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RESEARCH PAPER

Enhancing acne treatment with novel ternary metal complexes embedded in solid lipid nanoparticles: Development, *in vitro* characterization, and clinical evaluation

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ABSTRACT

Objective(s): The aim of this study is to investigate the potential of Solid lipid nanoparticles (SLNs) to enhance the therapeutic effectiveness of ternary metal complexes of hydroxy acids in the treatment of acne.

Materials and Methods: Ternary complexes of Cu (II) and Zn (II) with glycine amino acid (Gly) as a primary ligand, and Hydroxy acids (salicylic acid (L1), lactic acid (L2) or glycolic acid (L3)) as a secondary ligand, were synthesized in a slightly acidic medium and isolated in different ratios. These ternary complexes were loaded into SLNs and evaluated for particle size, polydispersity index, Zeta potential, entrapment efficiency and *in vitro* release studies. SLN-encapsulated ternary metal complexes were clinically evaluated in acne patients.

Results: Scanning Electron Microscopy revealed that SLNs were spherical in shape and varied in size from 115 to 210 nm when measured with a Malvern Zetasizer. The zeta potential was ranged from -41.33 ± -2.5 to -47.32 ± -2.1 mV. The calculated entrapment efficiency (EE%) was 79 - 83% with slow release of the ternary complexes from the prepared SLNs. The *in-vivo* clinical study disclosed that Zn(L^1)(Gly) SLNs outperformed Cu(L^1)(Gly) SLNs in terms of acne spot reduction and patient satisfaction.

Conclusion: In conclusion, this study demonstrated that SLNs-encapsulated ternary metal complexes are a promising new treatment for acne.

Keywords: *Acne, Clinical study, Salicylic acid, SLNs, Ternary complex*

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INTRODUCTION

Topical administration of several drugs has recently emerged as a viable solution to the challenges posed by biological impediments to drug delivery [1]. The application of nanotechnology in the field of medicine is expected to increase promptly [2, 3]. One advantage of nanomaterials is their small particle size and larger surface area which enables their potential biological applications [4]. Solid lipid nanoparticles (SLNs) are colloidal lipid carriers having exceptional characteristics as biocompatibility, safety, tolerability, stability and ease of manufacturing [5]. Typically, SLNs are composed of surface active agents, co-surfactants and solid lipids [6]. Different preparation approaches can be implemented, such as high-pressure homogenization, solvent emulsification or solvent evaporation [7]. For the time being, many scale-up techniques for the manufacturing of SLNs are applied. These methods include, hot melt extrusion [8], high-pressure homogenization, precipitation by mixers, microchannels, and microemulsion evaporation [9]. The therapeutic effectiveness of many drugs can be enhanced when formulated as SLNs [10]. Because of their propensity to cross the blood-brain barrier, they can be employed to target brain illnesses [11]. SLNs provide tailored anticancer drug delivery and may

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increase the efficacy of the existing treatments [12]. They can treat antimicrobial resistant infections successfully [13]. SLNs can be prepared for effective ophthalmic drug delivery [14]. They also have the capability to be utilized in the fields of dermatology and cosmetics [15]. Acne is one of inflammatory skin conditions that has been widely investigated in terms of disease pathogenesis and treatment approaches [16]. Hyperkeratinization, obstruction of sebaceous follicles, and microbial colonization of pilosebaceous, which promotes perifollicular inflammation are the major pathogenic factors of Acne [17]. Many research studies have demonstrated that zinc (Zn) can help cure acne vulgaris [18]. The proposed mechanism of zinc in management of Acne is by inhibiting Propionibacterium acnes lipases and levels of free fatty acids, consequently inhibiting the inflammation of the patient's skin [19]. Copper (Cu) acts as an antioxidant and can treat Acne by neutralizing the hazards of free radicals [20, 21]. Metals have long been utilized to treat a variety of ailments, but their toxicity and limited absorption were the major restrictions [22]. The effects of alpha-hydroxy acids (glycolic acid, lactic acid) and beta-hydroxy acids (salicylic acid) on acne were investigated [23]. Salicylic acid has keratolytic action when applied in the appropriate concentration [24, 25]. Salicylic acid is also recognized as an effective ingredient in acne treatments due to its antibacterial properties [25]. The ternary complexes of Zn or Cu with amino acids and hydroxy acids have shown considerably increased antibacterial activity against microbial strains when compared to hydroxy acids or metals alone [26]. The goal of this study is to prepare ternary complexes of different trace metals with glycine as a primary ligand, and different hydroxy acids as secondary ligand which will then be loaded into solid lipid nanoparticles. SLNs are assumed to protect the metal ternary complexes from chemical degradation, improve their stability, control their release, and enhance therapeutic efficacy.

MATERIALS AND METHODS

Materials

Copper Carbonate, Zinc Carbonate, glycolic acid, lactic acid, Salicylic acid, Glycine, Ethanol (Merck, Darmstadt, Germany). Cetyl palmitate, Polysorbate 80 (Sigma-Aldrich St Louis, MO, USA), Germaben™ II (propylene glycol, methylparaben, propylparaben, and diazolidinyl urea) (Ashland, Germany).

Methods

Production of ternary metal complexes

The ternary complexes of metal carbonate, salicylic acid, lactic acid or glycolic acid and glycine in a molar ratio of 1:1:1 were prepared according to the previously published technique [26]. Briefly, the mixture consisting of Zn or Cu carbonate, hydroxy acid and glycine was heated to 80-90 °C for two hours. Then, ethanol was added till formation of thick precipitant and then filtered. The residue was rinsed with absolute ethanol and desiccated in an oven at 80-90 °C.

Preparation of solid lipid nanoparticles of the Cu (II) and Zn (II) ternary complexes

Solid lipid Nanoparticles dispersions were prepared by hot homogenization technique using high pressure homogenizer (EmulsiFlex™ - C5, Avestin Europe GmbH, Germany). The selected lipid (Cetyl palmitate) was heated to 85 °C. The surfactant (Polysorbate 80) was dispersed in the melted lipid till formation of clear dispersion. The aqueous phase containing 1% of ternary copper metal Glycinate complexes ($Cu(L^1)(Gly)$, $Cu(L^2)(Gly)$ or $Cu(L^3)(Gly)$), or Zinc metal Glycinate complexes ($Zn(L^1)(Gly)$, $Zn(L^2)(Gly)$ or $Zn(L^3)(Gly)$) was heated to 85 °C and was incorporated to the melted lipid under continuous stirring then the preservative (GermabenTM II) was added. The resulted dispersions were subjected to high pressure homogenizer for 5 cycles. The pressure was set at 500 bar. The developed dispersions of ternary complexes loaded nanoparticles were kept at refrigerator (2–8 °C) for further investigations.

Characterization of solid lipid nanoparticles

Transmission electron microscopy

Transmission electron microscopy (TEM) images were recorded using Transmission Electron Microscope (Jeol, USA, Model JEM2100). Ternary complexes loaded SLNs were diluted appropriately with distilled water. Few drops of the dispersion were placed on Cu grid, and then the image was captured.

Particles size and Zeta potential of the ternary complexes loaded-SLN

The dynamic light scattering technique was used to estimate the mean particle size, zeta

potential and the polydispersity index of the particles. The samples were diluted with distilled water and measured using a Malvern Zetasizer instrument (Serial Number: MAL500962, Malvern Instruments, U.K.) at 25 °C.

Entrapment efficiency measurement

The Cu (II) and Zn (II) ternary complexes content of the nanoparticles was determined by Atomic Absorption. The supernatant was separated by centrifuging the SLNs dispersions at 14000 rpm at −4 °C. The drug entrapment efficiency (EE) was calculated by the following equation:

$$EE = \frac{W_1 - W_2}{W_1} x100 \qquad \text{Equation 1}$$

where W1 is amount of drug added in the formulation and W2 is the amount of drug in the supernatant.

In-vitro release of the Cu (II) and Zn (II) ternary complexes from the loaded solid lipid nanoparticles

In-vitro release study was performed using apparatus 5 (paddle over disk) at 37 + 0.5 °C for 24 hr. The specified amount of SLNs from each formulation was placed in the disk. The dialysis membrane (Cellulose Ester (25 kDa)) was used to cover the disc. The receptor vessel was filled with 900 mL of phosphate buffer saline (PBS) pH 6.8. The rotation speed was set at 100 rpm. Five mL samples were withdrawn from the release medium and substituted with the equivalent volume of fresh medium. The samples were withdrawn after 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hr. The release kinetics of the selected formulae were assessed by fitting the data to zero-order and diffusion models using Excel spreadsheet. All experiments were performed in triplicate and statistically evaluated.

Assay of metals

Atomic Absorption Spectrometry was used to quantify the percentage of Cu and Zn, using a Perkin Elmer AAS 3100. A specific weight of the complex was continually heated with concentrated nitric acid almost to dryness. The metal nitrate produced was dissolved in distilled water and the solution was brought to volume in a measuring flask. The metal hollow cathode lamp belonging to the metal concerned is placed in the operating position. The current was adjusted to the recommended value and λ_{nm} is selected to give the maximum absorption using the appropriate monochromator slit width. The absorbance of the metal was determined and the concentration of the metal was calculated from the constructed calibration curve [27].

Stability of formulations

The stability of the selected formulations was carried out on fresh and stored samples (for 12 months at refrigerator (2–8 °C) by thermogravimetric analysis using a Perkin-Elmer TGA 7 thermal analyzer (Waltham, MA 02451, USA). The measurements were carried out under nitrogen atmosphere at a heating rate of 10 °C/min.

Clinical study

Study design

This clinical study is randomized open-label, prospective, within-subjects design. The study protocol was reviewed and approved by the Ethical Committee of Faculty of Pharmacy, Cairo University.

Sample size calculation

The sample size was calculated using SAS® OnDemand software, considering that there are two sample means with expected mean ratio = 1, CV = 0.3 and power = 0.9.

Demographic data of participants

Eighty participants of both sex who had previously been diagnosed with acne vulgaris were selected to be enrolled in the study and randomized into either Zn(L^1)(Gly) (N = 40) arm or Cu(L^1)(Gly) (N = 40) arm. Their age, body mass index, weight, height, and vital signs were recorded. All participants were required to submit their informed written consent, duly signed, before starting the study.

Inclusion and exclusion criteria

A clinical evaluation for skin conditions was performed by the dermatological investigator. Personal information, a description of symptoms, and details of past medical history (family history of acne) are all included in the baseline examination. Following that, all potential participants were subjected to a clinical evaluation and a complete skin examination to capture baseline open or closed comedones, erythematous papules and pustules, cysts, or nodules. The Exclusion criteria include patients with previous systemic illnesses

requiring long-term treatment, patients with genetic and/or endocrine problems, participants who decline to provide informed written consent, pregnant and/or nursing women or women using birth control tablets or hormonal intrauterine devices.

Clinical evaluation

Each participant was given one of the selected formulations of SLNs of the ternary metal complexes along with a mild soap bar to maintain comparable conditions that may impact the facial skin. Furthermore, participants will be advised to refrain from applying other skin creams or products which may affect the facial skin.

Participants were advised to wash their face using the mild soap bar, at least 10 min prior to the application of the anti-acne preparation. Participants were then be advised to apply the anti-acne preparation on the affected areas, twice daily for a period of 6 weeks. They were asked to visit the dermatologist every 3 weeks and return on Day 42 (After 6 weeks) for follow-up evaluation. During each visit, high-definition digital photos of both sides of the face were captured using consistent settings for uniformity.

Evaluation questionnaires were completed by the dermatologist including the parameters which have to be measured and assessed such as the number of blackheads and whiteheads per 3 cm^2 and participants' satisfaction level based on specific criteria as reduction in the number of blackheads and whiteheads, moisturizing and soothing effect of the formulations and an overall healing of acne. The information gathered from the clinical investigation and from the questionnaires were statistically evaluated using SPSS 16.0 statistical software.

RESULT AND DISCUSSION

Transmission electron microscopy

Fig. 1 depicts the results of TEM imaging of the ternary complexes of Cu and Zn-loaded SLN, indicating that the particles had nanometer-size spherical shapes. The particle size ranged from 100 to 200 nm.

Particle size and Zeta potential

Particle size and Zeta potential were determined using Malvern Zetasizer following these simple procedures; about 1 mg of SLN was dispersed in 1 mL of distilled water by sonication and then was subjected to Zeta potential analyzer. The zeta potential is an important and practical tool for determining particle surface charge, which could be used to predict and control the stability of colloidal suspensions. Because charged particles repel one another and thus overcome the natural tendency to aggregate, the higher the zeta potential, the more likely the suspension will be stable due to electric repulsion. The prepared SLNs are negatively charged. The negative charge of the lipid, combined with the steric stabilization of polysorbate 80, would increase the physical stability of the prepared SLN formulations. The zeta potential of ternary complexes of Cu (II) and Zn (II) Solid lipid nanoparticles **r**anged from -41.33 ± -2.5 to -47.32 ± -2.1 mV. The particle size was also measured by Malvern Zetasizer. Their size was determined to be in the colloidal range of 115 nm to 210 nm as presented in Table 1.

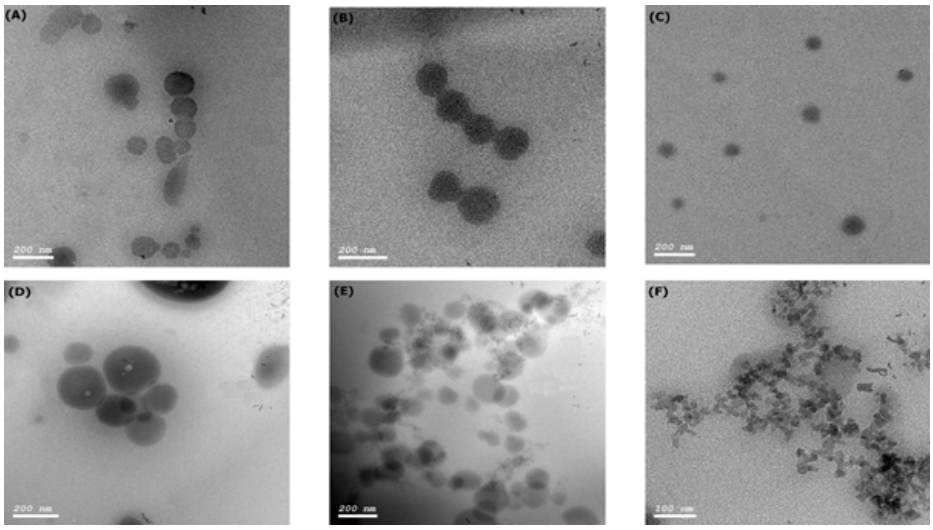

Fig. 1. Transmutation electron micrograph of different ternary metal complexes of hydroxy acid-loaded SLNs (A) [Cu (L^1)(Gly)], (B) [Cu (L^2)(Gly)], (C) [Cu (L^3)(Gly)], (D) [Zn (L^1)(Gly)], (E) [Zn (L^2)(Gly)], (F) [Zn (L^3)(Gly)]

Table 1. Characteristics of different ternary metal complexes of hydroxy acid-loaded solid lipid nanoparticles

SLN Formulation	Particle size, nm + SD	% EE + SD	Zeta potential, mV + SD
Cu(L^1)(Gly) SLN	200.0 + 3.0	80.0 + 6.9	-41.33 + 2.58
Cu(L^2)(Gly) SLN	195.0 + 7.5	79.0 + 19.3	-45.53 + 7.58
Cu(L^3)(Gly) SLN	202.0 + 46.5	81.0 + 7.8	-47.32 + 6.30
Zn(L^1)(Gly) SLN	210.0 + 8.4	82.5 + 8.7	-41.54 + 1.56
Zn(L^2)(Gly) SLN	200.0 + 40.2	83.0 + 14.6	-46.11 + 10.45
Zn(L^3)(Gly) SLN	115.0 + 31.6	80.3 + 18.3	-47.12 + 6.66

These findings were corroborated by transmission electron microscopy results. Statistically there is no significant difference among all formulations regarding particle size and zeta potential when tested by Analysis of Variance (ANOVA).

Entrapment efficiency of SLN (% EE)

The drug entrapment efficiency was calculated to be between 79.0 and 82.5%. ANOVA testing showed there is no significant difference between all formulae (P>0.05). The drug's solubility in melted lipid, the chemical and physical structure of the solid matrix, and the polymorphic state of the lipid all contribute to the drug's %EE in the lipid nanoparticles. The unentrapped drug that could be solubilized in the water surfactant phase made up the remaining percentage.

In-vitro drug release

In-vitro release samples were analyzed by Atomic Absorption after suitable dilution. The percentage of ternary complexes released from the SLNs was plotted as a function of time. The release profile of the ternary complexes was extended for 24 hr as shown in Fig. 2 and 3.

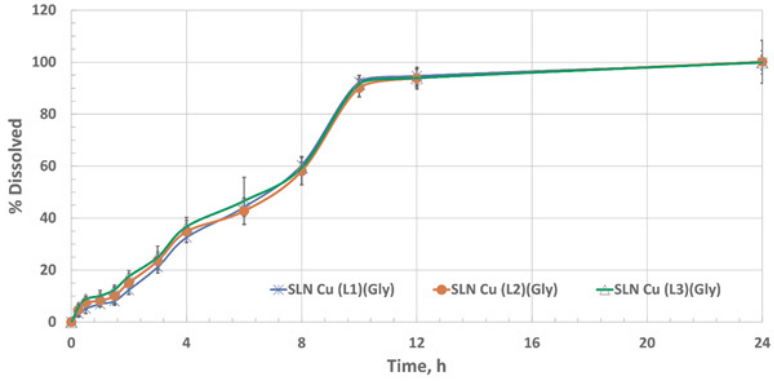

Figure 2. *In-vitro* drug release of Cu (II) complexes from SLNs

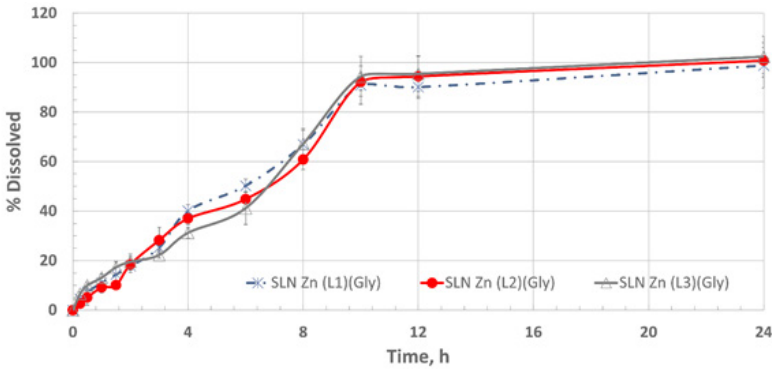

Fig. 3. *In-vitro* drug release of Zn (II) complexes from SLNs

The drug release rate was increased with time until it reached almost a plateau 10 hours later. There was no burst release, implying consistent encapsulation of ternary complexes in the solid lipid matrix [28].

The slow release of the ternary complex from the lipid nanoparticles can be explained due to its high solubility in the lipid components of the particles which account for its lipophilic nature [29]. There is no statistical difference (P>0.05) between the release data of all SLN formulations.

The *in-vitro* release data were fitted to zero-order ($M_t = M_0 + k_0.t$) and diffusion models ($Q = kH.t^{1/2}$) and to the Korsmeyer–Peppas equation ($\frac{M_t}{M_\infty} = k.t^n$) (to assess the release kinetics of the selected formulae, Zn(L^1)(Gly) and Cu(L^1)(Gly).

Where "M_t" denotes the amount released at the time "t", "M_o" is the initial amount of drug at zero-time, "M_∞" is the total amount of the entrapped complex, "Q" indicates the amount of drug released in time "t" per unit area, "k" is the release rate constant and "n" is the Korsmeyer–Peppas exponent [30].

The proposed mechanism of the release of Zn(L^1)(Gly) from the core of the particles is non-Fickian diffusion. This is demonstrated by the higher coefficient of determination (r^2 = 0.9622) compared to r^2 = 0.9002 for Zero-order kinetics. Also the Korsmeyer–Peppas exponent "n" was calculated to be 0.5911 which falls between 0.45 and 0.89, indicating anomalous, non-Fickian diffusion [31]. The same was concluded for the release of Cu(L^1)(Gly) as it showed greater r^2 (0.9483) indicating diffusion mechanism and also "n" = 0.6617 signifying anomalous, non-Fickian diffusion.

Although there is no statistical difference between ternary complexes of salicylic acid loaded SLNs and other hydroxy acid complexes, Zn (L^1) (Gly) and Cu (L^1)(Gly) SLNs were chosen for further investigations due to the superior chemical and physical stability of the SLNs formulations after storage for 12 months.

The assessment of the selected formulations revealed that there were no statistically significant differences observed between the fresh and stored samples in terms of physical or chemical stability. This was evidenced by the consistent maintenance of particle size, zeta potential, entrapment efficiency, physical state, and chemical drug content over the course of the storage period.

Also, it was reported that the salicylic acid peel lasted longer and had less side effects than glycolic acid [32]. Higher concentrations of lactic acid were needed to give the same keratolytic effect than lower concentrations of salicylic acid [33].

Clinical study

The proposed protocol for conducting a prospective, within-subjects clinical trial was set to evaluate the clinical efficacy of anti-acne preparation of Zn(L^1)(Gly) SLN and Cu(L^1)(Gly) SLN in the management of mild to moderate acne vulgaris.

With this design, the outcomes of one group before and after application of the therapy were typically compared without comparison to a negative control group for the benefit of the patients. This protocol was in accordance with the International Conference on Harmonization's Guidelines for Good Clinical Practice [34].

Salicylic acid is usually applied topically as peeling agent, it can get rid of lipids between cells, diminish keratinocyte adhesion, and promote loosening and ensuing separation of these cells [35]. Salicylic acid is used in many skin conditions such as scalp psoriasis [36], dandruff [36], seborrheic dermatitis [37] and acne [38]. Salicylic acid also possesses fungicidal properties and is used topically in the treatment of dermatophyte skin infections [39]. Topically administered salicylic acid has the potential to induce local adverse effects such as erythema, exfoliation, crusting, dryness, pigmentary dyschromias, or contact sensitization, as well as severe systemic side effects such as hypoglycemia or systemic toxicity (salicylism) [40, 41]. Chemical modification of salicylic acid postulates that the ternary complexes may display higher levels of activity [42, 43] and stability [44] with less side effects.

By comparing the data collected before and after application of Zn(L^1)(Gly) SLN, the mean number of acne spots before treatment is greater than after treatment and is statistically significant (P<0.05). The number of acne spots on the face decreased markedly as shown in Fig. 4. The comparable results were obtained after application of Cu(L^1)(Gly) SLN as presented in Fig. 5. Based on the statistical data acquired before and after treatment, it is reasonable to assume that the therapy is unquestionably successful.

It is obvious that Zn(L^1)(Gly) SLN is more efficient than Cu(L^1)(Gly) SLN. The average acne reduction of Zn(L^1)(Gly) SLN was 10.55 to 2.13 / 3 cm^2 with 79.81% reduction in number of spots. Cu(L^1)(Gly) SLN had an average acne reduction of 8.90 to 3.65 / 3 cm^2 with a 58.99% reduction in the number of acne spots.

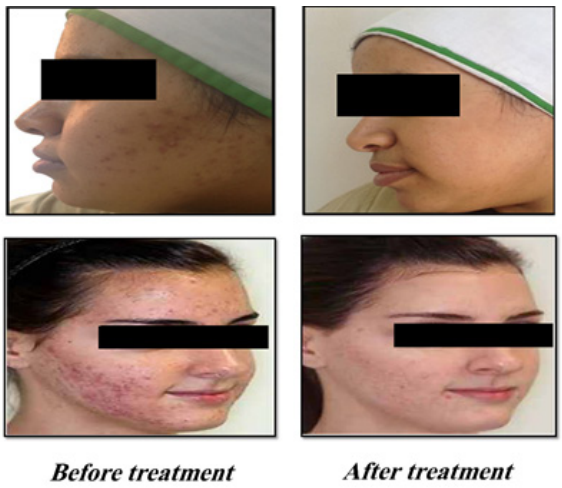

Fig. 4. Photos of participants before and after treatment with Zn(L^1)(Gly) SLNs

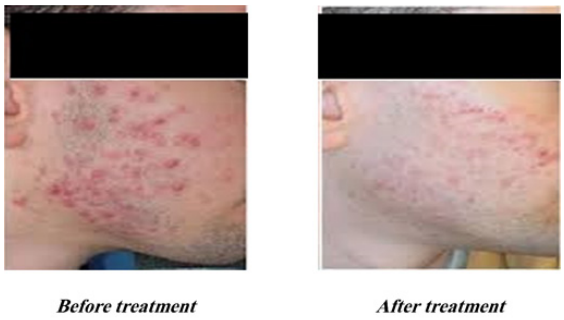

Fig. 5. Photos of participants before and after treatment with Cu(L^1)(Gly) SLNs

According to the assessment survey it was found that 95% of patients treated with Zn(L^1)(Gly) SLN experienced moisturizing and soothing effect on their faces upon regular application of the formulation. The majority of participants (85%) were satisfied with the treatment of acne using Zn(L^1)(Gly) SLN formulation, showing overall healing of acne without leaving dark spots whereas 7.5% of them were unsatisfied. For patients treated with Cu(L^1)(Gly) SLN, it was found that 62.5% had noticed a significant reduction in the number of blackheads and whiteheads. About 67.5% were pleased with the treatment, with 17.5% neutral in their opinion while 15% of them were frustrated.

CONCLUSION

Based on the findings, it is possible to conclude that salicylic acid could interact with Zn or Cu along with amino acids to form ternary complexes, which can then be formulated in solid lipid nanoparticles. Primary clinical data suggests that SLNs of Zn ternary complex are safe and effective in treating acne in the face region in most of participants when used for at least 6 weeks. However, this study has limitations as small sample size, short-term study duration, and a lack of a placebo control group. Further research including clinical studies on greater number of patients with various types of acne including severe cysts or nodules affecting different body regions is warranted.

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DATA AVAILABILITY

Data will be provided upon request.

FUNDING

The authors declare that there is no outsource funding of this project.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests in this study.

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