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Development of Tea Tree Oil Based Nanoemulgel Loaded with Azithromycin for Enhancing the Antibacterial Activity

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Abstract: Azithromycin (AZ) is an azalide macrolide antibiotic that is frequently employed for treating bacterial skin infections. It suffers from limited oral bioavailability, which results from incomplete absorption or extensive first-pass metabolism. Therefore, preparing azithromycin formulations for topical administration is highly recommended to avoid first-pass metabolism and to boost the concentration of the drug on the skin. The objective of our investigation was to formulate and evaluate the efficacy of AZ-loaded nanoemulgel as an antimicrobial drug. The physical appearance, spreadability, viscosity, particle size, in vitro drug release, ex vivo permeation investigations, and antimicrobial efficiency of the prepared formulations were evaluated. The prepared formulation loaded with AZ exhibited good physical quality. AZ-loaded nanoemulgel had a greater ex vivo drug permeation across rabbit skin than other formulations (AZ-loaded gel and AZ-loaded emulgel), revealing improved drug permeation and greater transdermal flux in addition to enhanced antibacterial efficacy ($p < 0.05$). Overall, our findings imply that tea-tree-oil-based nanoemulgel would be a promising delivery system for enhancing the antimicrobial efficiency of azithromycin.

Keywords: Azithromycin; anti-bacterial activity; nanoemulgel; emulgel; tea tree oil

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1. Introduction

E. coli is a Gram-negative bacterium that is typically found in feces and the lower intestine of warm-blooded animals. The most common cause of food- and water-borne diarrhea in people is *E. coli*. Moreover, *E. coli* is a frequent reason for infection in surgical wounds, especially those following abdominal operations, where it is frequently found combined with other gut bacteria. Compared to a number of multi-drug resistant bacteria (MDR), *E.coli* (*Escerichia coli*) strains have developed progressively stronger resistance to antibiotics [1]. Rai et al. argue that infections caused by MDR bacteria are more challenging to treat and need broad-spectrum antibiotics, which are harmful and expensive [2].

Azithromycin is an azalide, which is a subclass of macrolide antibiotics with a broad spectrum that has bacteriostatic activity against a variety of Gram-negative and Gram-positive bacteria [3]. Azithromycin inhibits the synthesis of bacterial proteins by blocking both the assembly of the 50S ribosomal subunit and the transpeptidation/ translocation process of protein synthesis [4]. Unlike erythromycin, azithromycin has a methyl-substituted nitrogen atom in the macrolide ring; this property makes it more stable, tolerated, and effective than erythromycin [5]. Azithromycin has limited oral bioavailability, which results from incomplete absorption or extensive first-pass metabolism [6]. Therefore, preparing azithromycin formulation for topical administration is highly recommended to avoid systemic side effects, including nausea, diarrhea, and abdominal pain. It also avoids first-pass metabolism, enhances patient acceptance, and reduces dose via direct contact with the pathologic site [7].

Essential oils are natural compounds derived from natural sources, and because of their efficiency and safety, they have been widely used in many formulations [8]. Tea tree oil is an essential oil that is derived from the leaves of *Melaleuca alternifolia*, a small tree that is indigenous to New South Wales and Queensland, Australia [9]. It is commonly known as melaleuca oil, which refers to its place of origin. Tea tree oil demonstrated broad-spectrum action, including effects against bacterial and fungal skin conditions, preventing infection, and promoting healing [10]. It demonstrates anticancer and antioxidant activity, in addition to its known antimicrobial and disinfecting activity [11], which can be attributed to terpoen-4-ol, which is the major constituent of tea tree oil. The formulation of this essential oil in a suitable drug delivery system could be beneficial because of the several advantages of tea tree oil.

Many pharmacological substances, especially those that are poorly water-soluble and have a narrow therapeutic index, have been transported via the skin using the transdermal drug delivery system (TDDS) [12,13]. TDDS enables the pharmacological agent to cross the epidermis reach the circulatory system, then be transported to the body via the bloodstream [14]. The transdermal administration route may offer benefits, such as lengthening the duration of action, avoiding first-pass hepatic metabolism, improving pharmacological efficacy, reducing undesirable side effects, and enhancing patient compliance. [15]. Therefore, the transdermal system development for the treatment of numerous clinical disorders, such as skin infection, has a feasible potential.

TDDSs include transdermal patches, gel, emulgel, and nanoemulgel [16]. Conventional dosage forms, including ointments, creams, gels, emulsions, and emulgels, have limited therapeutic applications due to the low drug permeability through the skin because of their large particle size. Therefore, the nanoemulgel concept was developed as an approach to solving the permeability issue [17]. Nanoemulgels effectively help the delivery of both lipophilic and hydrophilic medications, compared to hydrogels, which have a limitation in the transportation of lipophilic pharmaceuticals [18]. Additionally, nanoemulgels have a larger surface area than emulgels, which permits the rapid permeation of the drug through the pores of the skin and more effective transport of the drug into the circulatory system.

In the current study, we investigated the effectiveness of a nanoemulgel in strengthening the antimicrobial activity of azithromycin transdermal administration. The inclusion of azithromycin into nanoemulgel effectively increases its percutaneous penetration, which, in turn, enhances its antibacterial activity against skin infection. As far as we are aware, there has not been much information regarding the combination of tea tree oil and azithromycin in a nanoemulgel formulation meant to treat skin infections. The combination of azithromycin and tea tree oil into a nanoemulgel formulation intended for skin infection.

2. Materials and Methods

2.1. Materials

Tea tree oil was obtained from NOW® Foods, USA. Azithromycin (AZ), hydroxypropylmethyl cellulose (HPMC) ethyl alcohol, PEG 400, and Tween 80 were purchased from Sigma-Aldrich (St Louis, MO, USA).

2.2. Formulation of Topical Preparations Loaded with Azithromycin

2.2.1. Formulation of Gel

A suitable amount of HPMC (4% *w*/*w*), a synthetic gelling agent [19], was added to water, and the mixture was agitated until the formation of a hydrogel base using a magnetic stirrer. A known quantity of the medication was mixed with ethanol and vortexed for five minutes. To create a uniform gel, the initial mixture was subsequently added to the prepared hydrogel base [20].

2.2.2. Emulgel Preparation

Emulgel is a type of gelled emulsion made by adding gelling agents to an emulsion. For the oily phase preparation of emulsion, a known quantity of Azithromycin was dispersed in tea tree oil. The aqueous phase was created by adding the weighted amount of Tween 80 (surfactant), PEG 400 (co-surfactant), and ethanol (solvent) to water. This mixture was vortexed for five minutes. The primary emulsion was then created by continuously stirring the aqueous phase with the oily phase. On the other hand, as was previously noted, a gelling agent was used to prepare the gel base. A homogeneous emulgel was developed by adding the previously prepared emulsion that was loaded with AZ to the gel base and thoroughly mixing it for five minutes [21].

2.2.3. Nanoemulgel Preparation

For the nanoemulgel, the nanoemulsion is mixed with a gelling agent. The same method that was used to create the emulsion loaded with AZ was also applied here, followed by the homogenization with a homogenizer mixer for 10 min at 6000 rpm (20 high-speed digital, Ika-Eurostar, Staufen, Germany) to obtain nanoemulsion. A homogenous AZ-loaded nanoemulgel was created by adding the AZ-loaded nanoemulsion to the hydrogel base (4% HPMC) and blending for 10 min. The different topical AZ-loaded formulations' compositions are outlined in Table 1.

Table 1. Constituents of 2% *w*/*w* AZ-loaded formulations.

Formulation	Azithromycin (%*w*/*w*)	Gelling Agent (%*w*/*w*)	Tea Tree Oil (mL)	Ethyl Alcohol (mL)	Tween 80 (mL)	PEG400 (mL)	Water Up to (mL)
Gel	2	4	_	1	_	_	50
Emulgel	2	4	5	1	1	1	50
Nanoemulgel	2	4	5	1	1	1	50

2.3. Characterization of the Formulated AZ-Loaded Topical Formulations

2.3.1. Organoleptic Assessment

The color, homogeneity, and appearance of the topical preparation loaded with azithromycin were examined visually. Moreover, pH values were checked at RT, utilizing a pH meter (PCT-407, Taipei City, Taiwan) [22].

2.3.2. Viscosity

The Brookfield viscometer (Model DV-II, USA, Spindle number 06 at 10 rpm, at 25 °C) was used to assess the formulations' viscosity. The viscosity was measured in triplicate [23].

2.3.3. Spreadability Measurement

The spreadability regulates the area on the skin where the formulation can spread freely after use. One gram of the developed preparations was sandwiched between two glass slides under a standard load. The spreadability value was determined by calculating the formulations' spreading areas diameter [21].

2.3.4. Extrudability Determination

The extrudability is the weight in grams required to extrude a preparation from a collapsible tube of at least a half-centimeter ribbon in 10 s. Ten grams of the AZ-loaded formulations was packaged in a collapsible tube, and the preparation was forced out when the cap was removed by pressing the tube's crimped end [24]. The following equation was used to determine the extrudability (g/cm^2):

$$\text{Extrudability} = \frac{\text{Applied weight to extrude 0.5 cm ribone like gel from collipsible tube in 10 seconds (Gram)}}{\text{Area } (cm^2)}$$

2.3.5. Size and Size Distribution

The polydispersity (PDI) and particle size of emulgel and nanoemulgel loaded with AZ were measured using Malvern Zetasizer Apparatus (Worcestershire, UK). The assessments were carried out at a scattering angle of 90° and 25 °C [25].

2.3.6. Morphological Assessment

One milliliter of distilled water was employed to dilute ten-milligram samples, and then, a sample of one drop was coated with gold after being dried at room temperature and checked using a scanning electron microscopy [21].

2.3.7. Determination of Drug Content

Half a gram of the generated formulations was dissolved in 100 mL of PBS, pH 7.4, and stirred continuously for a half-hour. The final product was filtered through Whatman filter paper. The ultraviolet-visible spectrophotometer was used to detect the solution's absorbance at 232 nm, and the following equation was used to determine the amount of AZ included [22].

$$\text{Drug content percentage} = \frac{\text{Drug actual amount in the formuation}}{\text{Drug theoretical amount in the formulation}} \times 100$$

2.4. In Vitro Release of Azithromycin-Loaded Preparations

Using the previously described process by Abdallah et al. [22], with certain modifications, USP dissolution apparatus II was used for assessing the azithromycin in in vitro release profiles. Briefly, a half gram of the generated preparation containing ten milligrams of AZ was placed within glass tubes with a cellophane membrane (MWCO 14,000) attached to one end. The tubes were attached to the dissolution apparatus, and they were permitted to revolve in 100 mL of phosphate buffer at 50 rpm while maintaining a temperature of 37 ± 0.5 °C (pH 7.4). Samples were taken out at predetermined intervals and replaced with fresh media to maintain the sink condition throughout the investigation. Spectrophotometry was used to analyze samples at a maximum wavelength of 232 nm [26].

2.5. Stability Studies of the Developed AZ-Loaded Formulations

The samples were kept in tightly closed containers for three months at 4 ± 0.5 °C in the refrigerator and at 25 ± 0.5 °C. The samples' physicochemical properties and in vitro drug release at the predefined duration were assessed.

2.6. Ex Vivo Skin Permeation Experiment

2.6.1. Rabbit Skin Preparation

Skin permeation experiments were conducted using white albino male rabbit skin because of its comparatively low cost and availability. An electric clipper was used to shave the white albino male rabbits' abdominal skin. The abdominal skin was detached, and the adipose tissue was then removed after scarifying the animals. The skin samples were stored at 4 °C overnight in PBS (pH 7.4).

2.6.2. Azithromycin Permeation from Various Formulations

Using locally fabricated diffusion cells, a skin permeation investigation of AZ was conducted through male albino rabbits' skin [22]. The donor compartment faced the stratum corneum side of the mounted rabbit skin, which was placed in between the donor and receptor compartments. Half a gram of the formulation corresponding to 2.5 mg of AZ was applied to the skin in the donor compartment. The receptor medium, which contained PBS pH 7.4 (100 mL) and sodium azide (0.02% *w/v*), was constantly swirled at 100 rpm while being maintained at a temperature of 37 ± 0.5 °C using the dissolution apparatus II [27]. At regular intervals, samples (5 mL) were collected and analyzed spectrophotometrically at λ_{max} 232 nm, using the appropriate receptor media as a blank to prevent any interference. To keep a constant volume of receptor media, an equal volume of fresh buffer was added. Ex vivo permeation characteristics of AZ through rabbit skin were estimated, including

$$J_{ss} = \frac{Amount\ of\ permeated\ drug}{area\ of\ permeation\ X\ time}$$

$$ER = \frac{J_{ss\ (test)}}{J_{ss\ (control)}}$$

where J_{ss} is steady state transdermal flux (μg/cm^2.h), and ER is the enhancement ratio.

2.7. Antibacterial Investigation

The antimicrobial efficiency of the generated nanoemulgel loaded with AZ against *Escherichia coli* (*E. coli*), Gram-negative bacteria was evaluated utilizing the agar diffusion technique according to Zafar et al. with some adjustment [28]. Initially, the Petri dishes were sterilized at 160 °C for 60 min in a hot-air oven [29]. Afterward, ten milliliters of sterile nutrient agar media, sterilized in an autoclave at 121 °C for 15 min, were added to each plate. The microorganism was introduced into the plates after solidification under aseptic conditions [28]. A cork borer was used to make three millimeter-diameter cups in the plate, and the samples (placebo nanoemulgel, AZ-loaded gel, and AZ-loaded nanoemulgel) were then inserted to test their effectiveness [30]. The Petri dishes were left for thirty minutes at room temperature for efficient drug diffusion; then, they were incubated for a twenty-four-hour period at 37 °C [31]. The antibacterial efficacy was assessed by measuring the diameter of the inhibitory zone using a graded scale.

2.8. Statistics

Mean ± standard deviation (n = 3) was used to express the collected data. One-way ANOVA (GraphPad Prism version 5) was used for the statistical analysis. The difference was considered statistically significant if p was less than 0.05.

3. Results

3.1. Determination of pH and Organoleptic Assessment

The physical characteristics of the various topical AZ preparations (gel, emulgel, and nanoemulgel) that were effectively generated are provided in Table 2. Homogenous, smooth, and white formulations were produced, free from coarse particles. All formulations had pH levels that ranged from 5.8 to 6.4, which were deemed to be appropriate and would not irritate the skin when applied.

Table 2. Organoleptic evaluation of AZ-loaded formulations.

Properties	AZ-Loaded Gel	AZ-Loaded Emulgel	AZ-Loaded Nanoemulgel
Color and homogeneity	White homogenous	White homogenous	White homogenous
pH	5.8 ± 0.1	6.4 ± 0.2	6.3 ± 0.3
Viscosity (cP)	70,700 ± 1868	99,100 ± 1015 *	84,500 ± 1400 *,#
Spreadability (mm)	60.33 ± 2.5	51.33 ± 2.08 *	42.67 ± 1.53 *,#
Extrudability (g/cm^2)	154.67 ± 4.62	135.33 ± 5.03 *	106.67 ± 6.11 *,#
Drug content (%)	97.76 ± 2.92	98.92 ± 1.75	96.74 ± 3.23

compared to AZ-loaded emulgel, * compared to AZ-loaded gel ($p < 0.05$).

3.2. Viscosity Determination

The data regarding viscosity determination are demonstrated in Table 2. It was demonstrated that the emulgel's viscosity (99,100 ± 1015 cP) was much higher than that of gel and nanoemulgel (70,700 ± 1868 and 84,500 ± 1400 cP). These results were consistent with those of Mohamed et al., who concluded the highest viscosity of the topical emulgel loaded with atorvastatin compared to gel and nanoemulgel [32].

3.3. Spreadability and Extrudability Determination

The spreadability and extrudability are important criteria for topical preparations. They are considered essential aspects for the uniform application of topical preparations and patient compliance. The AZ-loaded gel, emulgel, and nanoemulgel formulations had spreadability values of 60.33 ± 2.5, 51.33 ± 2.08, and 42.67 ± 1.53 mm, respectively, and extrudability values of 154.67 ± 4.62, 135.33 ± 5.03, and 106.67 ± 6.11 g/cm^2, respectively (Table 2). These results indicate that the generated formulations have great skin spreadability and can be easily extruded by pressing down with the thumb. These findings are equivalent to those attained by Ali et al., who estimated extrudability and spreadability importance as the crucial factors for uniform distribution and patient compliance in the gel preparations [24,33].

3.4. Assessment of Drug Content Percent

The AZ-loaded gel has a 97.76 ± 2.92% drug content percentage; the AZ-loaded emulgel has a 98.92 ± 1.75 drug content percentage, while AZ-loaded nanoemulgel has a 96.74 ± 3.23 drug content percentage. This outcome demonstrated that the drug content was within the specified range (100 ± 10%). This revealed that the drug was dispersed uniformly throughout the generated formulations.

3.5. Polydispersity Index and Particle Size

The average particle size of emulgel loaded with azithromycin was 707.3 ± 17 nm, while the average particle size of AZ-loaded nanoemulgel was 227.8 ± 12 nm (Figure 1). Additionally, the polydispersity index (PDI) of the nanoemulgel and the emulgel loaded with AZ were estimated. AZ-loaded emulgel had a PDI of 0.631, while AZ-loaded nanoemulgel had a PDI of 0.395. Therefore, our findings supported the uniform size distribution of the nanoemulgel formulations. Additionally, the nanoemulgel's smaller particle size and lower PDI value are considered positive indicators of formulation stability and contribute to the enhanced drug penetration in the skin [34].

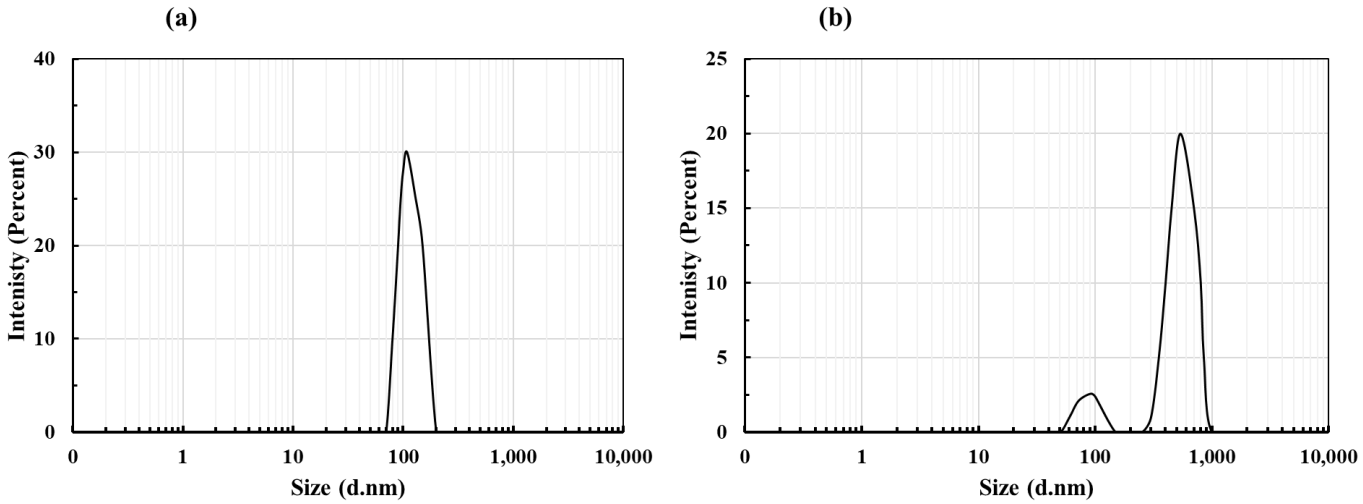

Figure 1. Size distribution of formulated (**a**) AZ-loaded nanoemulgel; (**b**) AZ-loaded emulgel.

3.6. Morphological Assessment

The morphology of the nanoemulgel loaded with AZ was estimated using SEM. Small spherical vesicles of nanoemulsion are dispersed throughout the macromolecular polymer network, as shown in Figure 2. Additionally, no visible AZ crystals were found, indicating adequate drug solubility.

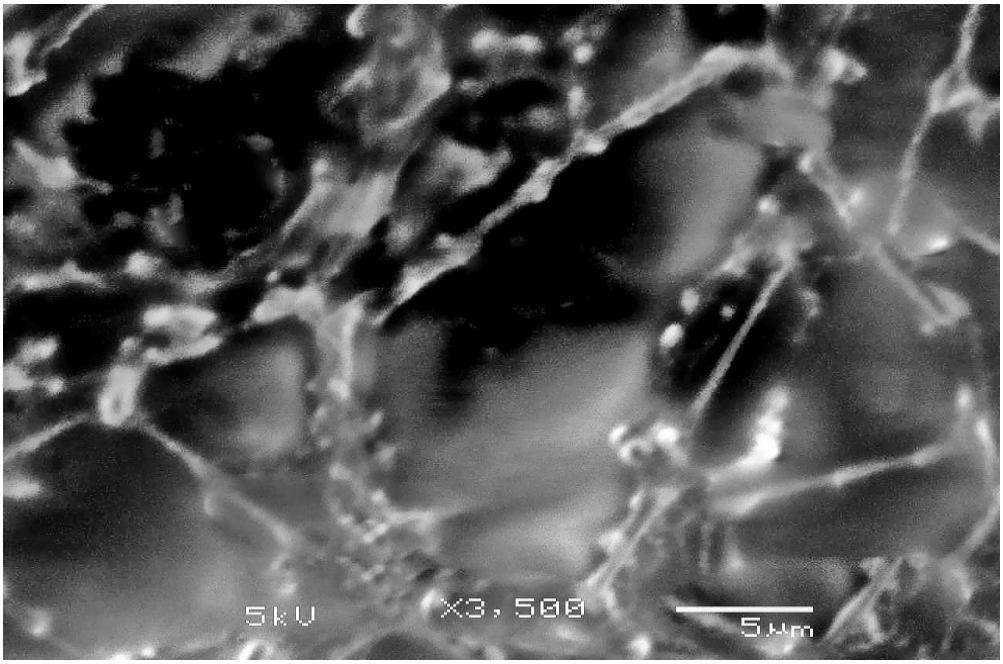

Figure 2. Observation of AZ-loaded nanoemulgel using a scanning electron microscope (SEM).

3.7. Azithromycin In Vitro Release Investigation

The percentage of AZ released after 6 h was 70.93 ± 2.21, 60.33 ± 2.82%, and 51.78 ± 2.70 for the gel, nanoemulgel, and emulgel, respectively, while AZ suspension showed a 97.94 ± 2.04% release after 4 h (Figure 3). In comparison to the free drug, all of the formulations' percentages of AZ released were significantly ($p < 0.05$) reduced. In addition, a significantly greater AZ amount was released from gel than from other formulations ($p < 0.05$) (nanoemulgel or emulgel). This is a result of the increased water proportion of the gel formula, which facilitates the drug's diffusion into the releasing medium. However, the significantly decreased release of AZ from nanoemulgel and

emulgel, in contrast to the gel formulation, was explained by the higher viscosity, which could hinder the drug diffusion in addition to the decreased aqueous composition and tea tree oil incorporation [35]. It is interesting to note that the nanoemulgel's significantly larger drug release ($p < 0.05$) may be due to its smaller average particle size than the emulgel, as illustrated in Figure 1.

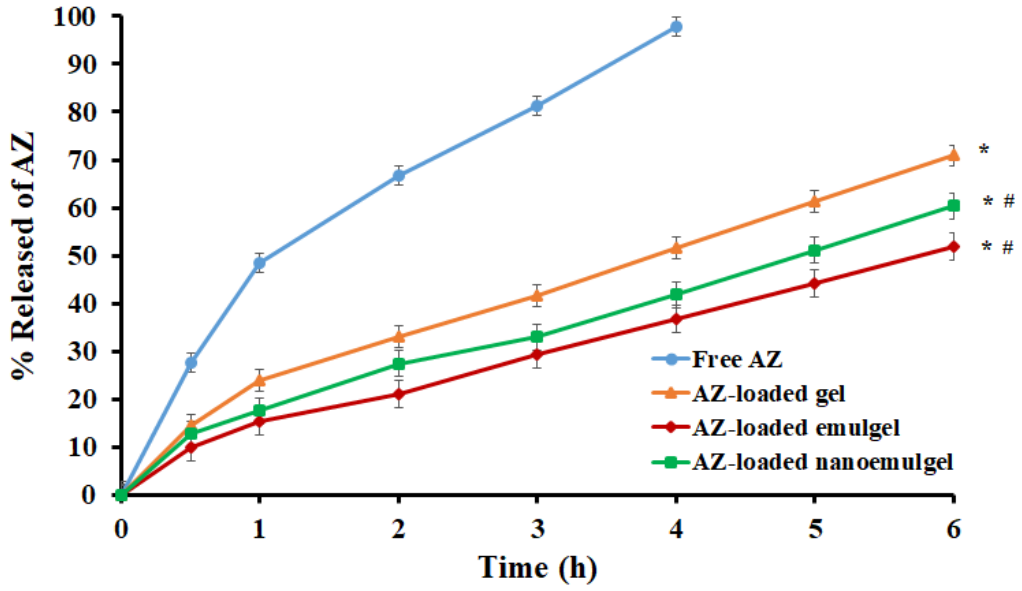

Figure 3. In vitro release study of AZ from different AZ-loaded formulations. # compared to AZ-loaded gel, * compared to free AZ ($p < 0.05$).

3.8. Study of Stability

The physical characteristics and the percent of AZ released from all preparations under investigation were calculated during a 6-month period at 25 °C and 4 °C; the findings are demonstrated in Table 3 and Figure 4. Our findings demonstrated insignificant variation in the homogeneity, color, viscosity, extrudability, or spreadability after six months of storage at either temperature. Furthermore, compared to the corresponding fresh preparations, there were no noticeable variations ($p < 0.05$) in the released amount of AZ from the preserved formulations at either 25 °C or 4 °C (Figure 4). In fact, HPMC used for gel preparation may be responsible for the stability of the AZ-loaded gel. Elmataeeshy et al. reported similar results and claimed that thickening agents are essential for the stability of the preparation [36].

Table 3. Physical properties of AZ-loaded formulations after storage for 6 months at different storage conditions.

Condition	At 4 °C			At 25 °C		
Formulation	**Color and Homogeneity**	**Spreadability (mm)**	**Viscosity (cp)**	**Color and Homogeneity**	**Spreadability (mm)**	**Viscosity (cp)**
AZ-loaded gel	White homogenous	60.67 ± 1.53	72,100 ± 1418	White homogenous	62.67 ± 1.15	71,000 ± 1127
AZ-loaded emulgel	White homogenous	53.33 ± 0.58 *	100,100 ± 1386 *	White homogenous	54.33 ± 0.58 *	98,600 ± 964 *
AZ-loaded nanoemulgel	White homogenous	44.00 ± 1.73 *,#	85,100 ± 1039 *,#	White homogenous	43.67 ± 1.15 *,#	83,500 ± 361 *,#

compared to AZ-loaded emulgel; * compared to AZ-loaded gel ($p < 0.05$).

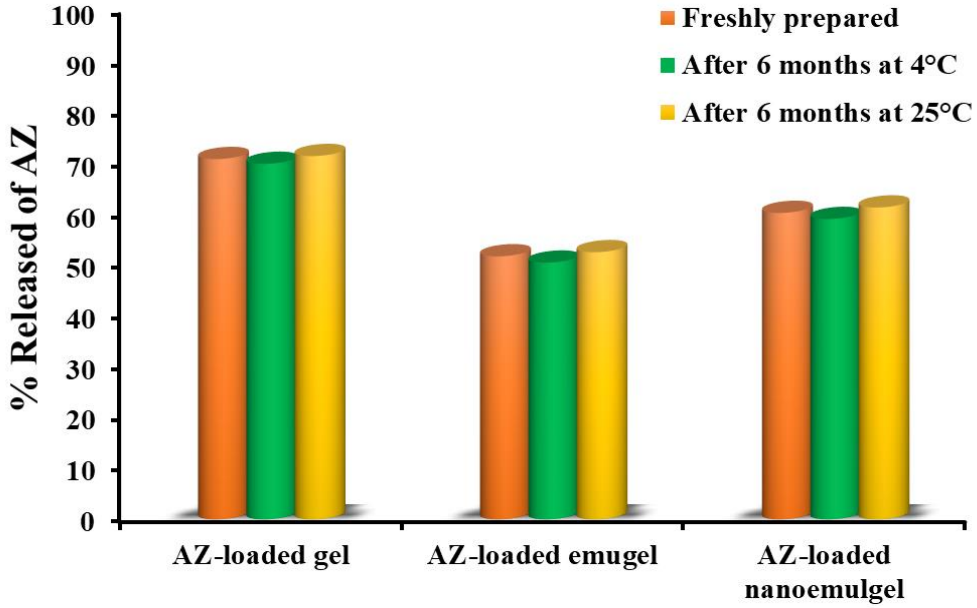

Figure 4. Study of the AZ in vitro release from different formulations upon storage for 6 months at different storage conditions.

3.9. Ex Vivo Study

Figure 5 estimates the skin permeation of AZ from different topical formulations compared to AZ suspension permeation. It was obtained that the cumulative amount of AZ (μg/cm^2) penetrated from nanoemulgel through rabbit skin was significantly larger compared to the AZ-loaded emulgel, AZ-loaded gel, or AZ suspension (Figure 5). The SSTF flux (J_{ss}) of the AZ suspension was noticeably lower than those of the other generated formulations under evaluation, as indicated in Table 4. Contrarily, gel formulation increases AZ permeation by 1.72 folds with a J_{ss} of 148.04 ± 6.09 μg/cm^2.h compared to free drugs. This increase could be related to the gel's colloidal characteristics [37]. Interestingly, nanoemulgel formulation could significantly ($p < 0.05$) enhance the skin permeability properties of AZ compared to AZ-loaded gel and emulgel since it had the largest SSTF value (206.68 ± 7.99 μg/cm^2.h) and the largest ER (2.41 ± 0.17). Actually, the AZ flux from emulgel and nanoemulgel might be improved by the addition of the penetration enhancer (oil) and surfactant. Additionally, the external water phase may hydrate the stratum corneum, cause cell swelling, and facilitate the drug transport [21]. Moreover, the improved permeation of nanoemulgel containing AZ could related to the nano-scaled particles, which provide a greater surface area for AZ penetration and facilitate the release of a large portion of the drug [36]. Our findings were consistent with those of Shehata et al., who found that niosomal emulgel considerably increased the skin penetration of insulin compared to insulin solution and niosomal gel [38].

Table 4. Skin permeation characteristics of various AZ-loaded preparations after ex vivo studies.

Formula	SSTF μg/cm^2.h	ER
Free AZ	85.93 ± 5.29	1
AZ-loaded gel	148.04 ± 6.09 *	1.72 ± 0.05 *
AZ-loaded emulgel	176.95 ± 4.38 *,#	2.06 ± 0.11 *,#
AZ-loaded nanoemulgel	206.68 ± 7.99 *,#,$	2.41 ± 0.17 *,#,$

Compared to AZ-loaded gel. * compared to AZ suspension. $ compared to AZ-loaded emulgel ($p < 0.05$).

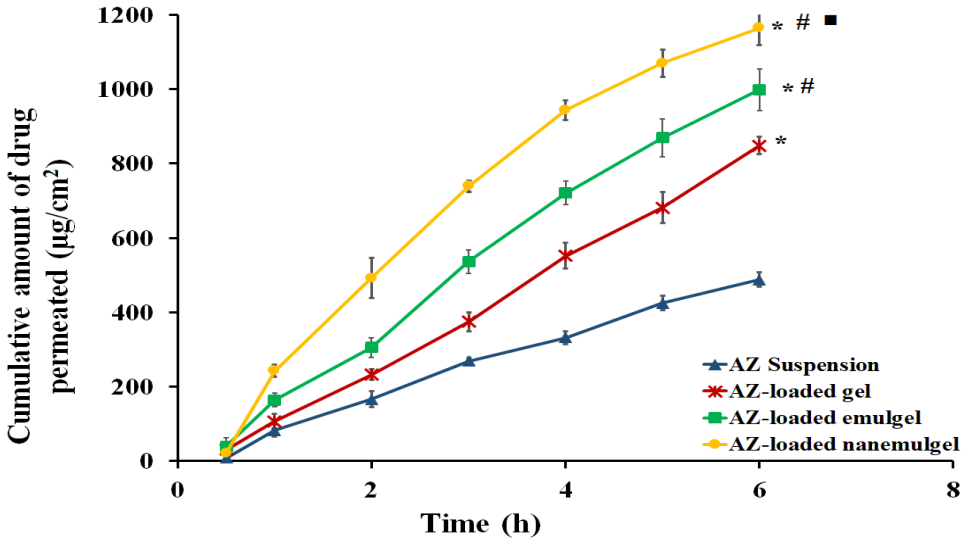

Figure 5. Permeability pattern of AZ from various AZ-loaded formulations. # compared to AZ-loaded gel. * compared to AZ suspension. ■ compared to AZ-loaded emulgel ($p < 0.05$).

3.10. Investigation of Antibacterial Properties

Figure 6 displays the results of the antibacterial activity of the generated formulations against *Escherichia coli* (Gram-negative bacteria) using the agar diffusion technique by measuring the zones of inhibition. The macrolide azithromycin works by preventing the production of proteins in bacterial cells [39]. Azithromycin suppresses protein production by reversibly trussing to 50S ribosomal subunits of affected microorganisms, which effectively prevents the growth of bacteria. Azithromycin has the capacity to penetrate bacterial outer membranes, which is a kind of bacterial self-defense [39].

The results showed that AZ-loaded nanoemulgel was effective against *Escherichia coli* and that it produced a zone of inhibition that was significantly greater than that produced by placebo nanoemulgel and the AZ-loaded gel formulation ($p < 0.05$). The AZ-loaded nanoemulgel displayed zones of inhibition of 16 ± 1.8 mm (after 12 h) and 22 ± 1.7 mm (after 24 h) against *E.coli*. The zones of inhibition against *E.coli* for the AZ-loaded gel were 10 ± 1.3 mm (twelve hours) and 15 ± 1 mm (twenty-four hours). Furthermore, the placebo formulation (nanoemulgel free from AZ) showed that the inhibition zones against *E.coli* were 6 ± 0.5 mm and 8 ± 1 mm after twelve hours and twenty-four hours, respectively. It is interesting that the placebo nanoemulgel formulation with tea tree oil demonstrated a considered bacterial growth inhibition, which was undoubtedly caused by the antibacterial effects of the tea tree oil. The use of tea tree oil, which has antibacterial properties, may have contributed to the fact that the placebo formulation (nanoemulgel free from AZ) showed a certain suppression of bacterial growth. Moreover, the higher antibacterial activity of AZ-loaded nanoemulgel may be due to the antibacterial synergism between AZ and tea tree oil. The action of terpinen-4-ol compound, the main constituent in tea tree oil, may be responsible for the observed antibacterial efficacy of the placebo formulation [40]. It has been demonstrated that tea tree oil prevents respiration in the cells of *E. coli*, and by rupturing the microbial membrane's permeability, the oil inhibits bacterial cell growth and causes the cells to die [41]. According to previous studies, tea tree oil combined with different antibiotics, such as doxycycline [42] and neomycin [43], exhibits additional efficiency against *E. coli*. These conclusions were confirmed by the results of our current investigation, which also demonstrated the antibacterial efficiency of tea tree oil combined with Azithromycin.

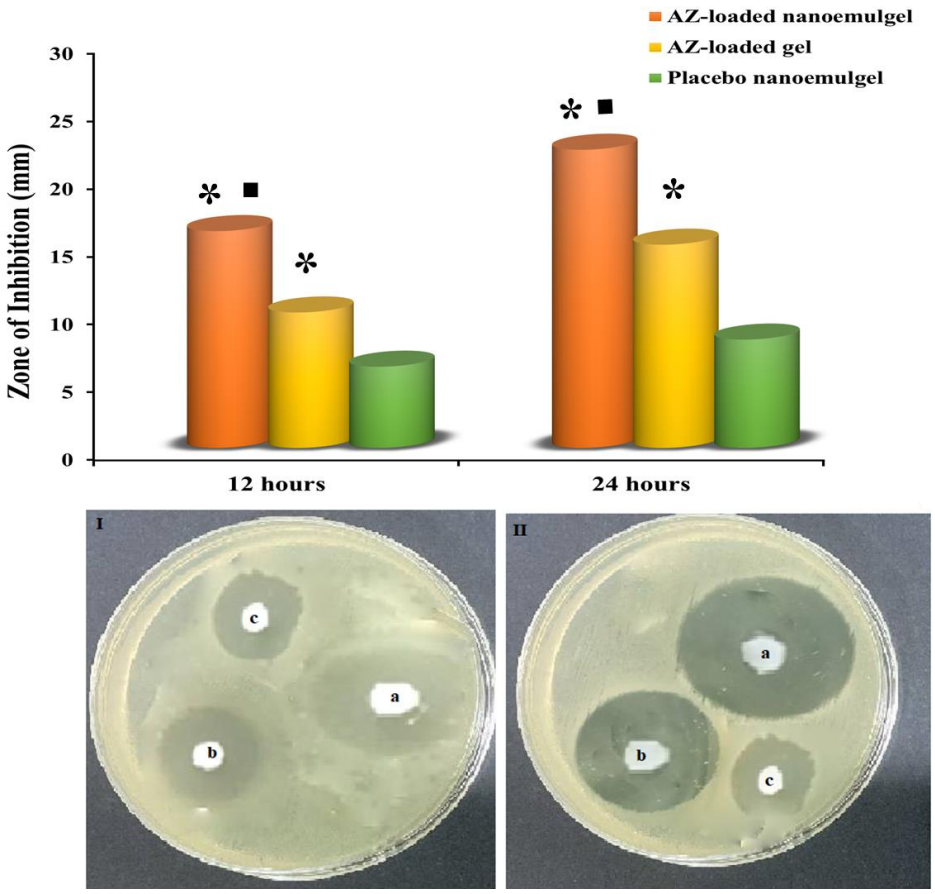

Figure 6. Antimicrobial assessment of (a) AZ-loaded nanoemulgel, (b) AZ-loaded gel, and (c) placebo nanoemulgel against *E.coli* after (**I**) 12 and (**II**) 24 h. ■ versus AZ-loaded gel ($p < 0.05$); and * versus placebo nanoemulgel ($p < 0.05$).

4. Conclusions

In the current study, a nanoemulgel formulated with azithromycin was evaluated for its ability to reduce bacterial growth. The developed azithromycin-loaded nanoemulgel displayed favorable physical properties, viscosity, pH, extrudability, spreadability, and particle size to be applied topically. Additionally, compared to AZ-emulgel or AZ-gels, AZ-nanoemulgel demonstrated better antibacterial efficiency. Therefore, the current study may offer a novel method for the efficient therapy of bacterial infection using azithromycin and a natural product (tea tree oil) formulated into nanoemulgel.

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