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Antidermatophytic activity of Apamarga-Mulaka seeds specific to Sidhma (Pityriasis versicolor) in children - A clinical study

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Background: *Apamarga* (*Achyranthes aspera* Linn.) and *Mulaka* (*Raphenus sativus* Linn.) drugs are being used by Ayurveda to cure the *Sidhma (*~Pityriasis versicolor*)* for many centuries in children. Objective: To assess the comparative effect of 10% glycerin preparation-*Apamarga* and *Mulaka* Seeds' dried water extract and-1% clotrimazole lotion in Sidhma (Pityriasis versicolor) disease affected children, aged from 1-16 years irrespective to sex. **Material & Method:** A total of 40 cases, of both sex of Sidhma (Pityriasis versicolor) were taken and randomly allocated, irrespective to severity of disease, into – a trial group -A (n=20) and another control group-B (n=20) based on the 10% glycerin based trial drug Apamarga-Mulaka Seeds dried water extract and control drug-1% clotrimazole lotion applied over the skin lesion, respectively, for 42 days. Skin lesion characteristics of both groups were observed at first, second, third and fourth follow up. **Observation and Results:** The analysis of gathered data in respect to types and distribution of rashes, color of rashes, size of rashes, itching and scaling of Pityriasis versicolor lesions in children shows statistically significant (<0.001) effect in trial group when compared to the control group drug on 1st & subsequent follow up. During the study period, no adverse effect was noted. **Conclusion:** Early & significant effect of the trial drug suggests its safer and effective use in treatment of *Sidhma* (Pityriasis versicolor).

Keyword: *Apamarga*, *Mulaka*, Aqueous extract, Pityriasis versicolor, *Sidhma*.

1. Introduction

Pityriasis versicolor (P. versicolor) is one of the common disorders of pigmentation seen in the tropics and sub-tropical regions especially during the summer months. It is a superficial fungal infection characterized by skin pigmentary changes due to colonization of the stratum corneum by a dimorphic lipophilic fungus known as malassezia furfur [1]. Increase in humidity, temperature and carbon dioxide tension are important predisposing factors [2, 3] for worldwide distribution with a high rate (20-50%) in tropical and subtropical regions [4]. According to Acharya Charaka [5], Sidhma is characterized by branny desquamation having white and copper color. The lesions are very thin. The colour of the lesion is similar to the flower of Alabu (*Lagenaria siceraria* (Mol.) Standl). These features of Sidhma are almost similar to P. versicolor in terms of scaling, site and color of the lesion.

Pityriasis versicolor, also known as Tinea versicolor, is a superficial chronic recurring fungal infection of the stratum corneum, characterized by scaly, dyspigmented irregular macules most often occurring on the trunk and extremities [6]. Pityriasis versicolor is caused by Malassezia yeast, a dimorphic fungus [7]. and a member of normal skin flora of human beings

which are under certain conditions, the commensally yeast transforms into filamentous pathogenic forms [8].

Many herbal drugs combinations such as Apamarga and Mulaka Seeds are effectively used for the treatment of *Sidhma* [9, 10, 11, 12, 13] since *Samhita* period [12], (~2nd BC). Presently used drugs like Imidazoles [14] and 1% clotrimazole are also effective in treatment of pityriasis versicolor respectively with efficacy of 85% [15] and 86.7% [16], when applied locally.

Many *in-vitro* studies on herbal drugs [17-21] (*in vitro*) has been carried out against cutaneous dermatophytosis but no *in-vivo* study are carried out on Apamarga and Mulaka Seeds recipe in P. versicolor in children, i.e. aqueous extracts of Apamarg + Mulak exhibited good inhibitory activity (*in vitro*) against *Candida albicans* (16mm) *Cryptococcus neoformans* (14 mm) and *T. mentagrophytes* (12 mm) at 60µg/ml [17]. So with the expectation that this study will make a track for evidence based and rational use of Ayurvedic therapy against P. versicolor (dermatophyte) in children.

2. Materials and Method

Total of 40 cases, suffering from P. versicolor were registered aged from one year to sixteen year irrespective to sex and severity of disease and then randomly allocated into - trial group -A (n=20) & control group-B (n=20) 10% glycerin based Apamarga-Mulaka Seeds and 1% clotrimazole lotion applied over the skin lesion of patients of group A and group B respectively. The whole study was executed after getting clearance from the Institute Ethical committee (Institute of Medical Sciences, Banaras Hindu University Varanasi reference no. Dean/2007-08/1399 dated on 28-1-2008). Diagnosis of P. versicolor was confirmed by KOH preparation of skin scraping, demonstrating thick walled spores and characteristics hyphae.

2.1 Inclusion Criteria

The children's whose parents had given their written informed consent and intended to reside within the city for at least 2 months of duration, along with above said criteria in respect to age, sex and diagnosis.

2.2 Exclusion Criteria

The children, who were suffering from the acute systemic illness, topical hemorrhagic, pustular and vesicular rashes due to other fungal or secondary bacterial infection, as well as who have not attended 1st and subsequent follow up were excluded from the study.

2.3 Examination of Rashes to Assess the Severity

The rashes in each patient were examined thoroughly, The severity of the lesions was observed for the purpose of diagnosis at the time of case screening, to evaluate the drug's effect on subsequent follow up in term of lesion characteristics like distribution of rashes, colour of rashes**,** size of rashes**,** individual lesion, primary lesion**,** secondary lesion**,** involvement of axillae and groin as per scoring system table no. 1.

2.4 Adverse reaction

During the study erythema, stinging, blistering, peeling, edema, pruritis, urticaria, or general irritation of the skin was observed.

2.5 Preparation of Drugs

The Apamarga was collected from the BHU campus field and Mulaka Seeds which were purchased from the market and their authenticity was confirmed by department *of Dravyaguna, Ghana Satava* (Dried-water extract) of these drugs was prepared in dept of *Rasa Shastra*, Faculty of Ayurveda, Institute of Medical Sciences, BHU, Varanasi.

Apamarga Panchanga (leaf, flower, stem, root, and fruit) and Mulaka Seeds were cleaned and dried, and crushed till the *Yavakutta* (Barley sized pieces of drug). Crushed *Mulaka* Seeds and *Yavkutta* (~chopped into barley size pieces) *Apmarga* were collected in a vessel, filled with 4 times water of drugs and kept overnight. Next day morning container was heated till total quantity of water reduced upto 1/4th, then contents were filtered 2-3 times by the four

folded cotton cloth, Again, this filtered decoction was boiled slowly, until decoction got the consistency equal to honey. At this stage vessel was taken out from the fire, contents were dispersed uniformly in a tray and dried in oven at 30 ^{0}C. Thereafter it was taken out from the oven; a fine powder was prepared through the fine sieve [22].

2.6 Mode of dispensing

A combination of dry extract (ointment) of *Apamarga & Mulaka* Seeds with glycerin 10% and Clotrimazole 1% were given to the patient of group-A and group- B, respectively.

2.7 Method for application

Patients of group-A and group-B were advised to apply a layer of the drug topically in inverse direction of hairs, over the lesion, twice in a day for 42 days, after cleaning the lesion with sterilized cotton swab, prepared by boiling for 30 minutes. Drug dose was depending on the area of lesions. The patients were advised to take congenial routine diet. They were advised also to abstain from spicy, oily food & oil massage.

2.8 Follow up

During the study, four follow-ups were carried out. The interval between registration and 1st follow up, 1st FU to 2nd FU was one week thereafter, duration between two follow-ups i.e. 2nd to 3rd FU and 3rd to 4th FU was kept two weeks apart. The total duration of follow up was 6 weeks (42 days).

2.9 Assessment Criteria for Rashes

The following scoring system was developed for assessing-

(I) The skin lesion characteristics at registration and on subsequent follow-ups.

(II) The effect of drug during the trial period in disease (Pityriasis versicolor).

Table 1: Scoring system to evaluate the severity of lesion

Skin Lesion Characteristics	Score			
	1	2	3	4
Type and Distribution of Rashes.	Normal Skin/Macular Rashes Subside completely	Macular rashes distributed over the trunk only	Macular Rashes distributed trunk and neck	Distributed extensively over the neck, trunk and limbs
Color	Normal Skin Color	Color of the lesion nearer to normal skin	White / Brown/c pink,	coppery brown
Size	Not Measurable	< 5 cm, Multiple	5-10cm, Multiple	>10cm, Multiple
Scaling/Itching	No Scaling and Itching	No Scaling but Occasional Itching	Scaling and Itching: Reduced Scaling And Itching Both.	Scaling, Itching Present

Reduction in scores suggests improvement in skin lesions, while increase in scores indicates the deterioration. Interpretation of score was done as follows:

a. Excellent improvement in disease was considered when the cumulative skin lesion score was observed < 5.
b. Moderate improvement in disease was considered when the cumulative skin lesion score was observed in between 6-10.
c. Mild improvement in disease was considered when the cumulative skin lesion score was observed in between 11-15.
d. No improvement in disease was considered when overall skin lesion score was observed >15.

3. Observation & Result

In all registered 40 cases of P. versicolor in group A, 7 cases were drop out after registration. Rest of the children of group A & B were observed on subsequent follow ups after topical application over the rashes for the effect of trial and control drugs.

Table 2: Effect of Apamarga-Mulaka Seeds and Clotrimazole on the Type of Rashes and distribution in Pityriasis versicolor

Statistical Parameters *(Intragroup Comparison using Paired 't' test*).*	**Apamarga + Mulaka Seeds** (Group A) (n=13)			**Clotrimazole (Group B)** (n=20)		
	Mean±S.D.	**'t'**	**'p'**	**Mean±S.D.**	**'t'**	**'p'**
At Registration	2.46±0.77	-	-	2.85±0.98		
1st FU	2.46±0.77	-	-	2.75±0.97	1.00	0.33
2nd FU	1.15±0.37	4.98	<0.001	2.70±1.12	0.33	>0.05
3rd FU	1.15±0.37	4.98	<0.001	1.00±0.00	13.50	<0.001
4th FU	1.00±0.00	6.79	<0.001	1.00±0.00	14.91	<0.001

On statistical analysis, no significant change in rashes was observed in any group after 1st follow up, whereas on 2nd follow up, combination of *Apamarga and Mulaka* Seeds has shown highly significant (P<0.001) effect than clotrimazole group. But on subsequent follow ups, highly significant improvement was observed in children of both groups (Table 2).

Table 3: Effect of Apamarga-Mulaka Seeds and clotrimazole on the color of rashes in Pityriasis versicolor

Statistical Parameters *(Intragroup Comparison using Paired 't' test*).*	**Apamarga + Mulaka Seeds** (Group A) (n=13)			**Clotrimazole (Group B)** (n=20)		
	Mean ± S.D	**'t'**	**'p'**	**Mean ± S.D**	**'t'**	**'p'**
At Registration	3.15±0.55			3.15 ± 0.36		
1st FU	3.07±0.27	0.56	>0.50	2.00 ± 0.00	-	-
2nd FU	2.15±0.55	6.24	<0.001	2.00±0.00	-	-
3rd FU	2.15±0.55	6.24	<0.001	2.00±0.00	-	-
4th FU	1.61±0.87	8.40	<0.001	1.60±0.50	10.76	<0.001

Significant and consistent change in color of rashes in children of group A was observed after 2nd follow up while in children of group B it was observed highly significant from 1st follow up & achieved near normal score at 4th follow up as evident from table no.3.

Table 4: Effect of *Apamarga-Mulaka* Seeds and Clotrimazole on the Size of Rashes in Pityriasis versicolor

Statistical Parameters *(Intragroup Comparison using Paired 't' test*).*	**Apamarga + Mulaka Seeds** (Group A) (n=13)			**Clotrimazole (Group B)** (n=20)		
	Mean ± S.D	**'t'**	**'p'**	**Mean ± S.D**	**'t'**	**'p'**
At Registration	3.15±0.55	-	-	2.85±0.74	-	-
1st FU	2.61±0.76	3.74	<0.02	2.60±0.68	2.39	<0.05
2nd FU	2.30±0.63	5.50	<0.001	1.80±0.52	3.63	<0.02
3rd FU	1.46±0.87	8.12	<0.001	1.85±0.36	10.49	<0.001
4th FU	1.30±0.75	12	<0.001	1.00±0.00	10.49	<0.001

Change in mean scores of rashes size at 1st FU and 2nd FU was observed moderate to highly significant in patients of Group-A, while the patients of Group-B achieved moderately significant effect after 1st FU. Thereafter, patients of both groups have shown similar improvement (<0.001) as mentioned in table no.4.

Table 5: Effect of *Apamarga-Mulaka* Seeds and clotrimazole on the scaling/itching of rashes in Pityriasis versicolor

Statistical Parameters *(Intragroup Comparison using Paired 't' test*).*	**Apamarga + Mulaka Seeds** (Group A) (n=13)			**Clotrimazole (Group B)** (n=20)		
	Mean ± S.D	**'t'**	**'p'**	**Mean ± S.D**	**'t'**	**'p'**
At Registration	4.00±0.00			4.00±0.00		
1st FU	2.84±0.55	7.50	<0.001	3.00±0.00	-	-
2nd FU	1.00±0.00	-	-	1.00±0.00	-	-
3rd FU	1.00±0.00	-	-	1.00±0.00	-	-
4th FU	1.00±0.00	-	-	1.00±0.00	-	-

Table no. 5 revealed that change in mean score of scaling/itching was highly significant (p<0.001) after first follow up, while most of the patients of both groups have achieved normal mean scores at 2nd FU (Table no.5).

Table 6: Overall effects of trial drugs in Pityriasis versicolor children after the follow-ups

Statistical Parameters *(Intragroup Comparison using Paired 't' test*).*	**Apamarga + Mulaka Seeds** (Group A) (n=13)			**Clotrimazole (Group B)** (n=20)		
	Mean±S.D	**'t'**	**'p'**	**Mean±S.D**	**'t'**	**'p'**
At Registration	12.00±0.00			12.50±0.57		
1st FU	10.33±0.57	5.00	<0.001	10.75±1.25	2.78	<0.001
2nd FU	7.33±0.57	14.00	<0.001	7.55 ±0.95	7.75	<0.001
3rd FU	6.00±1.72	6.00	<0.001	5.75 ±0.50	14.10	<0.001
4th FU	4.66±0.57	22.00	<0.001	5.00±0.00	25.98	<0.001

Change in overall mean score of lesion characteristics of P. versicolor in patients of both groups has shown statistically highly significant (<0.001 effects).

In all follow ups of study there was no erythema, stinging, blistering, peeling, edema, pruritis, urticaria, or general irritation of the skin was observed.

Statistical Analysis: To analyze the data, Intragroup comparison was adopted by using paired't' test except the condition where S.D. was 0.00 at any follow-ups for any characteristic.

4. Discussion

Type of Rashes and distribution, color of rashes, size of rashes, scaling/itching these criteria were adopted to assess the effect of drug in the all P. versicolor and result show highly significant as compare to clotrimazole. Group-A (*Apamarga & Mulaka*) has achieved near to normal scores earlier than clotrimazole group at 2nd follow up, which suggest better and earlier response in term of types of rashes.

The *Lepa* (~ herbal drug containing ointment) formulation was applied in recommended upward or reverse direction of the hairs over the skin was to get the quick and effective response after the drug application. Because of this, Lepa enters in to *Romkupa* (~hair follicles) and further gets absorption through *Swedavahi Srotas* (~Pilosebaceous Route) and *Shiramukha* (~skin pores) leading to desired effects [23-24]. it has been proved that maximum absorption of some compounds occurred at sites with higher follicular density [25].

The probable scientific explanation for the drug absorption, its metabolism and action has been considered that the stratum corneum serves as the main (Transepidermal) pathway for permeation and penetration [26]. In addition to the transepidermal route, pilosebaceous unit, comprising of hair follicle and sebaceous glands (transfollicular) may contribute significantly to transdermal delivery. [27] As only about 0.1% of the total skin surface area is occupied by the orifices of hair follicle [28], their role in percutaneous transport appears to be limited. However, the hair follicle is an invagination of the epidermis extending deep into the dermis, thus providing a greater actual area for potential absorption. Topical liposomes have been shown to target the drug to the pilosebaceous unit. [29]

After targeting the drug, biotransformation is due to drug-metabolizing enzymes are expressed in the skin. Evidence for expression of cytochromes P450, flavin monooxygenases, glutathione-*S*-transferases, *N*-acetyl transferases, and sulfo-transferases presented in human skin cells [30]. This probable mechanism of the drug action may be responsible for the effect of Lepa to cure the P. versicolor.

Michalowski *et al* and Terragni *et al* found an increased incidence of P. versicolor cases during the warmer months [31, 32], while another study suggest incidence of 71.2% cases of P. versicolor in the hot months of May to October [33]. It has been noticed also that P. versicolor lesions over the face were smaller than those present on the trunk.

Findings of these studies support the present study, that has shown marked increase in the incidence of P. versicolor during the summer and monsoon with a sudden fall in December to February.

The result of the present clinical study supports the rational usage of Lepa prepared from the Apamarga and Mulaka seeds by the Ayurvedic physicians for centuries and result of in vitro study carried out in past.

5. Conclusion

The extracts of *Apamarga & Mulaka* possesses cutaneous antifungal properties can be used as alternative cutaneous antifungal agents against the Pityriasis versicolor without any side effects.

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