Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(12); 1414-1420
doi: 10.25258/phyto.v9i11.11184
ISSN: 0975-4873

Research Article

Evaluation of the Therapeutic Efficacy of Tea Tree Oil in Treatment of Onychomycosis

Dina AbdelHamid[1], Amal H A Gomaa[2], Ghada F Mohammed[2*], Moustafa M K Eyada[2], Mohamed Aly El_Sweify[3]

[1]*Department of Dermatology and Venereology, Ministry of Health, Cairo, Egypt*
[2]*Department of Dermatology and Venereology, Suez Canal University, Ismailia, Egypt*
[3]*Department of Microbiology and immunology, Suez Canal University, Ismailia, Egypt*

Received: 16th Sep, 17; Revised 8th Nov, 17, Accepted: 24th Nov, 17; Available Online:25th Dec, 17

ABSTRACT

Background: Onychomycosis is a fungal infection that affects toenails or fingernails and may involve any component of the nail unit. Tea tree oil (TTO), or *Melaleuca* oil, is a pale yellow to nearly colorless, clear oil with a fresh camphoraceous odor that has antifungal effect if used from 5-100% concentration according to site of fungal affection. Aim: This study aimed to provide a new line for treatment of onychomycosis. Patients and methods: A randomized double blind interventional cohort prospective effectiveness trial, in which 66 patients with onychomycosis were recruited after their written consent was obtained. Patients on local or systemic treatment or with coexisting inflammatory skin disease were excluded. TTO efficacy in treatment of onychomycosis was evaluated by application of 100% TTO for 6 months with pre and post treatment nail culturing. Results: After 6 months treatment with TTO, 27% of patients were completely cured, 65% were partially cured and 8% had no response according to appearance of the index nail (the nail with the greatest fungal burden at the time of entry into the study). Calculation of P value by Chi-Square test, it equal 0.001 which is highly significant. Conclusion: TTO may play a role in treatment of onychomycosis without side effects of medications or surgical hazards caused by surgery.

Keywords: *Melaleuca alternifolia*, Onychomychosis, Tea tree oil.

INTRODUCTION

Many allopathic modalities are available for the treatment of onychomycosis, but they require a prolonged course of treatment and frequently have serious side-effects [systemic and topical antifungal medication or a combination of topical and systemic therapy, debriding to the healthy nail, nail matrixectomy, chemical avulsion (keratinolysis)][1]. Onychomycosis may lead to significant psychological distress and a significant impact on quality of life. These concerns have resulted in a multibillion dollar effort to reverse the condition[1]. *Melaleuca alternifolia* is a well-known functional food and traditional herbal medicine. It is native to the northeast coast of New South Wales, Australia**.** From its leaves, Tea tree oil (TTO), or *Melaleuca oil*, is extracted. It is a pale yellow to nearly colorless, clear oil with a fresh camphoraceous odor. TTO contains the essential oil, called terprenoids or isoprenoids, organic chemicals presenting naturally which are acquired through steam distillation. The plant terprenoids are used extensively for their aromatic qualities, while at the same time it is nontoxic to apply as a topical solution on affected areas. The organic terpinen-4-ol in the tea tree leaf is powerful antifungal compound[2]. TTO has an antifungal agent, effective against multiple dermatophytes affecting on the skin[3]. Its therapeutic effect was approved in comparison to the usual medications used in many studies on fungal infections as onychomycosis[10]. TTO has an antifungal agent, effective against multiple dermatophytes affecting the skin[3]. Its therapeutic effect was approved in comparison to the usual medications used in many studies on fungal infections as onychomycosis[4]. This study was performed to evaluate the efficacy of TTO as a herbal therapy in treatment of onychomycosis.

Study Design and Patient Population

Approval for this a randomized double blind interventional cohort prospective effectiveness trial was obtained from the Suez Canal Research Ethical Committee, and the study was conducted in accordance with the guidelines of the Declaration of Helsinki. Patients were recruited from the outpatient clinic of the dermatology department of Suez Canal University Hospital, between July 2011 and May 2012. Eligible participants were those who sought treatment for onychomycosis and had no evidence of any systemic or dermatologic disease. Excluded from the study were pregnant women, lactating women, and patients who were receiving treatment. Sixty-sex patients were eligible for participation, and 66 completed the study. Prior to the study, a complete medical history was obtained from each patient, and all patients underwent a general physical examination.

**Author for Correspondence: Dr_Ghada77@hotmail.com*

Table 1: Differentiation of fungal species by culture before and after 6 months of treatment.

variables	Before treatment		After treatment	
	frequency	%	frequency	%
Asergillus Niger only (A.N)	7	11%	1	2%
Aspergillus Fumigatus only(A.F)	11	17%	2	3%
Candida Albicans only	30	45%	3	4%
A.N+ A.F	4	6%	1	2%
A.N+ Candida albicans	7	11%	0	0%
A.F+ Candida albicans	4	6%	0	0%
Combination of 3 species	3	4%	0	0%
Total	66	100%	7	11%

Table 2: In vitro activity of tea tree oil on different fungus species on broth according to turbidity.

	Conc. of TTO	AN	AF	CA
1	0.5%	No turbidity	No turbidity	No turbidity
2	0.25%	No turbidity	No turbidity	No turbidity
3	0.125%	No turbidity	No turbidity	No turbidity
4	0.0625%	No turbidity	No turbidity	No turbidity
5	0.0312%	No turbidity	No turbidity	No turbidity
6	0.015%	No turbidity	No turbidity	No turbidity
7	0.007%	No turbidity	No turbidity	No turbidity
8	0.0035%	No turbidity	No turbidity	No turbidity
9	0.0017%	No turbidity	No turbidity	No turbidity
10	0.00085%	No turbidity	No turbidity	No turbidity
11	0.000425%	No turbidity	No turbidity	No turbidity
12	0.00021%	No turbidity	No turbidity	No turbidity

Table 3: In vitro activity of tea tree oil on different fungus species by culturing to broth in different doses according to growth.

Conc. of TTO	AN	AF	CA
0.5%	No growth	No growth	No growth
0.25%	No growth	No growth	No growth
0.125%	No growth	No growth	No growth
0.0625%	No growth	No growth	No growth
0.0312%	No growth	No growth	No growth
0.015%	No growth	No growth	No growth
0.007%	No growth	No growth	No growth
0.0035%	No growth	No growth	No growth
0.0017%	No growth	No growth	No growth
0.00085%	No growth	No growth	No growth
0.000425%	One colony	Two colonies	One colony
0.00021%	Three colonies	Three colonies	More than 10 colonies

Assessment Methods

Clinical examination and investigations including microscopic examination of potassium hydroxide (KOH) treated smear and culture on specific media for Dermatophyte and Candida, with two types of agars was used, Sabouraud Dextrose agar supplemented with gentamicin in concentration of 0.1 g/L (Memphis/Schering, Oman) and chloramphinicol in concentration of 50 mg/L (Alcon, U.S.), to specifically inhibit the growth of bacteria for isolation of Candida, and Sabouraud Dextrose agar with added cyclohexamid in concentration of 400 mg/L (Bioshop, France), which inhibits saprophytic fungi for isolation of dermatophytes. Gentamicin and chloramphinicol were directly added to the hot medium after sterilization by autoclave. Cyclohexamid was in powder form that was kept at -15°C till usage. 100µg of cyclohexamid dissolved in 1ml acetone were added to the hot media after sterilization. Cultures were incubated at 30ºC. Cultures were observed up to 22 days for growth. Identification of growth is based on age of growth, morphology of both surface and back of colonies, as well as microscopic examination of mycelium before and after staining by methylene blue (Muby chemicals, India).

Patients of onychomycosis who was approved by KOH test and culture were treated by application of TTO 100% twice daily on the affected nail for 6 months. During period of treatment, debridement and clinical assessment to the patients was performed at 0, 1, 3, and 6 months. Cultures were obtained at 0 and 6 months. Photographic imaging was done at every visit. At the 1-, 3-, and 6-month visits, "full," "partial," or "no" resolution by

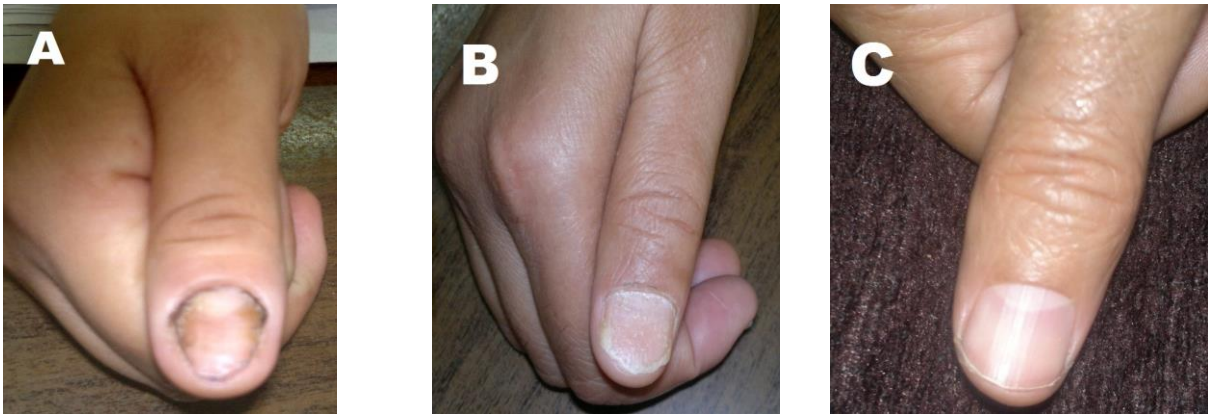

Figure 1: The right hand of male patient with onychomycosis in thumb in 0 month [A], in the 1st month of treatment with TTO which shows partial improvement [B], and in 3rd month of treatment with TTO which shows full cure of the thumb [C].

Figure 2: The right foot of male patient with onychomycosis in all toes nails in the 0 month of treatment [A], and in the 6th month of treatment with TTO with partial improvement [B].

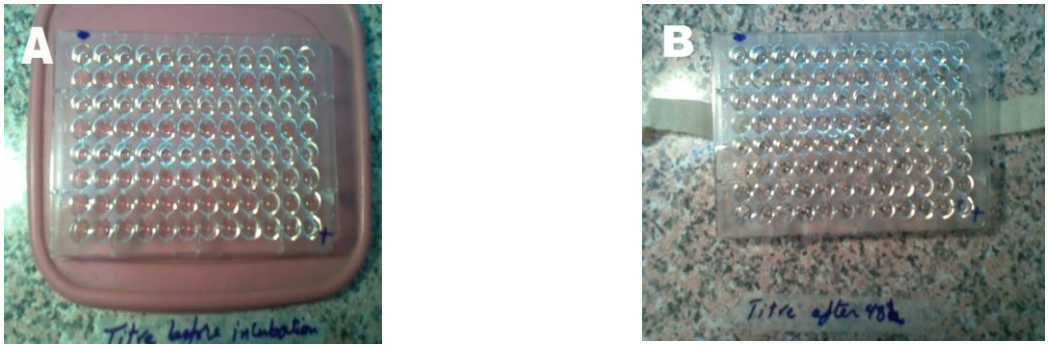

Figure 3: shows broth microdilution test of TTT to 3 species of fungi (AN, AF, CA) before incubation [IN VITRO ACTIVITY] [A], and after incubation for 48h in 35$_o$C which shows no turbidity in any well [B].

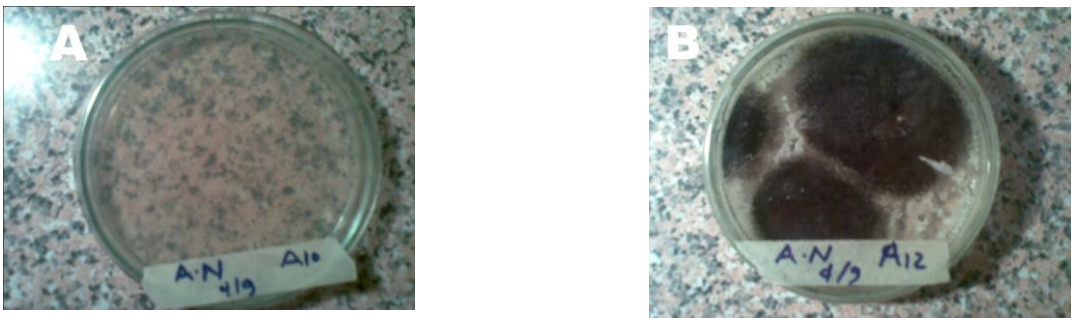

Figure 4: shows post culturing to AN (concentration of TTO= 0.00085%) which shows no turbidity in broth and no colonies growth on SDA within 7 days incubation [A], and post culturing to AN (TTO concentration = 0.000425%) which showed no turbidity in broth but shows one colony growth on SDA within 7 days incubation [B].

appearance of the index nail (the nail with the greatest fungal burden at the time of entry into the study) was recorded. In addition, all patients were examined 3 months after the end of treatment to assess whether their nail appearance and symptomatology (pruritus and pain) had resolved, improved, not changed, or worsened[4]. Also, side effects were assessed (Dermatitis, Ulceration, Respiratory symptoms or Other side effects).

Pre-treatment cultures to fungus revealed 3 fungal species (*Aspergillus niger (AN), Aspergillus fumigatus (AF)* and *Candida albicans (CA)*). Post-treatment cultures were taken of all patients after using treatment for 6 months by the end of the 6th month, using Sabouraud Dextrose agar supplemented with gentamycine and choramphenicol. Post-treatment cultures were incubated at 30ºC and observed for up to 22 day for any growth.

In vitro activity of tea tree oil against fungal species

Fungal isolation

the 3 species of fungi that were isolated from the pre-treatment cultures of nails were subcultured on Sabouraud Dextrose agar for seven days at 30°C[5].

Preparation of fungal inoculum

Inocula were prepared by growing isolates on Sabouraud Dextrose agars. Filamentous fungi (AN and AF) inoculum concentration was $5.0X10^4$cfu/ml[6]. Which was adjusted by using spectrophotometer at wave length 530nm with optical density 0.09-0.11 according to CLSI standards[7]. *CA'* inoculums concentration was $5.0X10^4$ cfu/ml, which is equal to 0.5 McFarland, according to CLSI standard nephlometer for yeasts[8].

3-Broth microdilution method

It was done according to the reference method recommended by NCCLS, in which a series of doubling dilutions of TTO (Vitacost, US) in saline ranging from 8% to 0.004% (v/ v) was prepared in 96- well microdilution tray, with a concentration of 0.001% (v/ v) Tween 80 to enhance TTO solubility[6]. After addition of 10µ inoculate, trays were incubated for 48h at 35°C. Minimum inhibitory concentration (MIC) of TTO on fungus according to NCCLS was 0.004% to 0.25%[6]. So, 12 concentrations of TTO were used in this study from 0.5% to 0.0002%, based in doubling method of concentration to each well added fungal suspension, which showed no turbidity in all concentrations of TTO. Minimal fungicidal concentrations (MFCs) of TTO were determined at the lowest concentration resulting in no growth on subculture, were determined by subculturing 10 µL from wells with no visible turbidity and spot inoculating on Sabouroaud Dextrose agar plates. MFCs in study was in concentration of 0.00085% of TTO[6].

Statistical analysis was done using SPSS computer software tests. The difference in effect of TTO among patients was tested using the Chi-square test and considered significant when $p< 0.05$. The comparative analysis among ratios were calculated by (ANOVA) test considered significant difference when $p< 0.05$.

RESULTS

Study Population Characteristics

This study included 66 patients [13 (20%) male and 53 (80%) females] aged between 18 and 60 years with mean age 29.1±6.3. Most of patients (48%) aged between 28 – 37 years and most of them (70%) had fingers affection, 27% has toes affection and 3% has both fingers and toes affection. More than 80% had less than one year duration of onychomycosis. Onychomycosis only presented in 73%, and onychomycosis associated with symptoms presented in 27% [it was associated with pain and tenderness only in 15%, pruritus only in 6%, and pain, tenderness and pruritus in 6%].

Fungi isolated from the studied patients were only Yeasts and Non-dermatophytes [*CA* 59%, *AF* 26% and *AN* 15%]. At the end of 6th month, 27% showed full curing, 65% partial curing, and only 8% not cured (the difference was highly significant p value = 0.001*). [Figures 1, 2]

After 6 months of treatment with TTO all associated symptoms resolved (100%) and there weren't any side effects except dermatitis in 6%. After following up the dermatitis disappeared through 2 months. Fungal species by culture before and after treatment with TTO decreased significantly P value < 0.05 [Tables 1-3] [Figures 3-6].

DISCUSSION

Onychomycosis may involve any component of the nail unit[1], treatment of onychomycosis is challenging because the infection is embedded within the nail and is difficult to reach. So, new line of treatment using TTO as an antifungal was challenging nowadays.

This study was conducted on 66 subjects complaining of onychomycosis categorized randomly with matched age and gender. They were instructed to apply TTO on the affected nails twice daily for 6 months. This study showed female predominance as those conducted in United Kingdom and Spain[9]. But, a study was done in Canada revealed that males were more affected than females especially in old age[10]. The discrepancy between the female and male percentage in the current study and other studies is due to different cultures, different types of work between males and females in the western and eastern countries [Most eastern females are housewives who deal more with water than males in comparison to western countries].

All subjects were divided to 4 groups of job which 56% their job was dealing with water only, 3% was dealing with animals only with 27% of subject deal with animals and water, 3% of subjects had mechanical jobs that allow them to face traumas, chemicals, etc. The 5th group of job was subjected under name ''others'' for other causes may be detected and it resembled 11%. It is noteworthy that there no published data regarding the job as a risk factor in causing onychomycosis. Thus the current study may highlight new players in the pathogenesis of onychomycosis.

All subjects were divided into 5 groups of job, 56% were dealing with water only, 3% were dealing with animals only, 27% of subjects were dealing with animals and water, 3% of subjects had mechanical jobs that allow them to face traumas, chemicals. The 5th group of job was subjected under name ''others'' for other causes may

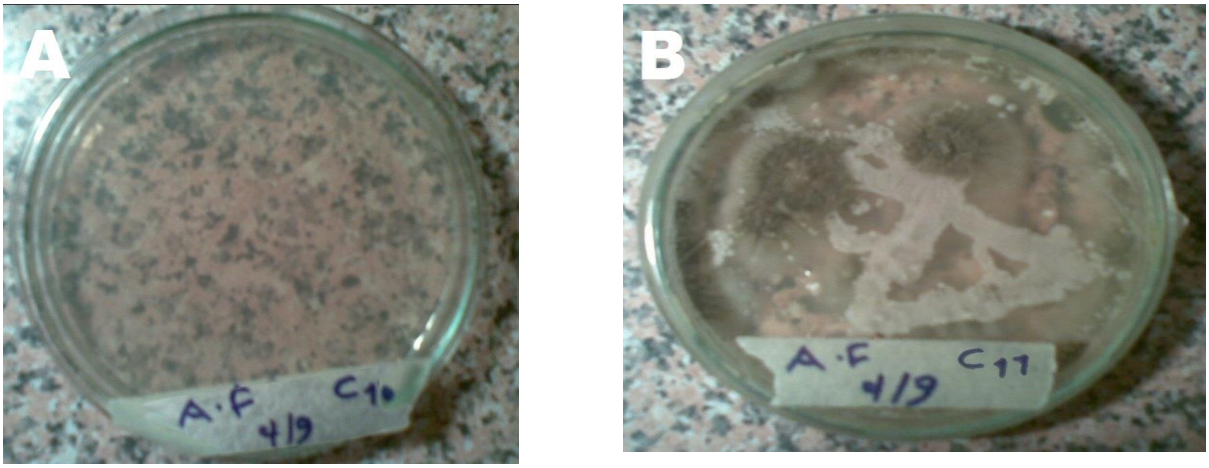

Figure 5: shows post culturing to AF (concentration of TTO= 0.00085%) which shows no turbidity in broth and no colonies growth on SDA within 7 days incubation [A], post culturing to AF (TTO concentration = 0.000425%) which showed no turbidity in broth but shows two colonies growth on SDA within 7 days incubation [B].

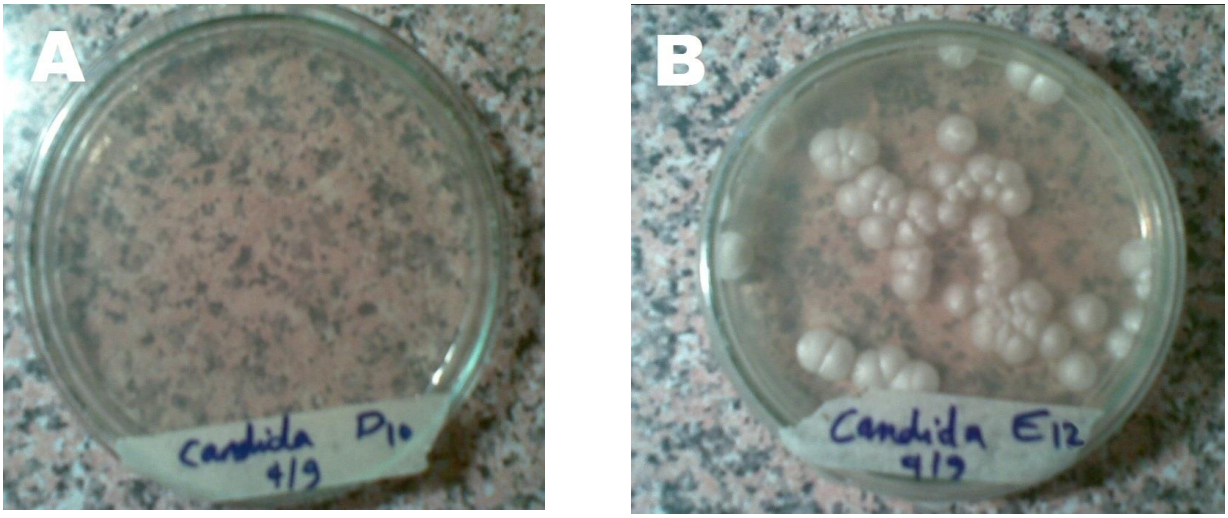

Figure 6: shows post culturing to CA (concentration of TTO= 0.00085%) which shows no turbidity in broth and no colonies growth on SDA within 7 days incubation [A], shows post culturing to CA (TTO concentration= 0.00021%) which showed no turbidity in broth but shows more than 10 colony growth on SDA within 7 days incubation [B].

be detected and it represented 11%. It is noteworthy that there is no published data regarding the job as a risk factor in causing onychomycosis. Thus the current study may highlight new players in the pathogenesis of onychomycosis.

Distribution of onychomycosis in current study agreed with a study was done in Hong Kong in 1997 on 2382 nail samples 1024(43.0%)toe, 1148(48.2%)finger, and 210 (8.8%) unspecified site)[11], these percentages between fingers and toes illustrate that fingers nails are more liable to fungal infection than toes nails, in spite of that toe nails are more liable to tinea pedies that lead to onychomycosis with a high percentage of who suffer from tinea pedies.

Twenty seven percent of subjects had associated symptoms with onychomycosis as pain/tenderness, pruritus, and by follow up of those symptoms it showed that 100% of them were resolved from pain/ tenderness and pruritus by the end of 6 months treatment with TTO, which approve that TTO has role in treatment of complicated onychomycosis.

Pre culturing to specimens were done and revealed that 15% were *AN*, 26% were *AF* and 59% were *CA*. Post culturing also were done and resulted in 11% of specimens had growth and by morphological differentiation it was 2% A.N., 3% A.F., 4% *CA* and 2% were combination of A.N. and A.F. This study was in agreement with study was done in United Kingdom in 108 patients with frequency of Candidal species 38%[12]. This current study was with discrepancy with other studies that reveal high percentage of Dermatophytes rather than other species of fungi causing onychomycosis especially in western than eastern countries[4,13].

In vitro activity to TTO was done on a broth of 3 fungal species detected in pre cultures with 12 concentrations (ranged MIC of TTO) of TTO by doubling microdilution method for 48h in 35°C and it revealed that no turbidity was found in all 12 concentrations, but by culturing to those 12 concentrations on Sabourauad Dextrose agar it revealed that concentrations number 11 and 12 had colonies growth although no turbidity which revealed that TTO has wide range of MFC. Assessing the side effects

that might be with using the oil, it showed no significance as only 6% had dermatitis and it decreased by using the oil with months of treatment till it was resolved in the 6th month of treatment. Clinically TTO was effective in treatment of onychomycosis as in the 1st month of treatment it showed only 3% full cure and 17% partial cure and in the end of the 3rd month of treatment the percentage was equal between patients with full cure and with those of partial cure (45.5%), in comparison to 6th month of treatment only 27% were fully cured and 65% were partially cured. Statistical analysis was done to this which resulted into P –value<0.001 which is highly significant compared to significant P- value <0.05. Findings that TTO in treatment of onychomycosis is significantly high was in agreement with the results of a study that was done on 117 patients with onychomycosis divided into 64 patients treated with TTO 100% for 6 months duration and 52 was treated with 1% clotrimazol solution which result on 60% of patients treated with TTO for 6 months had been fully cured or partially cured at the end of the 6th month[4].

Another study was done in 1999 on 60 patients of onychomycosis, 40 of them were using 2% Butanifine cream and 20 patients was using 5% TTO in form cream for 9 months duration which result in 80% of 20 patients used TTO had cured at the end of treatment duration[14].

In vivo activity of terpinen-4-ol, the main bioactive component of Melaleuca alternifolia Cheel TTO against azole-susceptible and -resistant human pathogenic Candida species was done in 2006 with reference to the treatment of vaginal candidiasis. However, there is a lack of *in vivo* data supporting *in vitro* results, especially regarding the antifungal properties of TTO constituents. Thus, the aim of this study was to investigate the in vitro and the in vivo anti-Candida activity of two critical bioactive constituents of TTO, terpinen-4-ol and 1,8-cineole. Oophorectomized, pseudoestrus rats under estrogen treatment were used for experimental vaginal infection with azole (fluconazole, itraconazole) -susceptible or -resistant strains of *CA*. All these strains were preliminarily tested for *in vitro* susceptibility to TTO, terpinen-4-ol and 1,8-cineole for their antifungal properties, using a modification of the CLSI (formerly NCCLS) reference M27-A2 broth micro-dilution method. *In vitro* minimal inhibitory concentrations (MIC90) values were 0.06% (volume/volume) for terpinen-4-ol and 4% (volume/volume) for 1,8-cineole, regardless of susceptibility or resistance of the strains to fluconazole and itraconazole. Fungicidal concentrations of terpinen-4-ol were equivalent to the candidastatic activity. In the rat vaginal infection model, terpinen-4-ol was as active as TTO in accelerating clearance from the vagina of all Candida strains examined[13].

In 2013 Antifungal activity of nanocapsule suspensions containing TTO on the growth of Trichophyton rubrum study was done which aimed to evaluate, for the first time, the antifungal efficacy of nanocapsules and nanoemulsions containing Melaleuca alternifolia essential oil TTO in an onychomycosis model. The antifungal activity of nanostructured formulations was evaluated against Trichophyton rubrum in two different in vitro models of dermatophyte nail infection. First, nail powder was infected with T.rubrum in a 96-well plate and then treated with the formulations. After 7 and 14 days, cell viability was verified. The plate counts for the samples were 2.37, 1.45 and 1.0 log CFU mL(-1) (emulsion, nanoemulsion containing TTO and nanocapsules containing TTO, respectively). A second model employed nails fragments which were infected with the microorganism and treated with the formulations. The diameter of fungal colony was measured. The areas obtained were 2.88±2.08 mm (2), 14.59±2.01 mm (2), 40.98±2.76 mm (2) and 38.72±1.22 mm (2) for the nanocapsules containing TTO, nanoemulsion containing TTO, emulsion and untreated nail, respectively. Nail infection models demonstrated the ability of the formulations to reduce T. rubrum growth, with the inclusion of oil in nanocapsules being most efficient[15].

CONCLUSION

Tea tree oil may play a role in treatment of onychomycosis without side effects of medications or surgical hazards caused by surgery.

CONFLICTS OF INTEREST

There are no conflicts of interest.

FUND FOR THE RESEARCH

There are no sponsor or fund for the research

REFERENCES

1. Rodgers P and Bassler M. Treating onychomycosis. *Am Fam Phys*. 2001; 63(4):663-673.
2. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) Oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev*. 2006 Jan;19(1):50-62.
3. Nenoff P, Haustein UF, Brandt W. Antifungal activity of the essential oil of Melaleuca alternifolia (Tea Tree Oil) against pathogenic fungi in vitro. *Skin Pharmacol.* 1996;9(6):388-94.
4. Buck DS, Nidorf DM, Addino JG.. Comparison of two topical preparations for the treatment of onychomycosis: *Melaleuca alternifolia* oil and clotrimazole. *Journal of Family Practice*. 1994 Jun; 38:601-605.
5. Hammer K A, Carsonl C F, Riley T V. in vitro activity of *Melaleuca Alternifolia* (tea tree oil) against dermatophytes and other filamentous fungi. *J Antimicrob Chemother*. 2002 Aug; 50(2):195-9.
6. *National committee for clinical laboratory standards*. (1998). Refrence method for broth dilution antifungal susceptibility testing of conidium-forming filmentous fungi: proposed standard M38-P. NCCLS. Wayne, PA, USA.
7. CLSI. Refrence method for broth dilution antifungal suscepility testing of filmentous fungi; approved standard. CLSI document M38-A [ISBN 1-56238-470-8]. CLSI, Pennsylvania, USA. 2002.

8. CLSI. Refrence method for broth dilution antifungal suscepility testing of yeasts; approved standard-second edition. CLSI document M27-A2 [ISBN 1-5623-469-4]. CLSI, Pennsylvania, USA.2002.
9. Elewski BE, Charif MA. Prevalence of Onychomycosis in Patients Attending a Dermatology Clinic in Northeastern Ohio for Other Conditions. Arch Dermatol. 1997 Sep;133(9):1172-3.
10. Gupta AK1, Jain HC, Lynde CW, Macdonald P, Cooper EA, Summerbell RC. Prevalence and epidemiology of onychomycosis in patients visiting physicians' offices: A multicenter Canadian survey of 15,000 patients. *J Am Acad Dermatol.* 2000 Aug;43(2 Pt 1):244-8.
11. Kam KM, Au WF, Wong PY, Cheung M. Onychomycosis in Hong Kong. *International Journal of Dermatology*. 2008 JUN; 36 (10): 757–761.
12. Dorko E, Jautová J, Tkáčiková L, Wantrubová A. The frequency of Candida species in onychomycosis. *Folia Microbiologica.* 2002 Dec.; 47 (6): 727-731.
13. Mondello F1, De Bernardis F, Girolamo A, Salvatore G, Cassone A. In vitro and in vivo activity of tea tree oil against azole-susceptible and -resistant human pathogenic yeasts. *J Antimicrob Chemother*. 2003 May;51(5):1223-9.
14. Sayed TA, Qureshi ZA, Ali SM, Ahmad S, Ahmad SA. Treatment of toenail onychomycosis with 2% butenafine and 5% Melaleuca alternifolia (tea tree) oil in cream. *Trop Med Int Health*. 1999 Apr;4(4):284-7.
15. Flores FC, de Lima JA, Ribeiro RF, Alves SH, Rolim CM, Beck RC, da Silva CB. Antifungal activity of nanocapsule suspensions containing tea tree oil on the growth of Trichophyton rubrum. Mycopathologia. 2013 Apr;175(3-4):281-6.