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Photodynamic therapy of tinea capitis in children using curcumin loaded in nanospanlastics: A randomized controlled comparative clinical study

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ABSTRACT

Tinea capitis is a common scalp dermatophyte infection in children. Treatment necessitates oral antifungal therapy which represents a therapeutic challenge. Photodynamic therapy (PDT) has emerged as a new antifungal therapeutic approach. The efficacy of PDT depends on the proper choice of the photosensitizer and its delivery system. Curcumin, a natural polyphenol, is well studied as a photosensitizer, however, its hydrophobicity hinders its clinical use. This study aims to improve the use of curcumin in PDT by loading it to a novel vesicular nano spanlastics (NVS) and examine their efficacy in PDT of tinea capitis.

A prospective, randomized, controlled comparative study was conducted. Fifty-two children with Tinea Capitis were randomly divided into four groups; Cur-PDT group, oral griseofulvin group, topical curcumin group, and blue light group. Clinical and dermoscopic assessments were done, at baseline, every two weeks and after 6 months for follow-up. The results were confirmed by potassium hydroxide (KOH) 10% and fungal culture before and after the treatment. Complete cure was based on clinical, dermoscopic, and mycological cures.

Cur-PDT group showed complete clearance in 46% of children with variable response among fungal species, compared to 100% in the griseofulvin group. No effects were observed in curcumin or light groups. Cur-PDT group showed very mild tolerable burning pain. Griseofulvin group experienced systemic side effects in some cases. Cur-PDT can be a promising well-tolerated alternative treatment of tinea capitis, but with variable responses among fungal species.

1. Introduction

Tinea capitis is one of the commonest dermatophyte infections, mainly of the Microsporum and Trichophyton species, affecting the scalp and hair [1]. It occurs mostly in children and rarely in adults. However, recent studies show a higher incidence of adult infection than previously reported [2–4]. Tinea capitis prevalence differs according to the geographical distribution [4,5]. The patient can present a localized area of hair loss, scaling, grey patch, black dots, kerion, favus, agminate folliculitis, erythema, or impetigo-like lesions [6]. This variability is related to the causative organism, the immune response, or the type of hair infection [7].

Topical antifungals have a limited role in the treatment of tinea capitis except as an adjunctive to oral therapy [1]. Oral antifungals such as griseofulvin, terbinafine, itraconazole, and fluconazole are the mainstay treatment [8]. They are facing many therapeutic problems such as long duration of treatment, multiple drug interactions, many adverse effects, lack of a syrup form in some countries, the need for higher doses for certain species, resistance, chronicity, and recurrence of infection.

Photodynamic therapy (PDT) has been recently introduced as an alternative therapeutic modality for many microbial diseases. It depends on a group of light-sensitive compounds, called photosensitizers (PS). Upon irradiation by light of an appropriate wavelength in the presence of tissue oxygen, PS can be excited from their ground state to higher energy levels. When they relaxed to their ground state again, they undergo two types of photochemical reactions producing free radicals, reactive oxygen species (ROS), and singlet oxygen which have a

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devasting effect on microbial cells [9].

PDT has been used for its antifungal effect with the advantage of multiple cellular targets to the radicals that decrease the probability of resistant strains selection [9]. In addition, it demonstrates an anti-dermatophyte biofilm activity and modulates the sensitivity to conventional antifungal drugs decreasing its dosage, toxicity, and resistance if used in combination [10].

Curcumin (diferuloylmethane) is a polyphenol compound with low molecular weight isolated from Curcuma longa. Hundreds of clinical trials showed safety, tolerability, and effectiveness against various chronic diseases in humans. The therapeutic dose can reach up to 2 g twice a day in children and up to 12 g/day in adults for 3 months [11–13]. In addition, it has antimicrobial and antifungal effects [14]. Recent studies have proved that the antifungal effect is potentiated when curcumin is associated with blue light (absorption at 408–434 nm range) due to the production of reactive oxygen species that can destroy the fungi. However, the clinical use of curcumin is still limited due to its self-aggregation, high hydrophobicity, and low bioavailability. Many efforts are continuously exerted to develop novel, suitable nano-delivery carriers to improve curcumin delivery via different routes of administration, such as liposomes [15], gold nanoparticles [15], nanoemulsions [16], and PEGylated lipid nanoparticles (Polyethyleneglycol (PEG) coated nanoparticles) [17].

In this work, we developed a novel form of spanlastics as a nano-vesicular system. It is composed of a mixture of non-ionic surfactants and Cremophor as a penetrating enhancer to improve the topical delivery of curcumin. The aim is to develop a new therapeutic approach to Tinea Capitis with curcumin photodynamic therapy as an alternative treatment.

2. Patients and methods

2.1. Preparation and characterization of nano-spanlastics vesicles gel loaded by curcumin (NSV-Cur)

2.1.1. Materials

Curcumin was purchased from SD Fine-Chem limited India. Tween 80 and Cremophor (Kolliphore RH40) were purchased from Sigma Aldrich (a product of Germany). Span 80 was purchased from Oxford Lab Chem, India. Carboxymethyl cellulose Na (Na-CMC) and propylparaben were purchased from Normest Company for scientific development, Egypt. Absolute ethanol 99.9% was purchased from International Company for Sup. and Medical Industries, Egypt.

2.1.2. Preparation of nano-spanlastics vesicles loaded by curcumin (NSV-Cur)

The curcumin-loaded nano-spanlastics (NSV-Cur) were fabricated by the ethanol injection method as described by Badria et al. [18] with slight modification. Briefly, 0.2 g Span 80, 0.2 g Cremophor, 0.01 g Tween 80, and 10 mg curcumin were dissolved in 4 ml absolute ethanol. The ethanolic solution was injected dropwise into preheated water (70 °C) under magnetic stirring. Finally, a milky dispersion of the NSV-Cur vesicles was formed and its final volume was adjusted to 50 mL. The obtained dispersion was then sonicated for 5 min by Bath Sonicator Retsch (Retsch, Germany) to attain vesicles with homogenous size, and stored at 4 °C till further use.

2.1.3. Characterization of the prepared NSV-Cur

The morphology and the structure of the fabricated NSV-Cur were examined under the transmission electron microscope (JEM 100S, Jeol, Ltd., Tokyo, Japan). A diluted NSV-Cur suspension was spread on a carbon-coated copper grid and negatively stained prior to examination.

The unloaded curcumin was separated by centrifugation for 30 min, at 10,000 rpm at 8 °C (Centrikon T-42K, Kontron, Instruments, UK). The precipitated loaded vesicles were then dissolved in ethanol and curcumin concentration was measured from a previously established standard calibration curve in ethanol by double beam spectrophotometer (Rayleigh UV-2601) at 420 nm. The encapsulation efficiency (EE) was calculated as a ratio of the initially added drug amount.

After dilution of NSV-Cur suspension 100 folds with distilled water, the mean particle size and zeta potential (surface charge) were measured by diffraction laser scattering (DLS) using the Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK).

To study the in vitro drug release, 1 ml of NSV-Cur was placed in a 1 cm^2 piece of dialysis membrane (molecular weight cut off 12,000–14,000 Da). Afterward, the membrane was immersed in 50 ml receptor media and kept under stirring (100 rpm) at 37 °C to keep the sink condition. The receptor media consists of phosphate-buffered saline (PBS buffer, pH 7.4) containing 10% ethanol to enhance curcumin solubilization [18]. As a receptor medium, Aliquots of 1 ml were withdrawn at different time intervals and replaced by a fresh medium. The concentration of curcumin in the withdrawn samples was determined spectrophotometrically at 420 nm, as described above.

The fabricated NSV-Cur were incorporated into 5% carboxymethyl cellulose hydrogel as previously described by Ibrahim et al. [19]. Propylparaben (0.2%) was added as a preservative. The concentration of curcumin in the prepared gel was 1.25 mg/g gel.

2.2. Patients

Fifty-two patients with Tinea Capitis participated in this randomized, comparative study. They were recruited from Al-Haud Al-Marsoud Hospital, Cairo, Egypt. Their ages ranged from 2-to 12 years. Both sexes were included. The study was performed according to the Declaration of Helsinki principles and approval was obtained from the ethical committee of the National Laser Institutional Review board (Cu-NILES/19/21). The exclusion criteria were previous oral antifungal treatment one month before the study, photosensitive disorders, or any other systemic diseases. Before enrollment, full explanations and instructions about the products, device, purpose, treatment plan, benefits, and possible side effects were provided to the parents. Signed informed consent was obtained from the parents, including written informed consent for images to be published before the initiation of the treatment. Detailed history and full clinical examination were performed for each patient to assess the size, site, number of the lesion as well as the weight of the child.

Treatment started soon after the clinical diagnosis of Tinea Capitis, positive KOH, and dermoscopy. Fungal culture was used only as an additional tool to confirm the diagnosis and to determine the fungal species because of the long period challenge of the result. Fungal culture takes 3–4 weeks to give results, with consequent delayed treatment and an increase in the risk of horizontal transmission.

2.3. Treatment protocol

The patients were randomly selected and allocated into four groups using the closed envelope method, with 13 children in each group, as follows:

Griseofulvin group (control): The children were treated with oral griseofulvin as conventional antifungal therapy (Ultra Griseofulvin125 mg manufactured by KAHIRA PHARM and CHEM. IND.CO Egypt) 12.5 mg per kg body weight per day with fatty meals in one or two divided doses for 6–12 weeks.

Cur-PDT group: After gentle scrapping of the scales, a thin film of NSV-Cur gel (1.25 mg/g) was applied under occlusion by an opaque cap for 20 min. Afterward, the lesion was illuminated with blue light (120 mW/cm^2), for 16 min using Light Emitting Diode (LED) system (450 nm, 3 cm spot size) designed in the Technology center in NILES, Cairo, Egypt as shown in Fig. 1. The session repeated every two weeks for a maximum of 6 sessions.

Curcumin group**:** The children were subjected to topical application of NSV-Cur gel (1.25 mg/g) once daily for 6 weeks, without light exposure.

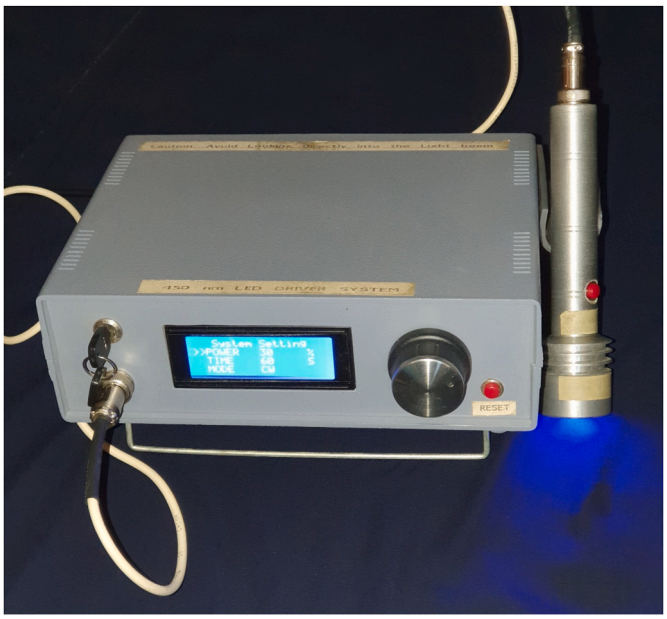

Fig. 1. light-emitting diode (LED) system, 450 nm, and 3 cm spot size.

Light group: The children were subjected to blue light therapy sessions with the same previously mentioned light dose. The session is repeated every two weeks for a maximum of 6 sessions.

2.4. Evaluation methods

Clinical, photographic, and dermoscopic assessments were done at baseline, every 2 weeks till the end of the treatment, and after 6 months for follow-up. The results were confirmed by potassium hydroxide (KOH) 10% and fungal culture before and after the end of the treatment.

2.4.1. Clinical assessment

Tinea Capitis was diagnosed clinically and assessed using the Total Signs and Symptoms Score (TSSS) [20]. It was performed at baseline, every visit, and at the end of the study. TSSS is a composite score (range, 0 to 9) of the signs and symptoms of erythema, desquamation/scaling, and papules/pustules, each of which was evaluated on a scale of 0–3 (0 = absent, 1 = mild, 2 = moderate, and 3 = severe). Clinical cure was defined as TSSS = 0 and the appearance of new hair growth. Assessment of pain was done for each patient using the numeric rating scale (NRS-11) for patient self-reporting of pain [21]. The disappearance of clinical signs of Tinea Capitis, TSSS = 0, and the appearance of new hair growth was considered a clinical cure.

2.4.2. Photographic evaluation

Standardized photographs using identical settings of the camera (Kodak 14 megapixel, 4x zoom; Kodak, New York, NY), patient positioning, and lighting were performed at baseline, at every visit and after 6-months post-treatment for follow up.

2.4.3. Dermoscopic evaluation

The dermoscopic evaluation was done before starting the treatment, at each visit, and at 6 months post-treatment for follow up using (DermLite DL4; 3 Gen, San Juan Capistrano, USA, magnification x10) and coupled to a camera (Kodak 14 megapixel, 4x zoom; Kodak, New York, NY). The presence of specific dermoscopic features of Tinea Capitis as comma hair, corkscrew hairs, zigzag hairs, and morse code hairs) were considered a positive dermoscopy. The presence of a single feature (corkscrew hair, comma hair, zigzag hair, Morse-code-like hair, or whitish sheath) is predictive of Tinea Capitis [22]. Other nonspecific signs also were recorded as broken hair, bent hair, black dots, and perifollicular scaling. The dermoscopic cure was defined as the disappearance of the previous features and the appearance of new hair growth.

2.4.4. Detection, isolation, and characterization of fungal pathogens

Direct microscopic examination of scalp scrapping using 10% potassium hydroxide (KOH) was done. A portion of the sample was placed on a slide and a drop of an aqueous solution of 10% (w/v) potassium hydroxide (KOH), was added. After 5 min, the wet mount was examined under low (× 10) and high (× 40) power magnification for the presence of fungal elements such as arthrospores. The other sample was sent to the laboratory to be cultured on Sabouraud's Dextrose Agar (SDA) with 0.05 mg of chloramphenicol and 0.05 mg of Cycloheximide and incubated aerobically at 27–30 °C, for at least 3 weeks before being discarded as a negative result. The identification of the dermatophytes from the positive cultures was based on the macroscopic colonial characteristics and the microscopic appearance of the conidia. The treatment started after the initial diagnosis of tinea capitis by the combined positive result of clinical, dermoscopic, and positive KOH examination. Negative KOH and culture results were considered a mycological cure.

2.5. Patient satisfaction

The parents rated their satisfaction at the end of the study for the improvement in the quality of life of their child, side effects, and overall degree of satisfaction at the follow-up visit. This was done by 10-point Visual Analogue Scale (VAS) (0–10; where 0 refers to "not satisfied and 10 refers to "completely satisfied").

2.6. End of the study

Primary endpoint: Complete cure (clinical, dermoscopic, and mycological cure by KOH or culture.

Secondary endpoint:

- Improvement of clinical signs of tinea capitis.
- Treatment safety and tolerability.

2.7. Statistical analysis

Data were collected, tabulated according to the randomization list, then analyzed using SPSS v. 25 to investigate and detect if there were statistically significant differences in the efficacy of different treatment modalities for Tinea Capitis. A test for normality was done to detect the distribution of data. Results were expressed as mean ± standard deviation (SD), comparison between the mean values of results by using paired student test within each group to compare between before and after treatment. Comparison between groups was done using ANOVA test followed by a post hoc test to determine the effect of each treatment modality for normally distributed data, otherwise, non-parametric tests were used. Results are considered significant if P-value is less than 0.05.

3. Results

3.1. Characterization of the prepared NSV-Cur

Curcumin was successfully loaded in the fabricated spanlastics vesicles (NSV-Cur) with high encapsulation efficiency (85% ± 5.1). The fabricated NSV-Cur exhibited a mean particle size of 168.6 ± 19.2 nm. TEM images obtained (Fig. 2) showed spherical, non-aggregated vesicles with the same particle size range obtained by Dynamic light scattering (DLS). The measured zeta potential was −42.2 ± 7.2, indicating high colloidal stability. The drug was released from the vesicles in a controlled manner with 85% ± 6.3 released after 24h (Fig. 3).

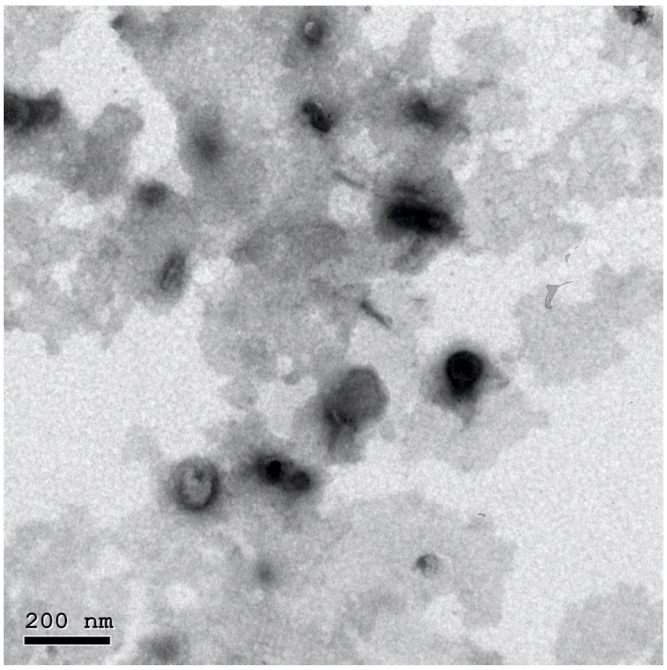

Fig. 2. Transmission electron microscopy (TEM) image showed spherical, non-aggregated vesicles with a nano-particle size range.

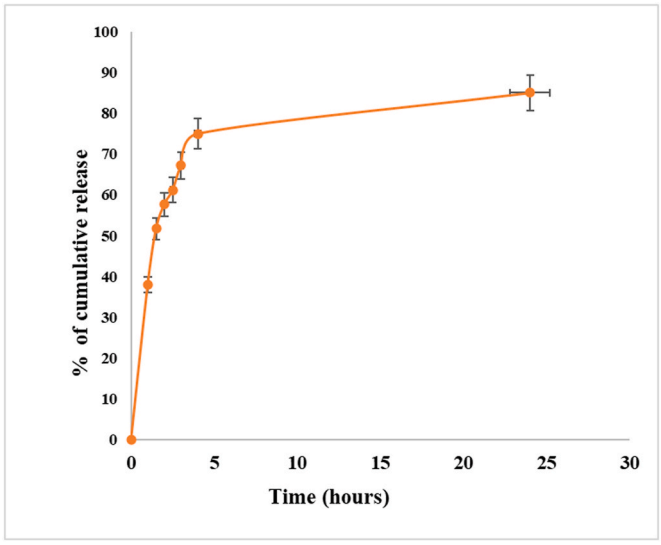

Fig. 3. In vitro drug release. The drug was released from the vesicles in a controlled manner with 85% ± 6.3 after 24h.

3.2. The clinical outcome

Fifty-two children with Tinea Capitis participated in the study. Forty-six continued the study. In the curcumin and light groups, six children dropped out because of incompliance.

The descriptive data of the patients for age and weight among groups are shown in (Table 1). The frequency distribution of consanguinity, animal exposure, gender, family history, fungal species, lesion type, and site were shown in (Table 2).

A promising successful therapeutic effect was observed in (46.2%) of the children in the Cur-PDT group with excellent complete cures as shown in (Figs. 4–6). The oral griseofulvin group (the conventional treatment of Tinea Capitis) showed a 100% cure. On the other hand, no effect was elicited in the curcumin or the light group (Fig. 6). The

Table 1
The descriptive statistics for age and weight among treatment groups.

Groups		Minimum	Maximum	Mean	Std. Deviation
Total	Age (years)	2	12	6.88	2.68
	Weight (kg)	10	42	23.09	7.41
Cur-PDT	Age (years)	2	12	7.46	3.479
	Weight (kg)	10	42	24.92	10.91
Griseofulvin	Age (years)	3	9	5.96	1.76
	Weight (kg)	14	33	21.69	5.72
Curcumin	Age (years)	3	12	7.30	2.49
	Weight (kg)	14	32	23.30	5.71
Light	Age (years)	3	12	6.90	2.76
	Weight (kg)	14	32	22.30	5.59

comparison of the treatment effect mean ranks among different groups using the Kruskal-Wallis test showed a significant statistical difference ($p = 0.0001$) between groups as shown in (Table 3).

A noticeable inflammatory reaction with variable degrees was observed in all groups except the light group. It was observed after 2 weeks (on the 2nd visit) and improved in cured cases towards the end of treatment. It ranged from mild erythema to pustule formation. Papules and pustules were noticed in only one patient in the Cur-PDT group after two weeks and improved with treatment. Furthermore, a decrease in scaling was observed in all groups except the light group. Consequently, improvement in TSSS was observed in cured cases of the Cur-PDT group ($p = 0.004$) and the griseofulvin group ($p = 0.0001$) but not in other groups. The comparison of the mean rank between groups regarding TSSS, scales, and erythema before treatment, at 2 weeks, and after treatment is shown in (Table 4).

There was no statistically significant difference in the therapeutic effect among groups in relation to age, sex, family history, consanguinity, clinical presentation, lesion location, animal exposure, and duration of the disease ($p > 0.05$). The only exception with a statistically significant difference in this regard was the therapeutic effect of Cur-PDT in relation to the history of animal contact ($p = 0.013$).

The isolated dermatophytes in our study were T violaceum, T tonsurans, and M canis. The comparison of the therapeutic effect on the isolated dermatophyte species in each group showed no statistically significant difference in all groups ($p > 0.05$) except in the PDT group ($p = 0.012$). PDT showed a successful complete cure in one (8%) T violaceum positive child. In addition to 5 (38.46%) children with positive KOH and dermoscopy, but the species failed to be determined by culture. However, the dermoscopic picture of these five cured cases points to Trichophyton species. Microsporum Canis species showed resistance to Cur-PDT where no cure occurred in 6 (46.15%) children as shown in Table 5 and Fig. 7.

Regarding the local and systemic side effects of treatment, no pain was reported in griseofulvin, curcumin, or light groups, however, all cases in the Cur-PDT group experienced mild tolerated burning pain sensation during the procedure. The mean degree of pain was 2.2 ± 0.6 SD and the range was 2: 3. Moreover, mild tolerated itching was reported in 2 cases in the curcumin group only. On the other hand, 3 cases reported GIT troubles in the griseofulvin group only in the form of nausea, colic and one case discontinued treatment after 2 days because of diarrhea.

There was no statistically significant difference regarding treatment effect among different groups about all dermoscopic signs (comma hairs, corkscrew, zigzag, bent hairs, broken hair, black dots, and perifollicular scaling) $p > 0.05$. However, the morse code sign within the Cur-PDT group was absent in all cured cases and present in all non-cured cases with a significant statistical difference of $p = 0.0001$ between cured and non-cured cases in the Cur-PDT group.

Treatment duration was slightly lower in cured cases of the PDT group with a mean of (5 ± 1.1) weeks in comparison to the griseofulvin group (6.77 ± 1.23) weeks. However, the results indicated no statistically significant difference between the two groups in this regard. The

Table 2
Frequency distribution of consanguinity, animal exposure, gender, family history, fungal species, lesion type, and site among treatment groups.

	Groups	Total		Cur-PDT		Griseofulvin		Curcumin		light	
		Fr	%	Fr	%	Fr	%	Fr	%	Fr	%
Consanguinity	No	38	83	11	84.6	9	69.2	8	80	10	100
	Yes	8	17	2	15.4	4	30.8	2	20		
Animal exposure	No	33	72	9	69.2	7	53.8	6	60	7	70
	Yes	13	28	4	30.8	6	46.2	4	40	3	30
Gender	Male	35	76	11	84.6	10	76.9	6	60	8	80
	Female	11	24	2	15.4	3	23.1	4	40	2	20
Family history	No	33	72	10	76.9	7	53.8	8	80	8	80
	Yes	13	28	3	23.1	6	46.2	2	20	2	20
Fungal species	M canis	13	28	6	46	3	23.1	2	20	2	20
	T tonsurans	12	26	0	0	6	46	3	30	3	30
	T violaceum	1	2	1	7.6	0	0	0	0	0	0
	No growth	20	34.3	6	46	4	30.8	5	50	5	50
Lesion type	Scaly	33	72	11	84.6	8	61.5	7	70	7	70
	Multiple	9	19	1	7.7	2	15.4	1	10	2	20
	Inflammatory	4	9	1	7.7	3	23	2	20	1	10
Lesion site	Frontal	5	11%	3	23.1	1	7.7	0	0	1	10
	Occipital	13	28%	3	23.1	3	23.1	3	30	2	20
	Lt parietal	11	24%	1	7.7	4	31	1	10	2	20
	Rt parietal	10	21.70%	2	15.4	1	7.7	0	0	1	10
	Vertex	18	39%	4	30.8	4	30.1	6	60	4	40

Fr: frequency, M canis: microsporum canis, T tonsurans: trichophyton tonsurans, T violaceum: trichophyton violaceum, Lt: left, Rt: right.

number of sessions in the Cur-PDT group ranged (2:3) sessions with two weeks apart till the appearance of cure signs.

The overall satisfaction assessed by VAS, as an important reflection of improvement in quality of life, revealed a very high satisfaction rate in cured cases of Cur-PDT group with a mean of (9.5 ± 0.45) and griseofulvin group with a mean of (8.84 ± 0.66) while the remaining non cured cases of Cur-PDT group, curcumin and light groups were scored less than 2.

Considering recurrence, no recurrence occurred at 6 months follow up in all cured cases of Cur-PDT group compared to only one (7.1%) patient in the griseofulvin group.

4. Discussion

In the current study, PDT using curcumin as photosensitizer loaded in spanlastics showed excellent successful therapeutic effect with complete cure in (46.2%) of Tinea Capitis cases. Oral griseofulvin showed a 100% complete cure rate. However, the efficacy was achieved in neither curcumin nor light groups. Complete cure was proven clinically with the disappearance of all clinical signs, TSSS assessment = 0, and the appearance of new hair growth. The results were confirmed dermoscopicaly and mycologically by the disappearance of all dermoscopic markers of tinea capitis together with negative KOH and fungal culture. According to Gupta et al. systematic review, griseofulvin is often regarded as the gold standard treatment for Tinea Capitis since 1960 and the first-line treatment in Microsporum species with variable but considerable complete cure rates [23]. However, it has had a lot of systemic adverse effects [23] and rising resistance in the last decades [24]. PDT using curcumin as a photosensitizer was reported to be effective in photoinhibition of dermatophyte species in vitro murine model [25], and in onychomycosis [26]. No clinical studies have been reported on Cur-PDT in Tinea Capitis treatment. However, successful treatment trials using other photosensitizers such as ALA PDT was reported in two case report studies, one in a child with liver dysfunction due to oral antifungal drugs [27], and the other in kerion treatment combined with itraconazole [28].

In the current study, Cur-PDT was well tolerated and associated only with a very mild tolerated burning sensation during the session with no other local or systemic adverse effects. Whereas in griseofulvin, GIT troubles were reported in 23% of the children in the form of nausea, colic, and diarrhea. Our results were approaching previous Gupta et al., 2018 study that involved 295 children, 26.8% experienced transient mild to moderate adverse effects, the most common being gastrointestinal. In addition to other reported adverse effects such as elevated triglycerides, SGOT, anemia, allergic reactions, photosensitivity, fever, headache, weight gain as well as multiple drug interactions had occurred [23].

An inflammatory reaction was observed after 2 weeks during the 2nd visit in some cases ranging from mild erythema to pustules formation in all groups except the light group. A similar observation was reported in previous studies and was explained by the inflammatory nature of the causative species from the start or due to the development of the host's delayed-type hypersensitivity reaction and presumed T cell-mediated immunity to dermatophyte which correlates with recovery from the infection [20].

The overall treatment duration was slightly higher in the children treated with griseofulvin. 38% of the children required a prolonged treatment period of 7–10 weeks. This may reflect multiple factors, such as type of infection, host reaction, or drug-related factors. Earlier studies have reported a rising resistance to griseofulvin and a step-wise increase in the recommendations regarding the dose and duration. The reduction in the efficacy of griseofulvin over the years was attributed to various factors, including changes in patterns of epidemiology, long-term use of the drug, poor compliance arising from the long duration of therapy, fungal genetic mutations resulting in decreased susceptibility, and recently biofilm formation [24]. In the latter, the organisms produce an extracellular polymeric matrix acting as a physical barrier preventing the penetration of antifungal agents and the development of antimicrobial tolerance. Moreover, it decreases the interactions with the host immune system and increases the expression of efflux pumps and the secretion of proteins that lead to the filamentation [24]. PDT demonstrated antibiofilm activity through the generation of cytotoxic reactive oxygen species (ROS) attacking microorganisms nonspecifically, inhibiting the ability of the microorganisms to adhere to surfaces, destroying biofilm structures, damaging some organelles, and inhibiting efflux capacity [29].

The efficacy of topical Cur-PDT depends not only on the efficacy of curcumin as a photosensitizer but also on the efficacy of the used carrier. Preliminary result in one in vivo study suggested that curcumin nanoparticles has a predilection for the hair follicle [30], the clinical site of Tinea Capitis infection, and allowed for enhanced targeting and decreased adjacent toxicity. The nanoparticles interact with the fungal cell membrane physically without chemical alteration. This suggested mechanism of action together with multiple cellular targets and

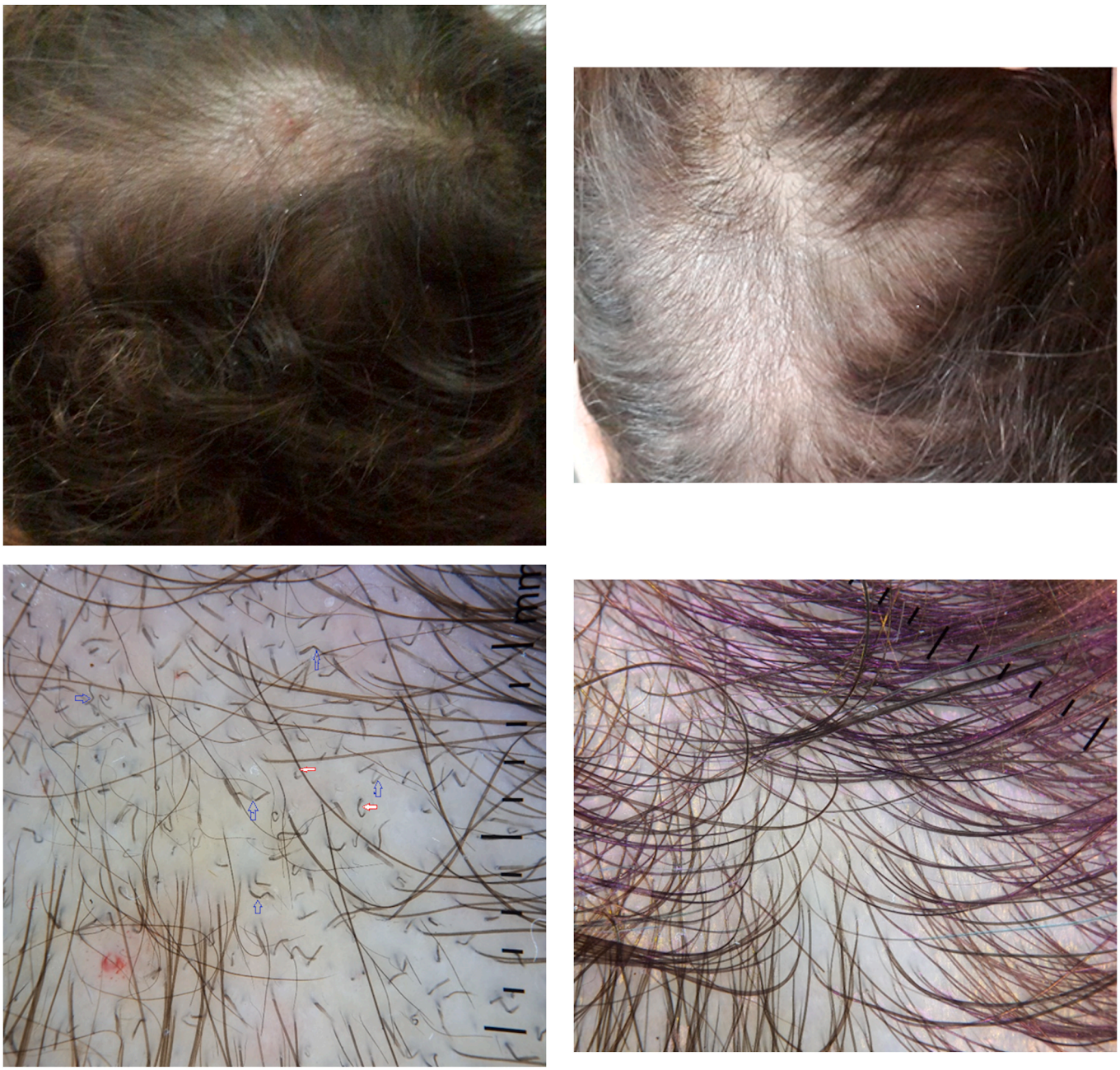

Fig. 4. Patient with tinea capitis subjected to PDT with complete cure after one session; (a) before treatment; showing well-defined grayish-white alopecic patch and mild erythema; (b) 2 weeks after the 1st session showing the disappearance of scaling and erythema, and the appearance of new hair growth; (c) dermoscopy before treatment showing multiple zigzag hairs (blue arrow), comma-shaped hair (white arrow); (d) dermoscopy after cure showing the disappearance of dermoscopic signs and appearance of normal hair. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

antibiofilm activity reduces the chance of developing a resistance [31]. In this study, curcumin was loaded with high encapsulation efficiency in nanospanlastic vesicles that were fabricated from a mixture of nonionic surfactant (Span 80), an edge activator (tween 80), and a penetrating enhancer (Cremophore). The high encapsulation efficiency may be attributed to the lipophilicity of the fabricated vesicles that facilitated the incorporation of a hydrophobic drug such as curcumin [18]. The non-ionic surfactant has formed the vesicles that provided sustained release of the drug. The addition of the edge activator and the penetrating enhancer improved the elasticity of the fabricated vesicles rendering them amenable to squeezing themselves through the tiny pores of the stratum corneum of the skin. Cremophore was found to enhance the skin deposition and the stability of the polymeric mixed micelles loaded with an antifungal drug (itraconazole) [32]. Collectively, the used ingredients enhanced the topical delivery of curcumin. Formulation of NVS-Cur in carboxymethyl cellulose (CMC) gel caused further enhancement of curcumin topical delivery.

Cur-PDT at optimal concentration and light dose can completely inhibit fungal growth via induction of reactive oxygen (ROS) and nitrogen species (RNS), causing fungal death by apoptosis [30]. However, curcumin alone has a minor inhibitory effect on dermatophytes [25]. This was supported by our findings that revealed a mild decrease in scaling in 3 cases of the curcumin group but not a complete cure. On the other hand, griseofulvin is a fungistatic medication that binds to microtubules and inhibits the contraction of the mitotic spindle hence mitosis. By binding to the keratin in keratin precursor cells, it makes

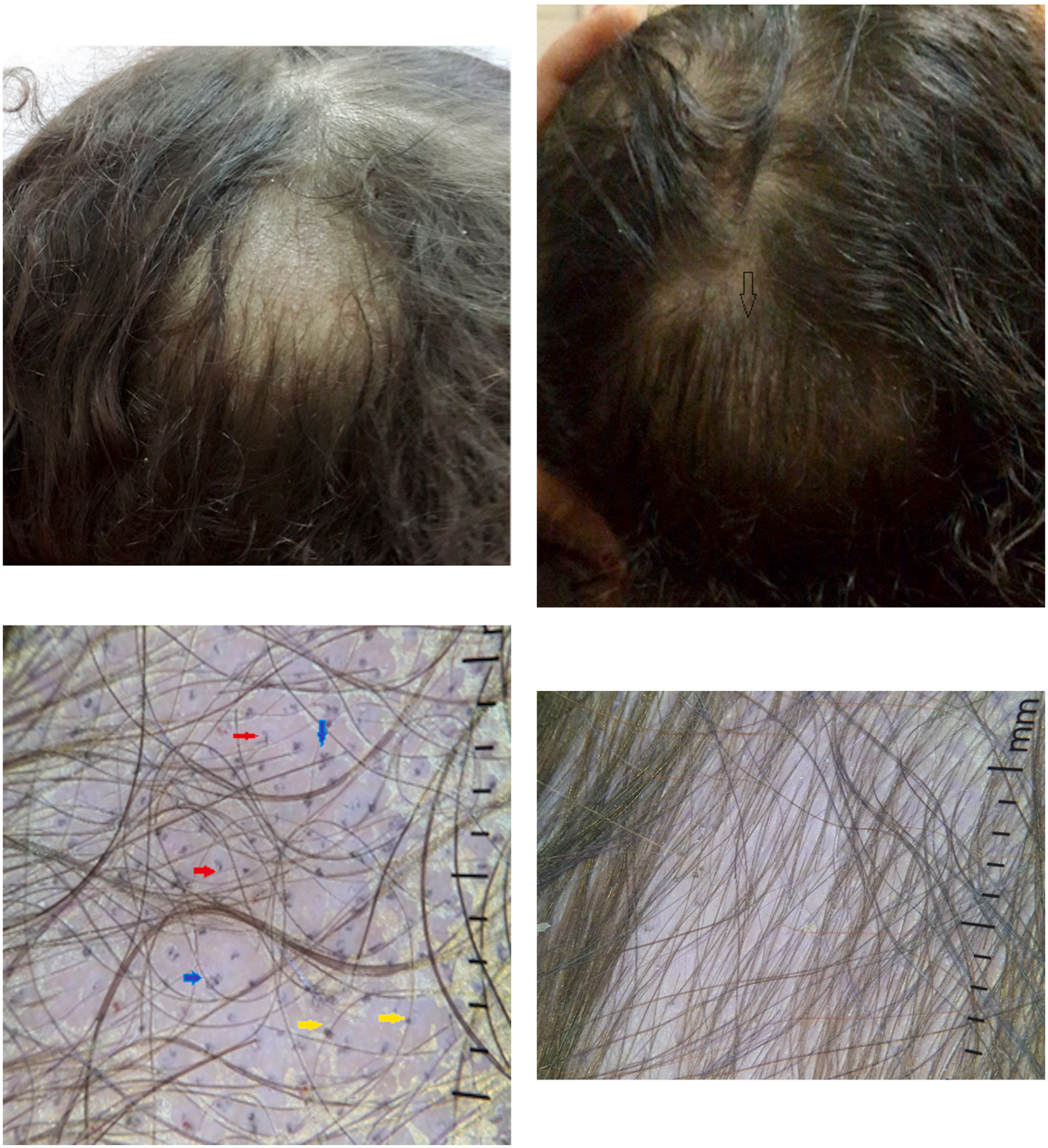

Fig. 5. Patient with tinea capitis subjected to PDT with complete cure after 3 sessions; (a) before treatment showing well-defined alopecic patch with black dots; (b) 2 weeks after the 2nd session showing the appearance of new hair growth; (c) dermoscopy before treatment showing multiple black dots (yellow arrow), comma-shaped hair (red arrow), and corkscrew hair (blue arrow); (d) dermoscopy after cure showing the disappearance of dermoscopic signs and appearance of normal hair. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

them more resistant to fungi. The drug spreads to its site of action only when skin or hair is replaced by keratin-griseofulvin complexes [8].

Our results also revealed some differences in the susceptibility to the photodynamic effects of curcumin. Successful complete cure occurred in Trichophyton species but was not achieved in Microsporum type. These species-related differences are supported by previous studies that tested curcumin's efficacy in vitro against more than 100 bacterial and fungal strains of pathogens belonging to 19 species and found that the efficacy of curcumin varied widely among microbial species and strains with very selective activity [33]. This can be explained by the different structure of the dermatophyte or the need for a higher curcumin concentration and light dose. This was in accordance with the previous in vitro results which reported that the maximum inhibition was for thin-walled T. rubrum conidia and the minimum inhibition was for

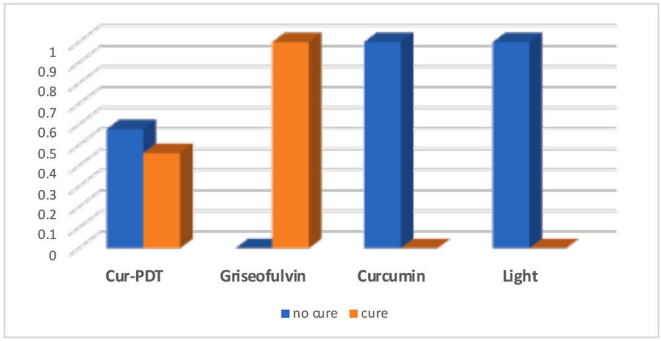

Fig. 6. Comparison between the percentage of cured and non-cured among different treated groups regarding treatment effect.

Table 3
Comparison of treatment effect mean ranks among the different groups using Kruskal-Wallis test.

	Groups	N	Mean Rank	p
Effect	Cur-PDT	13	24.62	**P =0.0001*****
	Griseofulvin	13	37.00	
	Curcumin	10	14.00	
	Light	10	14.00	

N: number, P considered significant if < 0.05.

thick-walled M. gypseum which in turn enhanced by a higher curcumin concentration [25]. Another explanation could be related to the photosensitizer nature.

The photodynamic inhibitory effect was successfully reported in M canis in two in vitro studies. One with hypericin-PDT against planktonic cells and in a murine model [34]. The other was with methylene blue PDT which showed a better response in M canis when compared to Trichophyton species [35]. Susceptibility differences have been reported as well with conventional antifungal treatment where griseofulvin was more effective than terbinafine against M Canis while terbinafine was found to be more effective than griseofulvin against trichophyton species [36].

The total fungal culture results in our study were positive only in 56.5% of cases. Wide variations in culture sensitivity were encountered in the previous studies with sensitivity values ranging from 23% to 84.6%. According to the meta-analysis of Agudelo et al., 2017, the overall sensitivity was 56% which is nearly similar to our results [37]. Levitt et al., 2010 considered using KOH or fungal culture as gold standards for diagnosis was problematic because they found that the culture could be negative even in the presence of active disease this was supported by his results where 59 patients out of 460 had negative cultures at day 28 of the study but turned positive at day 42 and suggested that culture could be used as a complementary test [38]. The possible reason was explained by one study due to the regular use of fungistatic oils [5]. However, another small study suggested a minimal role in this regard [39]. Although conventional culture is specific and sensitive, it has some important drawbacks of the long incubation period of cultivation (4–6 weeks) and development of atypical characteristics of dermatophyte strains which need great expertise and technical experience to accurately identify the species. Moreover, nonvital fungi can lead to false-negative results [40].

Despite the previously mentioned limitation, the causative species can be suggested by the dermoscopic criteria [22, 41, 36]. In the present study, 4 cases had the dermoscopic criteria of Trichophyton tonsurans due to the presence of Corkscrew, comma hair, and absence of the morse code-like hair [36]. The 5th case failed to be determined exactly due to a marked inflammatory reaction that masked the specific dermoscopic criteria. The other evident criteria (bent hair, black dot, and short broken hairs) were not specific nor conclusive to the causative species. This was consistent with the systematic review of Gupta et al. who reported that diagnosis of inflammatory Tinea Capitis can sometimes be difficult as microscopy and even pathology might give negative results [23].

Both griseofulvin and Cur-PDT using NVS-Cur provided a positive impact on the quality of life for cured children and their families. Children and their families were impressed by the disappearance of the lesion and the appearance of new hair growth and felt safe dealing with others with no transmission of infection.

4.1. Limitation

The total fungal culture results in our study were positive only in 56.5% of cases. However, the causative species can be suggested by the

Table 5
Comparison of treatment effect ranks associated with fungal cultures among the different groups.

Groups		culture	N	Mean Rank	
Cur-PDT	Effect	No	6	9.42	0.012
		M canis	6	4.00	
		T violaceum	1	10.50	
Griseofulvin	Effect	No	4	7.00	>0.05 (ns)
		M canis	3	7.00	
		T tonsurans	6	7.00	
Curcumin	Effect	No	5	5.50	
		M canis	2	5.50	
		T tonsurans	3	5.50	
Light	Effect	No	5	5.50	
		M canis	2	5.50	
		T tonsurans	3	5.50	

No: No culture, N: Number, M canis: Microsporum canis, T tonsurans: Trichophyton tonsurans.
This table showed no statistical significance, p > 0.05, except for PDT.

Table 4
Comparison of the mean ranks of TSSS, scales, and erythema before, 2 weeks, and after treatment among each group.

Groups		Mean Rank TSSS	p	Mean Rank Scale	p	Mean Rank Erythema	p
Cur-PDT	Before	2.38	0.004	3	0.0001	1.62	0.11
	2 weeks	2.27		1.54		2.38	
	After	1.35		1.46		2	
Griseofulvin	Before	2.79	0.0001	3	0.0001	1.86	0.002
	2 weeks	2.18		1.75		2.46	
	After	1.04		1.25		1.68	
Curcumin	Before	2.1	0.87	2.8	0.0001	1.7	0.05
	2 weeks	2		1.65		2.15	
	After	1.9		1.55		2.15	
Light	Before	1.9	0.4	2	1	2	1
	2 weeks	2.05		2		2	
	After	2.05		2		2	

TSSS: total signs and symptoms score, P considered significant if < 0.05.

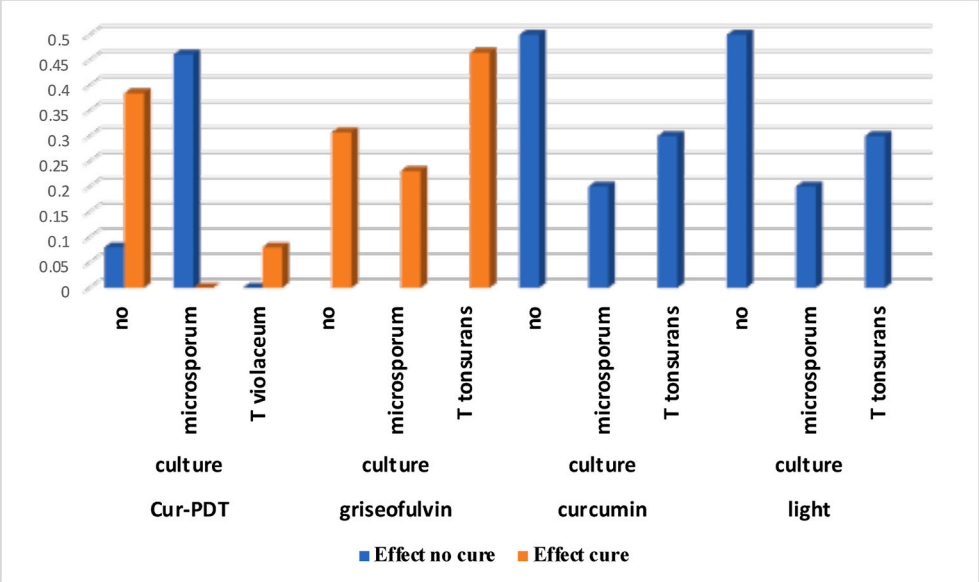

Fig. 7. Comparison between the effect of treatment and the isolated fungal species among different groups. (No: No culture).

dermoscopic criteria.

5. Conclusion

This study proved that Cur-PDT using curcumin loaded in the nano-spanlastics carrier can be a promising alternative treatment for Tinea Capitis. PDT using NVS-Cur is simple and well-tolerated with minimal local side effects. Moreover, PDT has the superiority of shorter duration of treatment than griseofulvin, but with variable response among fungal species. Griseofulvin showed systemic side effects in some cases. Neither curcumin nor blue light alone had a similar therapeutic effect. Further studies with different dermatophyte species, curcumin concentration, light dose, and duration of session are needed to establish the best effective parameters for this technique.

Author contributions

All authors contributed to the study's conception and design. The experimental part regarding the preparation and characterization of the NSV-Cur was performed by [Maha Fadel] and [Doaa A. Abdel Fadeel]. Patients recruitment, data collection, and analysis were performed by [Eman M. Abdullah], [Abeer Tawfik], [Jehan Alsharnoubi], and [Noha Abdallah]. The first draft of the manuscript was written by [Eman M. Abdullah] and [Doaa A. Abdel Fadeel]. All authors revised, commented, and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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