20	20	30
Lab stored	15	30	50	50	50	20	20	30

Table 11

Significant reduction in IC_{50} of all antifungals was observed against both clinical and laboratory stored strains after two generation of herbal conglomerate exposure,

Table 11

Isolates	Number	IC_{50} value in µg/ml/ antifungals + herbal conglomerate (40 µg/ml)						
		Zpto	Octo	Climb	Sel.disul	Flucyt	Fluco	Ketaco
Clinical	20	20	20	20	20	20	20	30
Lab stored	15	30	30	30	30	20	20	30

DISCUSSION

'Treatment pandemonium' or well-orchestrated 'indistinguishable antimicrobial onslaught' is the most preferred approach against superficial mycotic pathogens like dandruff and dermatophytoses is the scientific conviction, we believe and perpetuate. But to practice the above strategy, a clear scientific understanding of the antifungal agents to be used, the cross-reaction, synergy etc., are essential and only then such approach can be adopted.

Treatment of dandruff has gone awry due to heavy commercial interest and as a result, several antifungal agents have arrived in the market where some carry strict tag of drug while some are allowed in toiletry preparations.[9,10] When a normal flora of the scalp turns into pathogen, treatment must also focus on host specific factors than the pathogen centric because a normal flora cannot spontaneously over-turn its nature without either a compulsion or extra-advantage and more often that must have come from the habitat where it lives.

Therefore, the conventional board of treatment approach may not offer complete relief. In our long standing stature in research, we have established that several herbal extracts may potentiate treatment of fungal infections. The mere antimicrobial or antifungal efficacy of herbal preparations at laboratory level will never help us to convert such herbal preparations as drugs. Therefore, the use and relevance of all such antifungal herbal preparations either for treatment or to replace the conventional antifungal drugs is far from near. However, we have found that the diverse phytopharmaceutical agents exhibit action upon fungus at different levels such as interfering at biosynthesis of cell wall, modification of cellular inclusion, porosity in cell wall, cytoplasm leakage, genetic alteration etc. Although the herbal medicines exhibit all such actions, but the action has to be referred 'weak', 'subtle' and time-demanding. Prolonged exposure is required to achieve total or near total arrest or elimination of the fungus.

If we could comprehend both the strength and absolute limitation of the herbal medicines in dealing pathogenic fungus, how to 'use' the herbal medicines wisely can be strategized scientifically to complement the existing treatment line. We have adopted the above strategy where treatment, cure and relief to the patient is given paramount importance than proving whether herbal medicines are superior over allopathic drugs.

Our present study findings show that the incorporation of the herbal extracts along with conventional antifungals at in vitro level is quite effective in enhancing the effect of the antifungal agents several fold. We do not want to arrive or interpret such result either as synergy or fortification of the conventional antifungal agents instead we believe and argue that the herbal incorporation making the fungus vulnerable, weak and docile through their action and hence the conventional antifungal agents could exert its action quick. To confirm the above hypothesis, we have used two sets of isolates of P. ovale such as freshly isolated form clinical condition and the other being laboratory maintained culture. The sensitivity pattern of both sets of isolates were different where the clinical isolates show high susceptibility over laboratory maintained strains. This could be due to perfect vis-à-vis poor saprophytic adaptation of the above strains. But the question of why the herbal treatment could make them vulnerable remain unanswered and hence

we exposed all the strains to the herbal extracts in growth medium without any antifungal agents. After two generation of growth, the isolates were retested for their susceptibility towards several antifungal agents. Findings show that all most all strains became highly susceptible to antifungal agents when herbal exposure was given where the same strain that was not given such herbal exposure did not show such susceptibility.

The above findings help us to postulate that the herbal treatment may affect the fungus, the mechanism is yet to be elucidated, but make them vulnerable to antifungal agents. Interestingly such effect of herbal extract of the fungus does not seem to affect the growth phase and morphology of the fungus. Therefore, we believe that the herbal exposure may be creating 'pandemonium' to the fungus to identify and delimit the attack because the array of phyto-pharmaceutical agents and the nature of attack are quite quixotic which does not allow fungus to learn and adapt. Therefore, incorporating the herbal extracts along with conventional antifungal agents will certainly enhance the treatment success. The present study with Dano active AD oil has undoubtedly proved the above inference.

Declaration by Authors

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