Original Article

Safety and Efficacy of Fermented Olive Tree Leaf Extract for Treatment of Paronychia and *Neoscytalidium dimidiatum* Onychomycosis: A Pilot Study

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Objective: To evaluate the efficacy and safety of *Olea europaea* leaf extract for treatment of chronic paronychia and *Neoscytalidium dimidiatum* onychomycosis.

Materials and Methods: The present prospective pilot study was conducted. *O. europaea* leaf extract was applied twice a day. Assessment parameters were evaluated at baseline, 2-, 4-, and 6-months.

Results: A total of 9 chronic paronychia patients and 9 *N. dimidiatum* onychomycosis patients were included. Concerning chronic paronychia, thumb nail was common affecting site. *Candida albicans* revealed in 6 (66.7%). Comparing with baseline, severity of chronic paronychia was significantly reduced within 6 months after treatment (p = 0.021). For *N. dimidiatum* onychomycosis, all affected on big toenails. Mean normal nail length at baseline was 7.1 mm, which significantly improved after 6 months treatment to 8.9 mm (p = 0.016). Nail thickness significantly reduced after 6 months treatment (p = 0.001). No adverse events were observed in the present study.

Conclusion: *O. europaea* leaf extract has been shown to be an effective and safe alternative topical treatment for chronic paronychia from *Candida* spp. infection and *N. dimidiatum* onychomycosis. No side effect was found in the present study.

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Chronic paronychia and onychomycosis are common nail infections worldwide. However, most patients fail to recognize these nail abnormalities, which may lead to delayed treatment[1]. Candida infection of the nail folds or paronychia occurs frequently. The most common causative organism is *Candida albicans*. However, non-albicans species of candida [NAC] may be resistant to azole treatment have become more frequent causative organisms[2]. Topical azole is currently the first-line treatment. New treatments should be investigated to improve outcomes in paronychia patients.

Prevalence of toenail onychomycosis was found in 3.8% of patients attended the outpatient dermatology clinic at Siriraj Hospital. Non-dermatophyte [NDM] infection was found in 24.1% to 51.6% of onychomycosis patients, with *Neoscytalidium dimidiatum* commonly isolated in tropical countries[3,4]. NDM infections were found to be insufficient response to certain systemic antifungal drugs, including itraconazole, fluconazole, and terbinafine[5,6]. Many modalities have been used to treat NDM onychomycosis, including topical treatment, mechanical reduction, and device therapy[6,7]. Novel topical therapies should be studied to assess outcomes and safety.

Olea europaea leaf extract is a new option for topical treatment of chronic paronychia and onychomycosis. Phenol in *O. europaea* leaf was reported to demonstrate antifungal action against *C. albicans* and dermatophytes in vitro studies[8,9]. However, based on our review of the literature, no in vivo study has investigated the effectiveness and safety of *O. europaea* against NDM onychomycosis

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and NAC. Accordingly, the aim of this study was to evaluate the efficacy and safety of *O. europaea* leaf extract for treatment of chronic paronychia and NDM onychomycosis.

Materials and Methods

The present prospective pilot study was approved by the Siriraj Institutional Review Board, Siriraj Hospital, Mahidol University, Bangkok, Thailand. Eighteen immunocompetent patients presented with NDM onychomycosis or candida chronic paronychia between January and December 2015 study period were included. Patients were recruited from the outpatient dermatology clinic at the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Siriraj Hospital is Thailand's largest national tertiary referral center. The informed consent was obtained from all participants.

Potassium hydroxide examination [KOH] and fungal culture of infected toenails and fingernails were performed. Demographic information, clinical characteristics, and medical comorbidities were collected and recorded. *O. europaea* leaf extract packaged under the registered name NailProtex® (Alvogen, Inc., Pinebrook, NJ, USA) was given to patients with instructions to apply to affected nails twice a day, with self-mechanical reduction by nail emery board.

Chronic paronychia was diagnosed and severity of disease was graded by clinical examination and fungal laboratory identification. Chronic paronychia grading, microscopic examination, and fungal culture were performed and recorded at the first visit and at 2 months, 4 months, and 6 months after treatment. Chronic paronychia grading was defined, as followed: grade 1, redness or some swelling of the proximal nail fold with or without disruption of the cuticle; grade 2, redness and pronounced swelling of the proximal nail fold with disruption of the cuticle; grade 3, redness and swelling of the proximal nail fold with absent cuticle, early edema, and some nail plate changes; grade 4, redness and swelling of the proximal nail fold with absent cuticle, edema, and extensive nail plate changes with pain; and grade 5, same as grade 4 plus acute exacerbation of chronic paronychia (acute paronychia)[10]. Treatment outcomes were evaluated as: 1) clinical improvement if an improvement in severity grading was observed, and 2) mycological cure if there was a negative result on fungal culture.

NDM onychomycosis was diagnosed according to Gupta criteria[11] as positive KOH and positive fungal culture for more than two consecutive times. Normal nail length and nail thickness were measured every visit by the same evaluator using a Vernier caliper. Outcome assessment made by 2 dermatologists at every visit was compared to baseline. Treatment outcomes were evaluated as: 1) clinical improvement if improvement was observed in normal nail length and nail thickness, and 2) mycological cure if there was a negative result on fungal culture.

Side effects, including erythema, irritation, and nail color change, were also evaluated at every visit.

Statistical analysis

Descriptive statistics, such as age, nail thickness, and nail length, are expressed as mean ± standard deviation [SD]. Demographic data and other categorical data are presented as number and percentage. Friedman test and Dunn multiple comparison methods were used to evaluate clinical improvement in chronic paronychia patients. Paired sample t-test was used to compare mean nail length and thickness between the first visit and each subsequent visit. Results with a p-value ≤ 0.05 were considered to be statistically significant. Statistical analysis was performed using SPSS Statistics version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 18 patients were included in the present study, 9 had candida chronic paronychia and 9 had NDM onychomycosis.

All 9 chronic paronychia patients were female. Mean ± SD age was 60.1±10.6 years. The affected sites included 7 thumb nails and 2 index finger nails. Mean severity grading at baseline was grade 3. Culture revealed *C. albicans* in 6 patients and NAC in 3 patients. Compared with baseline, severity of chronic paronychia was significantly reduced within 6 months after treatment with *O. europaea* leaf extract (p = 0.021). Representative photographs were presented in Figure 1. Mycological cure rate for chronic paronychia was 56% at 6 months after treatment.

For NDM onychomycosis, 6 male and 3 female patients were included. Mean age was 67.2±7.9 years. All patients had *N. dimidiatum* onychomycosis at the big toenail. Mean normal nail length at baseline was 7.1±1.2 mm. Significant improvement was observed at 4 and 6 months after treatment with nail lengths of 8.4±0.8 mm (p = 0.047) and 8.9±0.8 mm (p = 0.016), respectively. Mean nail thickness at baseline was

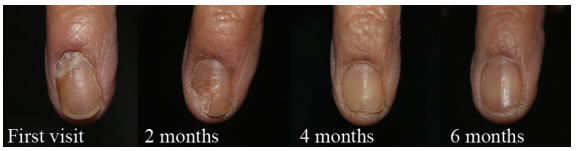

Figure 1. Clinical and treatment outcomes of chronic paronychia after applying *Olea europaea* leaf extract treatment in case number 7.

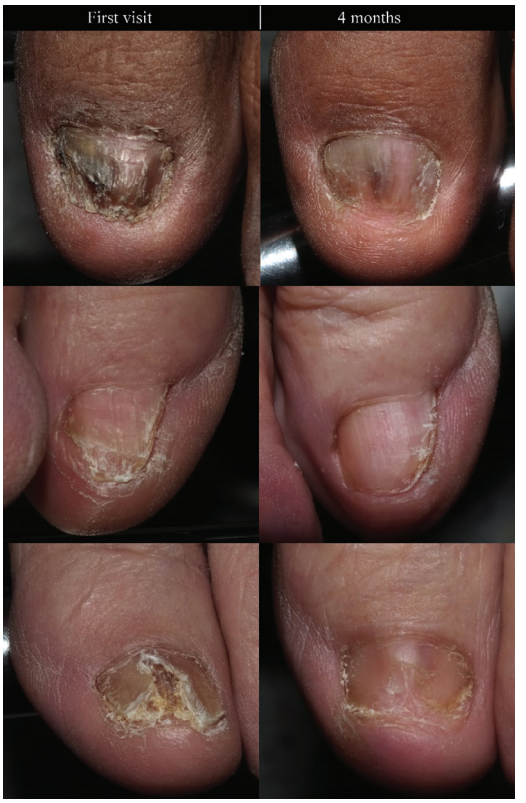

Figure 2. Clinical and treatment outcomes of non-dermatophytic onychomycosis after applying *Olea europaea* leaf extract treatment for 4 months in case number 10, 13, and 17.

1.1±0.4 mm. Significant improvement was observed at 4 and 6 months after treatment with nail thicknesses of 0.7±0.4 mm (p = 0.004) and 0.6±0.4 mm (p = 0.001). Representative images were shown in Figure 2. Mycological cure rate for NDMs onychomycosis was 33% at 6 months after treatment. No side effect was observed in the present study.

Discussion

The present study investigated the effectiveness and safety of *O. europaea* leaf extract for treatment of chronic candida paronychia and *N. dimidiatum* onychomycosis. For chronic paronychia, severity grading was significantly improved and 56% of patients achieved mycological cure. In *N. dimidiatum* onychomycosis patients, normal nail length, and nail thickness were significantly improved and 33% of cases had mycological cure. No adverse events were observed in any patient in the present study. The data supported that *O. europaea* leaf extract may be an effective and safe alternative topical treatment for *N. dimidiatum* onychomycosis and chronic paronychia from *Candida* spp. infection.

The in vitro antimicrobial activity of phenolic compounds from plants has been reported in several studies[12-17]. Mechanisms of actions against fungi have been proposed, including inhibition of the fungal 1,3-β-glucan synthase[18] and disrupting the cytoplasmatic membrane in *C. albicans*[19-22] and in dermatophytes[22]; modulating the antifungal activity of monocytes[23]; activating apoptosis in *C. albicans*[24,25]; and having an anti-biofilm effect[26,27].

Phenol compounds have also been studied in olive leaves (*O. europaea*) and their antimicrobial activity, especially against *C. albicans*, has been previously reported[8,28]. NailProtex® (Alvogen, Inc., Pinebrook, NJ, USA) is prepared from *O. europaea* leaf fermentation, which results in the isolation of several phenolic and phytochemical compounds (e.g., oleuropein, flavonoids, anthocyanins, and tannins) have been well-reported for their antioxidant activities. Pereira et al[8] reported that the antimicrobial activity in *O. europaea* leaf extract was most effective in *C. albicans*, with IC25 values lower than 1 mg/ml. Consistent with that finding, the authors' chronic paronychia patients with proven *Candida* spp. infection had significant improvement in clinical manifestations within 6 months, with 56% achieving mycological cure. These findings demonstrate that *O. europaea* leaf extract is effective in treating chronic paronychia caused by both *C. albicans* and NAC. Chronic paronychia may require longer duration of treatment to attain a higher rate of mycological cure.

Antifungal properties of phenolic compound from *O. europaea* leaves have not been studied in NDMs. All patients in the present study with *N. dimidiatum* onychomycosis had significant clinical improvement, including normal nail length and nail thickness after 4 months of treatment. Continued improvement was observed in those patients at 6 months after treatment. Clinical cure, which was defined as normal nail appearance, could not demonstrated in the present study due to the limited follow-up period. Mycological cure rate in *N. dimidiatum* onychomycosis patients was

33% after 6 months of treatment. A longer follow-up period may have yielded a higher rate of negative fungal laboratory results.

The present study has some mentionable limitations. First, this was a pilot study to evaluate the effectiveness and safety of *O. europaea* leaf extract on chronic paronychia and NDM onychomycosis. Accordingly, the sample size was small and not enough to draw conclusive, statistically supported determinations about the safety and efficacy of this agent. Second, patients were followed for only 6 months after treatment. Nail growth was slow in elderly patients and in patients with onychomycosis. A longer follow-up period would allow time to achieve clinical and mycological cure. Future larger studies include these factors will be needed to further explore outcomes in this novel dermatologic treatment.

In conclusion, *O. europaea* leaf extract was shown to be an effective and safe alternative topical treatment for chronic paronychia from *Candida* spp. infection and *N. dimidiatum* onychomycosis. No adverse events were observed in the present study.

Conclusion

O. europaea leaf extract was shown to be an effective topical treatment for *N. dimidiatum* onychomycosis and chronic paronychia from *Candida* spp. infection.

What is already known on this topic?

Phenol in *O. europaea* leaf was reported to demonstrate antifungal action against *C. albicans* and dermatophytes in vitro studies.

What this study adds?

O. europaea leaf extract was shown to be an effective topical treatment for *N. dimidiatum* onychomycosis and chronic paronychia from *Candida* spp. infection.

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Ethics consideration

The protocol for this study was approved by the Siriraj Institutional Review Board [SIRB]. The present study complies with the principles set forth in the Declaration of Helsinki (1964) and all of its subsequent amendments.

Informed consent

Written informed consent was obtained from all individual participants included in this study.

Potential conflicts of interest

The authors declare no conflict of interest.

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