A COMPARATIVE STUDY OF EFFICACY OF NEEM SEED OIL, TERBINAFINE CREAM AND A COMBINATION OF BOTH TERBINAFINE CREAM AND NEEM SEED OIL APPLIED TOPICALLY IN THE TREATMENT OF CLINICAL DERMATOPHYTOSIS

Bala Sharmin S*, Chincholkar Aparna, Wagh Ranjit, Mutalik Madhav, Siddiqui Waseem, Gupta Manjeeta

Department of Pharmacology, MIMER Medical College, Talegaon, Pune-410507.

ABSTRACT

Background: Dermatophytosis is the superficial parasitic infection of the skin, hair, and nails characterized by itching, scaling, erythema. Terbinafine hydrochloride is widely used in the treatment of dermatophytosis. Various side effects with available antifungal drugs and their rising costs emphasize the need for a novel, plant based drug for treatment of dermatophytosis. Many in vivo and in vitro animal studies have proved the efficacy of neem seed oil in the treatment of dermatophytosis. Aim: This study was undertaken to know the safety and efficacy of neem seed oil in the treatment of clinically diagnosed dermatophytosis.

Methods: Patients were randomly divided into three groups and were given terbinafine cream, neem seed oil or a combination of both. The patients were assessed clinically for a period of 6 weeks. Their skin scrapings were examined microbiologically. Results: The reduction in itching, scaling and erythema was similar in all the three groups at the end of 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks. The size (small and big) and number of lesions distributed in each group was the same. The time taken to heal lesions in all the three groups was the same. Lesions in all the three groups were healed to the same extent. Conclusion: Neem seed oil is as effective as terbinafine cream in the treatment of dermatophytosis. There is no additive effect observed when neem seed oil is combined with terbinafine cream in the treatment of dermatophytosis.

KEYWORDS: Dermatophytosis, terbinafine, neem seed oil.
MATERIAL AND METHODS
The study was approved by the institutional ethics committee. Pure neem seed oil and terbinafine cream was provided to the patient.

A. Materials available
1. Terbinafine Hydrochloride cream 1% w/w (TERBEST)
Manufacturer’s name- Mepromax Life sciences limited
Marketer’s name- Systopic (Dispensed at 15g tube)

2. Pure extracted neem seed oil
Manufacturer’s name: Shivkrpa agency, Baramati
(Dispensed as 20 ml in dark shaded plastic bottles)

3. Other materials used
Gloves, glass slides, cotton swabs, spirit, black chart paper for collecting specimens; urine dipsticks.

B. Sample size determination
Various studies have reported prevalence of dermatophytosis between 12% and 31%. Considering overall prevalence of 20% in general population\cite{1, 2} at \( \alpha = 0.01 \) and 80% power of test, the estimated sample size was 164. Sample size of 175 was initially included in the study. Simple random sampling (open label and parallel study) was done to avoid bias and patients were allotted in 3 groups (Table 1).

During the study, it was observed that patients who met with all the inclusion criteria were only a quarter of all the patients diagnosed as dermatophytosis. After obtaining permission from the institutional ethics’ committee, the sample size was thus reduced to 108 patients. Chi square test was the statistical test applied.

C. Inclusion Criteria
1. Clinically diagnosed new cases of dermatophytosis visiting Skin OPD
2. Both sexes
3. Age: 15-70 years
4. Patients who have signed the informed written consent.
D. Exclusion Criteria
1. Extensive lesions of dermatophytosis (total surface area >50cm²)
2. Any skin disease in addition to dermatophytosis (psoriasis, scabies, etc.)
3. Diabetic patients
4. Pregnancy or lactation

Urine dipstick test was performed for all patients who met with the inclusion criteria to rule out diabetes.

E. Clinical Assessment
Each patient was assessed clinically according to:
1. Presence of itching
2. Number and Size of Lesions
3. Presence of scaling, erythema.
Photos of the lesions of most patients were taken before and after the treatment with the consent of the patients to compare the lesions on subsequent visits.

F. Collection of skin scrapings for microbiological assessment
Skin scrapings were collected from the lesions of dermatophytosis. Skin sensitivity testing for neem seed oil was performed on each patient during the first visit.

G. Division of groups: As shown in table 1
Table 1

<table>
<thead>
<tr>
<th>Group A</th>
<th>GroupB</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbinafine cream 1%</td>
<td>Pure extracted neem seed oil</td>
<td>Pure extracted neem seed oil</td>
</tr>
</tbody>
</table>

All medications were to be applied over the lesion twice a day.

H. Instructions to the patients
1. To keep the affected area dry, clean
2. To maintain hygiene
3. To refrain from wearing tight clothes around the lesion.
4. Not to take any other medication
5. To notify if any adverse effect occurred with the given medication.
I. Microbiological Assessment

**KOH mount**

A positive finding by direct microscopic examination of the specimen established the diagnosis of dermatophytes. \[^3,4\] 

**Culture**

It was performed to identify species of the dermatophytes. \[^3,4\] 

Microscopic examination of fugal colonies- Growth on the media was identified by Lactophenol Cotton Blue preparation.

**J. Followup**

Each patient was advised to return for follow-up after one week, two weeks, four weeks, and six weeks.

**RESULTS**

![Figure 1: Bar graph showing distribution of lesions according to size](image)

<table>
<thead>
<tr>
<th>Chi-Square test result</th>
<th>Value</th>
<th>Degree of freedom</th>
<th>Exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>0.458</td>
<td>2</td>
<td>0.812</td>
</tr>
</tbody>
</table>

**Interpretation**

Since p-value for the Chi-square is greater than that of 0.05, it indicates that there is no association of size and groups.

Thus small and big lesions were equally distributed in all the three groups.
Figure 2: Bar graph showing Percentage Distribution of number of lesions in the three groups

Chi-Square test result

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Df</th>
<th>Exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>3.330</td>
<td>6</td>
<td>0.804</td>
</tr>
</tbody>
</table>

Interpretation

Since p-value for the Chi-square is greater than that of 0.05, it indicates that there is no association of number of lesions and groups.

The lesions of dermatophytosis are equally distributed in all the three groups with respect to number of lesions.

Figure 3: Bar graph showing percentage reduction in itching in the three groups in subsequent weeks of treatment
### Chi-Square Tests

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>.260²</td>
<td>6</td>
<td>1.000</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>.261</td>
<td>6</td>
<td>1.000</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>.004</td>
<td>1</td>
<td>.951</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>355</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 20.28.

### Interpretation

Since p-value for the chi-square is greater than that of 0.05 indicates that there is no association between time and groups for itching.

The symptom of Itching was cured to the same extent in the patients of all the three groups.

![% Reduction in Scaling](image)

Figure 4: Bar graph showing percentage reduction in scaling in the three groups in subsequent weeks of treatment:

### Chi-square test results

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>.555²</td>
<td>6</td>
<td>.997</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>.555</td>
<td>6</td>
<td>.997</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>.000</td>
<td>1</td>
<td>.985</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>325</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 21.03.

### Interpretation

Since p-value for the chi-square is greater than that of 0.05 indicates that there is no association between time and groups for scaling.
The symptom of scaling was cured to the same extent in the patients of all the three groups.

![Graph showing percentage reduction in erythema](image)

**Figure 5**: Bar graph showing percentage reduction in erythema in the three groups in subsequent weeks of treatment

Chi-square test results

<table>
<thead>
<tr>
<th>Chi-Square Tests</th>
<th>Value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>5.020^a</td>
<td>6</td>
<td>.541</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>5.047</td>
<td>6</td>
<td>.538</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>.554</td>
<td>1</td>
<td>.457</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>218</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a. 1 cells (8.3%) have expected count less than 5. The minimum expected count is 4.84.

**Interpretation**

Since p-value for the chi-square is greater than that of 0.05 indicates that there is no association between time and groups for erythema. The symptom of erythema was cured to the same extent in patients of all the three groups.

![Graph showing percentage of lesions healed and not healed](image)

**Figure 6**: Bar graph showing percentage of lesions healed and not healed in the three groups over a period of 6 weeks
Sharmin et al.  
World Journal of Pharmacy and Pharmaceutical Sciences

### Chi-Square Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>5.189a</td>
<td>6</td>
<td>.520</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>5.292</td>
<td>6</td>
<td>.507</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>.110</td>
<td>1</td>
<td>.740</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a. 1 cells (8.3%) have expected count less than 5. The minimum expected count is 4.84.

**Interpretation**

Since p-value for the chi-square is greater than that of 0.05 indicates that there is no association between time and groups for healing. Lesions of the patients in all the three groups healed to the same extent.

![Figure 7: Changes in appearance of the lesion in Group A over a period of 4 weeks](image)

![Figure 8: Changes in appearance of the lesion in Group B over a period of 4 weeks](image)
Microbiological assessment

1. KOH mount positive samples: 33
2. Samples which were positive for dermatophytes on culture by Sabouraud’s Dextrose Agar-23
3. Total number of samples microbiologically confirmed as dermatophytes - 45.
4. Percentage of samples which showed positive KOH mount:

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment</th>
<th>After 1 week</th>
<th>After 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>27%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B</td>
<td>30%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>Group C</td>
<td>34%</td>
<td>8%</td>
<td>3%</td>
</tr>
</tbody>
</table>

6. Out of the 23 specimens which showed the presence of dermatophytes on culture:
   a. Seven specimens showed the morphology of Trichophyton rubrum.
   b. Five specimens showed Trichophyton mentagrophytes.
   c. Four specimens showed the morphology of Micosporum Canis.
   d. Three specimens showed Epidermophyton floccosum.

DISCUSSION

The present study was conducted to find the efficacy of topical application of neem seed oil in the treatment of clinically diagnosed dermatophytosis. Neem seed oil was compared with the standard treatment of terbinafine cream applied topically. The combination of the two preparations was also evaluated.

Bhatia VK et al.[5] observed that the number of males exceeded that of females in an epidemiological study on dermatophytosis in human patients in Himachal Pradesh. Another study by Singh S et al[6] conducted on patients diagnosed with dermatophytosis yielded the
same results. These findings were similar to that of the present study. This could be the result of more outdoor activities, trauma, and common use of occlusive footwear in males.\textsuperscript{[7]}

Singh et al.\textsuperscript{[6]} observed that most of the patients which were diagnosed as dermatophytosis were young adults. According to Bhatia VK et al.\textsuperscript{[5]} the most affected age group for dermatophytosis was 21-50 years. These results are similar to the results of the present study. Agarwal US et al.\textsuperscript{[8]} in while studying the epidemiological aspects of dermatophytoes observed that most of the patients were students and labourers, which is comparable with the present study. Hanumanthappa et al.\textsuperscript{[9]} also observed in their study that majority of the patients were laborers.

All the patients who met with the inclusion criteria were divided into three groups for the treatment administered. Group A patients were given terbinafine cream, Group B patients were given neem seed oil. Group C patients were given both the preparations. The reduction in itching, scaling and erythema was similar in all the three groups at the end of 1 week, 2 weeks, 3 weeks, 4 weeks and 6 weeks.

The size (small and big) and number of lesions distributed in each group was the same. The time taken to heal lesions in all the three groups was the same. Lesions in all the three groups were healed to the same extent.

Agarwal US et al.\textsuperscript{[8]} and Bindu et al.\textsuperscript{[7]} in their study found that tinea corporis was the most common diagnosis followed by tinea cruris, which is comparable to the present study. Bhatia VS.\textsuperscript{[5]} et al also confirmed similar findings.

Singh S.\textsuperscript{[6]} found microbiological isolation rate of 44% which is similar to the present study. Bindu V.\textsuperscript{[7]} Singh S\textsuperscript{[40]} in their study found Trichophyton rubrum as the most common isolated species, which is similar to the present study. The result highlights the importance of both direct microscopy and culture in the definitive diagnosis of dermatophytosis.\textsuperscript{[5, 10]}

No side effects or adverse drug reactions were noted with the use of any of the three treatment regimens in any of the patients. In spite of the pungent odour of the neem seed oil, the patient acceptability was good.

**Strengths of the present study**
There was good patient acceptability of all the medications administered.
After possible extensive review of the literature through various sources, not many studies were found in which neem seed oil had been tested for its efficacy in fungal infections. A study was found in which neem seed oil was used and was effective against clinically diagnosed Pityriasis versicolor. However, it was not possible to find a mention of a reference where neem seed oil had been tried for its efficacy in the management of clinically diagnosed dermatophytosis.

Limitations
The efficacy of terbinafine cream, neem seed oil and their combination against the specific species of dermatophytes (Trichopyton, Microsporum, Epidermophyton) could not be estimated because the isolation rate was less. Further studies with a large sample size and a better isolation rate are required.

CONCLUSION
We conclude that purified neem seed oil, which is a non-synthetic naturally obtained medication is as effective as terbinafine cream in the treatment of clinically diagnosed dermatophytosis.

There is no additive effect observed when neem seed oil is combined with terbinafine cream in the treatment of dermatophytosis.

ACKNOWLEDGEMENTS
We express our gratitude to the microbiology department, dermatology department, Dr. Ganesh Pentewar, Shivkrpa agency.

REFERENCES