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Research Article

NATURAL OCCURRING COMPOUND SODIUM BORATE SHOWED MARKED ANTIMYCOTIC ACTION ON CLINICAL CASES OF ONYCHOMYCOSIS AND AGAINST ASSOCIATED FUNGAL SPECIES BY IN-VITRO TESTING *IN-VITRO*

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Abstract

Aim: The objectives of this study was to study the therapeutic antimycotic action of natural occurring compound borate on onychomycosis patients and also to observe in-vitro antimycotic properties of borate on fungal species isolated from clinical onychomycosis cases. **Methods**: This study was conducted from Aug.2017 to March 2018 involving 3 patients and 3 samples. Clinical sample (of clipped nails) were collected aseptically and preliminary microscopic examination using 20% potassium hydroxide (KOH) was conducted. Further, isolation and identification of mycotic species was performed using standard protocol using Sabouraud dextrose agar base media (SDA) and dermatophyte test agar media (DTM). Isolated fungi were tested for antifungal susceptibility against 3% concentration of borate testing using semisolid agar method. The percentage of inhibition was calculated by measuring the size of colonies after 7days and 14 days. Negative control with addition of borate and positive control in adding miconazole nitrate at the rate of 100 microgram/milliliter (Himedia) in the growth medium were include. Patients were treated with *shudha tankana malhar*. **Result**: All 3 patients of onychomycosis showed 80-100% recovery. The dermatophyte isolate belonged to *Trichophyton mentagrophyte*, showed 100% inhibition against sodium borate as compared to positive control i.e. *miconazole nitrate* after 7th and 14th day of post inoculation. **Conclusion**: Sodium borate can be inexpansive therapeutic option for treating cases onychomycosis, which is otherwise not easy to cure by standard drug therapy.

Keywords: Sodium borate, Onychomycosis, Antimycotic, Therapy.

INTRODUCTION

Onychomycosis is a fungal infection of nails which can be caused by dermatophytes, yeasts or non dermatophytic moulds (Weitzman and Summerbell, 1995). It is responsible for up to 50% of all nail diseases and 30% of all fungal infections (Scher, 1996). Clinically, onychomycosis is classified as distal lateral subungual onychomycosis, proximal subungual onychomycosis, white superficial onychomycosis and total dystrophic onychomycosis (Chander, 2002). Onychomycosis is not a life threatening condition. It causes cosmetic problem to the patient .the infected nail may serve as a chronic reservoir, giving rise to repeated mycotic infections thus posing animportant public health problem (Neupane *et al.,* 2009).

Study design and pattern

This study was carried out in the department of microbiology, Dr. G.C.Negi College of Animal and Veterinary sciences, CSKHPKV, Palampur. Clinically diagnosed cases of onchomycosis attending skin care unit OPD at R.G.G.P.G. Ayurvedic College and Hospital Paprola (H.P.) were included into this study. Patients who received topical or systemic antifungal up to four weeks preceding sampling were excluded from this study.

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MATERIAL AND METHODS

Sample (clippings of discolored nail plate and subungual debris) was collected from case of onychomycosis aseptically from the patients visiting the outpatient department of R.G.G.P.G. Ayurvedic College and Hospital Paprola (H.P.).Sample were initially screened by direct microscopic examination under 10% KOH mount on glass slide. Samples were screened using 40X objective. For isolation of involved fungal species, samples were inoculated in plates of SDA and DTM (HiMedia) by spot inoculation method and incubated at 25^0c for two weeks and observed daily for growth. Fungal species were identified based on their cultural characteristics and morphological features. Morphological features were studied by staining with Lactophenol cotton blue mount using slide cultural procedure. For testing susceptibility of fungal isolates from clinical cases against sodium borate (*Shudha Tankana)*, SDA medium plates containing 3% sodium borate dissolved in it were used. Negative control plates were without any additive and positive control platesmiconazole nitrate at the rate of 100microgram/milliliter. Sodium borate is water soluble compound. The dermatophyte strains were spot inoculated and incubated at 25^0c and examined every 24 hourly for growth, the colony diameter was measured (in cm) on 7th and 14th day after inoculation. The inhibition percentage was calculated using formula Vincent and co-worker (1945) and compared with negative and positive controls.

Table 1. Based on colony morphology, microscopic observations, one isolate was identified as *Trichophyton mentagrophyte*

S.No.	Organism	Growth rate	Media	Texture	Topography	Surface pigment	Reverse pigment	Under microscope
1)	*Trichophytonmentagrophyte*	slow	DTM	Powdery	Raised and heaped	Cream to white color	Purple to pinkish	Septatehyphae, sesilemicroconidia, macroconiaclub shaped

Human treatment and record observations…

Aims and objectives

1. Isolation of the species from the samples taken from the patients
2. suffering from onychomycosis
3. Identification of the isolated species
4. Assessment of sensitivity of these isolated species against the trial drug
5. *i.e., Suddha Tankana.*
6. To evaluate the effect of *Shudha Tankana* (sodium borate) locally in patients of onychomycosis (*kunakhroga*).
7. To evaluate the *Ayurvedic* principle.
8. To provide better and effective treatment for onychomycosis or *KunakhRoga*.
9. To establish new remedy in *Kunakh Roga.*

CASE REPORT

A 17 year old premenopausal female came to theOPD of skin care unit of R.G.G.P.G. *Ayurvedic* college and Hospital Paprola(H.P.) and was presented with Left hand, middle finger nail discoloration, ragged nail appearance along with subungual hyperkratosis for the last one year. There was no history of trauma, any connective tissue disorder, any vascular disease or any other major illness. Family history of any vascular disease or diabetes or hypertension was also absent
The patient was a resident of a place near Palampur, Himachal Pradesh and belonged to native 'Gaddi' community, traditionally involved in goat and sheep rearing.

Under Wood's lamp examination no fluorescence seen. KOH mount was done which came positive. Clinically, patient was anemic with haemoglobin 10.4 g/dl. Other blood/serum biochemical parameters in normal ranges were; total leukocyte count (TLC, 6500/mm^3), erythrocyte sedimentation rate (ESR, 10 mm/h), serum creatinine (0.9 mg/dl), serum uric acid (4.4 mg/dl), serum glutamic oxaloacetic transaminase (SGOT 24 unit/l), and serum glutamic pyruvic transaminase (SGPT 32 unit/l). The differential leukocyte counts revealed 69% neutrophiliaamd 23% lymphocytes. Patient was not treated with any other initial treatment before coming to the OPD of skin care unit of R.G.G.P.G. *Ayurvedic* college and Hospital Paprola (H.P.) Patient was diagnosed as a case of onychomycosis. Informed and written consent taken from patient. KOH mount positive test sample aseptically transported to and processed at the Microbiology laboratory of Dr. G.C. Negi College of Veterinary and Animal Sciences, Palampur (H.P.). At the Microbiology laboratory of veterinary sciences, primary inoculation of the collected sample (clipped nail) done on Dermatophyte Test Medium agar (DTM agar) and on Sabouroud's Dextrose agar (SDA) through spot inoculation method, and incubated at 25°C for 7-14 days. The growth of fungal organisms along with change in the color of the DTM from yellow to red within 14 days indicated the presence of dermatophytes, while there was no change in color of the SDA media. To avoid the contaminations, passaging was done to get the pure colony. The petriplates showing pure fungal growth were kept for further identification of dermatophyte and other fungal species. Isolated strains grown on Sabouroud's Dextrose agar (SDA) media were also studied for their cultural characteristics. For the identification, the colonies were examined for their morphology, texture and pigmentation on obverse and reverse sides and the confirmation was done by microscopic, morphological examination using wet mount technique and slide culture technique. To make slurry, 5% sodium borate was added in DTM agar/ SDA media and autoclaved (as melting point of sodium borate is very high i.e. 743^0c Suitable controls were also included. DTM agar/SDA media with Miconazole nitrate-100 microgram/ milliliter (Himedia) served as the positive control. DTM/ SDA plate without any drug served as negative control. A total 3 petriplateslabelled as of sodium borate, miconazole nitrate (positive control) and negative control were inoculated. Under aspectic conditions all three petriplates inoculated with a same isolated strain (except *Candida albicans*) with spot inoculation method in 0.5 cm diameter spot. The plates were then incubated at 25ºC and examined every 24 hourly for growth. The colony diameter of grown strains were measured (in cm) on 7th day and 14th day after inoculation.

Calculation of percentage inhibition

The percentage of inhibition were calculated according to the formula given by Vincent and co-worker(1945):

$$\text{Percentage inhibition} = \frac{(C-T)\times 100}{C}$$

Where C was colony diameter (cm) of drug free control *i.e*, negative control and T was colony diameter (cm) of treated fungi on drug containing plates.

RESULTS

Isolation and Identification of field strains:

Fig. a. *T. mentargrophyte* (on 20 X);

Fig. b. *T. Mentargrophyte* surface pigment (DTM);

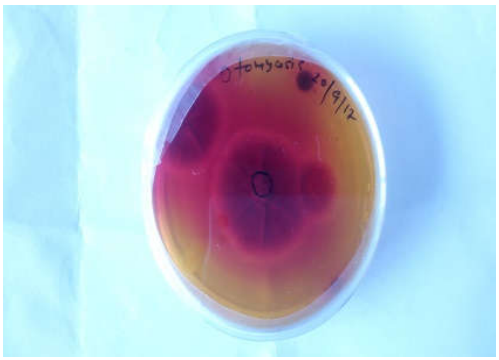
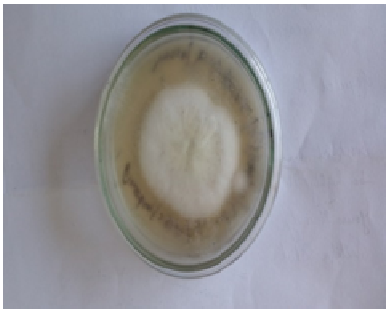


Fig. c. *T. mentargrophyte* reverse pigment (DTM);

In our study antidermatophytic activity of Shudha Tankana against *Trichophyton mentagrophyte* was obtained. On 7th and 14th day growth rate on negative control compare to positive control and sodium borate were measured which was as follow-

Table 2.

S.No	Organism	+ve control		Sodium borate		-ve control	
		7th day	14th day	7th day	14th day	7th day	14th day
1)	*Trichophytonmentagrophyte*	(-)	(-)	(-)	(-)	4.5cm	6.2cm

Types of plates	Colony growth diameter(SDA)	
	After 1 week	After 2 weeks
Negative control		
Sodium borate (5%)		
Positive control		

Sodium borate showed 100% zone of inhibition against *Trichophytonmentagrophyte* equivalent to miconazolenitrate

Conclusion

By observing the in--vitro study it can be concluded that *shudhatankana* have excellent antidermatophytic activity and it may be given in the patient (locally) suffering from onchomycosis.

MATERIALS

Shudha Tankana Malahara-(for local application)

Petroleum jelly was added to the fine and purified powder of *ShudhaTankana*, mixed properly to make a homogenous mixture.

Table 3.

S. No.	Drug Name	Chemical Name	Proportion of the Drug per 100 grams
1)	*Shudha Tankana*	Purified Borax	30 grams
2)	Petroleum jelly white	Parafine soft white	100 grams

Dose- Three times a day according to the lesion.

Duration- 3 months.

Criteria of assessment: The study will be based on OSI (onychomycosis severity index) score (Carney *et al.,* 2001).

Table 4. Area of involvement

Affected nail %	No. of points
0	**0**
1-10	**1**
11-25	**2**
26-50	**3**
51-75	**4**
76-100	**5**

Table 5. Proximity of disease to matrix

Amount of involvement from distal edge	No. of points
<1/4	**1**
¼-1/2	**2**
>1/2 – ¾	**3**
>3/4	**4**
Matrix involved	**5**

Table 6. Presence of dermatophytomaor subungual hyperkeratosis>2mm

Present	No. of points
No	**0**
Yes	**10**

The onychomycosis severity index is calculated as follows: the score for area of involvement is multiplied by the score for the proximity of disease to the matrix, and 10 points are added for the presence of dermatophytoma or subungual hyperkeratosis of greater than 2mm. a cumulative score of 0 indicates cured; 1 through 5, mild onychomycosis; 6 through 15, moderate onychomycosis; and 16 through 35, Severe onychomycosis.

Observation

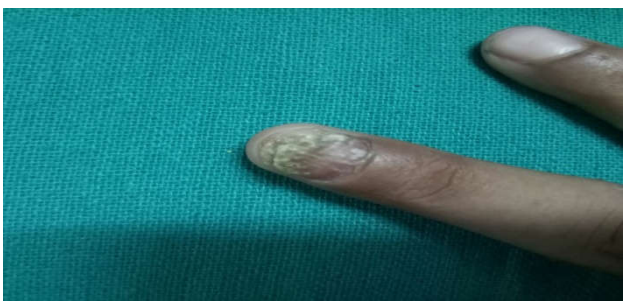


Figure 1- the left hand middle finger nail receives a score of 5 (for area) multiplied by 5 (for proximity of disease to the matrix owing tp matrix involvement) for a total of 25. Thick subungual hyperkeratosis is present, for which we add 10 points for a total of 35. This nail has severe involvement
OSI Score = 35

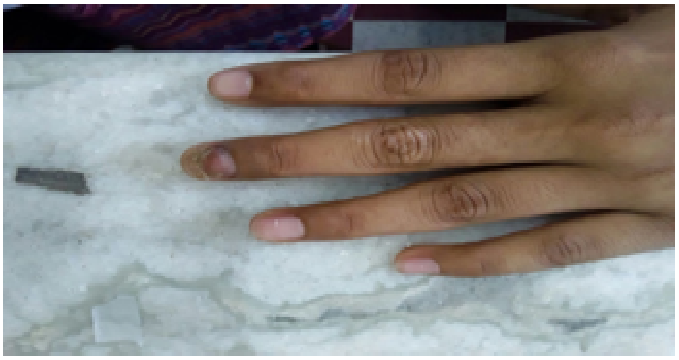


Figure 2- at 2nd month of treatment, affected nail receives a score 3 (for area) multiplied by 3 (for proximity of disease to the matrix) for a total of 9.Thick subungual hyperkeratosis is present, for which we add 10 points for a total of 19.
OSI Score = 19

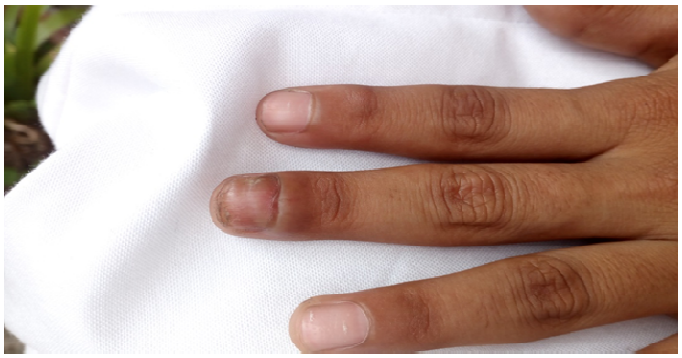


Figure 3- at 3rd month of treatment, affected nail receives a score 1 (for area) multiplied by 1 (for proximity of disease to the matrix) for a total of 1. Thick subungual hyperkeratosis is absent, for which we add 0 point for a total of 1.
OSI Score = 1

DISCUSSION

The prevalence rate of onchomycosis is determined by age, predisposing factors, social class, occupation, climate, living environment and frequency of travel (Williams, 1993). One should look for cutaneous signs of psoriasison the scalp, gluteal folds, elbows and knees and nails should be evaluated for other signs of psoriasis, especially for pitting and/or small salmon colored droplets evident on the plate. Approximately 10% of the patients with lichen planus have abnormal nails (Murray and Dawber, 2002; Sobhanadri *et al.,* 1970). Topical antifungal agents are of limited efficacy when used alone or with older antifungal agents to treat onchomycosis, but they may result in more rapid curewhen used in conjugation with the newer systemic compounds. They may also help to prevent the recurrence of tineapedis, which often accompanies fungal toenail infection. Improvement of the conventional formulations led to the development of an alcoholic solution containing 28% tioconazole and undecylenic acid, for instance, which has produced moderate results (Hay *et al.,* 1985).

In *Bhava Prakasha* reference is given as-

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dqu[k'psŸknk 'kSyk% lfyysIyOrsfipA

¼Bhava Prakasha Chi- 61@78½

The condition referred as onychomycosis in modern science can be co-related to *Kunakh Roga*as per *Ayurvedic* classical texts. Diagnosis was made on the basis of signs and symptoms as per literature along with investigations. On the basis of above reference *shudhatankana* selected as a trial drug to treat the diagnosed case of onchomycosis.

Total effect of therapy

Shudhatankana showed 100% inhibition effect against isolated strain of dermatophyte in vitro study, found cheap of cost as well as clinically effective in the treatment of onychomycosis.

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REFERENCES

Carney C, *et al.* 2011. *Arch Dermatol.,* 147(11):1277-82. Doi: 10.1001/ archdermatol.2011.267.

Chander J. Dermatphytosis. In: Textbook of Medical Mycology. 2nd ed. New Delhi: Mehta Publishers, 2002:100-04)

Hay RJ, Mackie RM, Clayton YM. 1985. Tioconazole (28%) nail solution: An open study of it's efficacy in onchomycosis. *Clin Exp Dermatol.,* 10:152-7.

Murray SC. And Dawber RP. 2002. Onychomycosis of toenails: orthopaedic and podiatric considerations. *Australas J Dermatol.,* 43:105-12.

Neupane S, Pokhrel DB, Pokhrel BM. 2009. Onychomycosis: A clinic- epidemiological study. *Nepal Med Coll J.,* 1

Scher P. 1996. Onychomycosis: A significant medical disorder. *J Am AcadDermatol.,* 35:S2-5.

Sobhanadri C, Rao DT, Babu KS. 1970. Clinical and mycological study of superficial fungal infections at Government General Hospital: Guntur and their response to treatment with Hamycin, Dermostatin and Dermamycin. *Indian J Dermatol Venereol.,* 36:209

Weitzman I, Summerbell RC. 1995. The dermatophytes. *Clin Microb Rev.,* 8:240-59).

Williams HC. 1993. The epidemiology of onychomycosis in Britain. *Br J Dermatol.,*129:101.

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