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Preparation and Evaluation of Berberine Chloride Loaded Poloxamer Gel for Transungual Delivery

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ABSTRACT

Oral antifungal medications are the most commonly used treatment for fungal infections, but these have severe side effects. To address these side effects, transungual delivery systems have been introduced. The objective of the present study was to formulate the gel of berberine chloride using Poloxamer as the gelling agent. Various gel formulations were prepared with different concentrations, using mercaptoethanol as a permeation enhancer. These formulations were assessed using different parameters, including spreadability, Fourier Transmission Infrared Radiations, Scanning electron microscopy, x-Ray diffraction, antifungal activity against *Candida albicans* and histopathology. Upon physiological evaluation, the gel exhibited a pH of approx. 5.5, adequate spreadability and met the specified requirements for adhesion and cohesiveness. The components of the gel formulation were found to be compatible with berberine chloride. The gel showed the highest zone of inhibition against *C. albicans*. Raman microscopy studies confirmed the uniform distribution of berberine chloride within the gel formulation. *Ex vivo* permeation studies of berberine chloride from the gel formulation across the bovine hoof revealed that the gels exhibited sustained release of berberine during 24 h study period. Furthermore, the gel formulations remained stable and exhibited promising properties over a 90-day period.

Key words: Poloxamer, berberine chloride, transungual delivery, formulation

INTRODUCTION

Nails fulfil several functions in our daily lives, including activities like scratching and grooming (Bhairy *et al.*, 2023). Unfortunately, nails are vulnerable to various types of fungal infections, with onychomycosis being a prevalent occurrence (Mohite *et al.,* 2022). Onychomycosis can be classified into four distinct types: distal subungual onychomycosis, proximal subungual onychomycosis, total dystrophic onychomycosis and white superficial onychomycosis. Onychomycosis affects approximately 13% of the global population, making it a substantial portion of all nail infections involving the nail plate and nail bed. As onychomycosis advances, it leads to nail thickening, discolouration, deformation, hyperkeratosis, cracking and deterioration. In its severe stages, it can result in complete nail dystrophy (Kesharwani *et al.,* 2022). This condition predominantly impacts older individuals who may encounter challenges in maintaining their nails and immunocompromised individuals, such as those with diabetes, HIV, or recurring injuries near their nails (Aggarwal *et al.,* 2020a). Managing the recurrence of onychomycosis presents a significant treatment challenge. Various antifungal products containing substances like fluconazole, itraconazole and terbinafine are available in the market for treating onychomycosis (Gupta *et al.,* 2023). These antifungals are therapeutic effective, but the main challenge lies in delivering these drugs to the specific target site, particularly the nail bed. Systemic antifungals come with several side effects and can be costly (Ullah *et al.,* 2022). Unfortunately, topical treatments often yield low success rates due to inadequate penetration of antifungal medications into the nail plate (Nair *et al.,* 2023). The rate at which drugs permeate the nail plate is influenced by several factors, including the thickness of the keratin layer, nail plate hydration, the physico-chemical properties of the drug and the formulation's composition. Keratolytic agents like urea and

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salicylic acid have been shown to be effective in enhancing drug penetration across the nail plate. These agents disrupt the keratin arrangement within the nail plate, reducing its barrier properties. Transungual gels are widely favoured by patients because they maintain transonychial water levels, promoting nail hydration and facilitating sustained drug diffusion across the nail. Transungual formulations have gained popularity due to their ease of application and the aesthetic appeal they offer giving nails a glossy and colourful appearance (Martins *et al.,* 2023). Patients widely embrace these formulations because they maintain transonychial water levels, enhancing water retention, hydrating the nail plate, and facilitating sustained drug diffusion. A promising approach in this regard is the direct delivery of drugs to the infected nail area through transungual drug delivery. This method has gained prominence in recent years due to its safe and effective application at the target site.

Berberine, an isoquinoline alkaloid, is derived from the *Berberis aristata* plant within the Berberidaceae family (Wang *et al.,* 2020). It is commonly found in various quaternary ammonium salt forms, such as berberine chloride, hydrochloride and sulfate (Gaba *et al.,* 2021). This compound exhibits a wide spectrum of antimicrobial properties and has proven highly effective in treating a variety of microbial diseases caused by bacteria, fungi, protozoa and helminths (Jamshaid *et al.,* 2020). Furthermore, berberine chloride has been demonstrated to have anti-candidal activity by interacting with the cell membrane of this yeast. Researchers have also explored derivatizing berberine chloride to enhance its oral bioavailability.

The aim of this study was to formulate a gel formulation loaded with berberine chloride for transungual delivery. The impact of a permeation enhancer was assessed through techniques like Scanning Electron Microscopy and the uniform distribution of the drug in the gel formulation was confirmed using Confocal Raman microscopy.

MATERIALS AND METHODS

Poloxamer 407 and berberine chloride were purchased from Sigma-Aldrich, Bangalore, Mercaptoethanol was obtained from Hi-Media laboratories Pvt. Ltd., Mumbai, India; Absolute ethanol was purchased from Research Lab Fine Chem Industries, Mumbai, India.

The Poloxamer 407 gel was prepared by cold preparation method on weight basis to yield different concentrations (18 to 24% w/w). A weighed amount of Poloxamer was gradually dissolved in cold distilled water at 4°C with continuous stirring until a clear homogenous solution was obtained. Ethanolic solution of berberine chloride and mercaptoethanol (5% v/v) was added to the above prepared solution. The solution was warmed at room temperature until a smooth gel was produced.

The pH of gel formulation was recorded using digital pH meter. One g of the gel was dissolved in 100 ml of distilled water and stored for a period of 2 h. Gel formulations were analyzed using triplicate samples. Gel preparations were checked visually for homogeneity. The gel was kept in glass container and allowed to set. Then formulation was tested for morphological appearance and aggregates.

Spreadability of formulated gel was determined by parallel plate technique. Breifly, 0.5 g of gel was poured on a glass plate at centre (one cm^2 area) of a glass plate, and on top of it, another glass plate was carefully positioned in a concentric manner. The initial diameter of the gel formulations was determined as the diameter of the circle within which the gels spread. Then known weights (20, 50, 100, and 150 g) were placed on the upper plate. Subsequently, the spreadability of the gels was evaluated by observing changes in the area covered when weights were applied. Spreadibility was determined using following Kumar *et al.* (2019) as:

$$S = \frac{W \times L}{T}$$

Where, S: Spreadability, W: Weight placed over the plate, L: Length of gel moved on the petri plate and T: Time.

Gel size of 3 × 4 cm (diameter × height) was used for texture profile analysis. It was performed by TA-TX2 (Stable Micro Systems Ltd., Surrey, UK) texture analyzer with a load cell (50 kg). Compression platen of 75 mm diameter was utilized to compress gel at the rate of 1.0 mm/s at 25°C. Test speed was

maintained at 1.0 mm/s and target mode distance was 10 mm. Trigger force was 5 g (Auto). Analysis completed in 100 s. For each sample of gel, three readings were taken and average value was taken for preparation of final result. Force-time graph was obtained and textural parameters, like hardness, cohesiveness, adhesiveness were calculated with the help of software (Shafiur and Al-Mahrouqi, 2021).

The *ex vivo* permeation studies of berberine chloride gel formulation were carried across the cleaned and washed bovine hooves as the permeation membrane. Bovine hooves had similar structure as that of human nails. So, these were selected for permeation studies (Aggarwal *et al.*, 2020b). Washed and cleaned hooves were hydrated in phosphate buffer saline pH 7.4 for 24 h. After hydration, hooves swelled with the presence of pores and these had more permeability. Hydrated hooves were sandwiched between the donor and receptor compartment of the Franz diffusion cell with receptor chamber containing 10 ml of PBS 7.4 and cross-sectional area (1.1304 cm^2) of diffusion cell. Poloxamer gel was applied on the membrane and the Franz diffusion cell assembly was placed on the magnetic stirrer with the continuous revolution of 400 rpm for 24 h at 37°C. One ml of sample was withdrawn from the receptor compartment using sampling port at a pre-defined time interval up to 24 h and replaced with fresh buffer solution. Aliquoted samples were analyzed using UV spectrophotometer at 420 nm and the cumulative amount of drug permeated was determined.

The compatibility between the drug and the excipients were studied by (Perkin Elmer Spectrum, BXII) FTIR spectrophotometer. The IR Absorption spectra were recorded in 4000 to 400/cm for berberine chloride, and Poloxamer 407 using KBr pellet.

XRD studies were carried out by the Rigaku, Mini Flex2 Goniometer on powder diffraction system. The X-ray generator was operated at 3.0 kV and 50 mA, using the CuKα line at 1.54056 Å as radiation source. The powdered sample was placed in a glass specimen holder. Sample was poured into the sample holder and scanned at 25°C. The samples were scanned from 5° to 80° (2θ) count time of 2.00 s, using an automatic divergence slit assembly. Spectra were analyzed to get final result.

Confocal Raman microscopy was employed to analyze the distribution of drug in the gel formulation. The Raman spectra were recorded in a Raman spectrometer (Alpha 300 RWI- Tec., Germany) consisting of a laser (785 nm) and UHTS 300 spectrophotometer and a CCD camera. The data acquisition was done by keeping the integration time of 5 s, with 100X scan resolution and grating of 300 g/mm.

To study the surface morphology of gel formulation scanning electron microscopy was performed. The optimised gel formulation was applied on the dorsal surface of cleaned bovine hoof which was placed in the sample holder and attached to aluminium stub with double sided mounting tape and coated with gold plated palladium alloy. The high magnification images were taken using SEM (JEOL/ 7610F Plus) at 35000X.

The antifungal activity of the berberine chloride gel was determined by agar diffusion technique against *Candida albicans*. The potato dextrose agar was used as the culture media for the growth of *C. albicans (*MTCC227). One ml of the fungal strain was inoculated into 25 ml of sterile potato dextrose agar at 40°C, which was then poured into sterile Petri plates and allowed to gel. A well of 6 mm was made in the centre of the potato dextrose agar plate using sterile micropipette tips. In each well the gel was added carefully and incubated at 37°C for 48 h. After incubation, the plates were evaluated for zone of inhibition (Yang *et al.*, 2019).

Bovine hooves were submerged in distilled water for 24 h to remove soft tissues and dust particles. Cleaned hooves were put in optimized gel formulation for 48 h, followed by fixation in 10% formalin solution for 24 h. The hooves were sliced by edge surface and flat portion was selected for study. It was dehydrated using absolute ethanol and embedded in the paraffin wax. Tissues were dissected into small pieces using microtome. The eosin and hematoxylin were used as staining agents. The fine section of hooves was examined fewer than 100X magnification.

The stability studies of berberine chloride loaded gel formulation were carried out at room temperature (25°C). The prepared formulation was evaluated for different parameters such as pH, spreadability and homogeneity.

RESULTS AND DISCUSSION

Poloxamer 407 is used for the preparation of transungual gel, because of its high water retention capacity. It may hydrate the nail plate to enhance the permeation of drug across the nail plate. Poloxamer is water soluble non-ionic triblock copolymer made of polar and non-polar blocks that give the polymer amphiphilic and surface active properties. The aqueous solution of Poloxamer goes through a Sol to gel transition when temperature is raised. Due to the amphiphilic nature of Poloxamer, it has tendency to improve the solubilization of drugs. Along with these properties, it is also having the potential for wound healing. Because of these qualities: Poloxamer was chosen for the formulation of transungual gel.

The pH of skin lies between 5.1 to 5.8. The pH of formulated gel was also found to be 5.1-5.3. The pH of 18, 20, 22 and 24% was found to be 5.54±0.12, 5.59±0.17, 70±0.31 and 5.78±0.25, respectively.

Spreadability is a crucial parameter for determination of consistency and ease of application of gel formulations on the skin surface. The spreabability of the formulation is influenced by the viscosity. Usually, the formulation with high viscosity has lower spreadability making it difficult to apply on the skin. The gel formulation with 18% Poloxamer was found to have more spreadability than other formulations while the formulation containing 24% Poloxamer exhibited limited spreadability (Table 1).

Table 1. Spreadability profile of Poloxamer gel loaded berberine chloride

Concentration (%) Poloxamer gel	Weight (g)	Spreadability (g/cm/sec)
18	20	17.90±1.11
	50	21.07±1.06
	100	37.39±0.78
	150	51.51±0.60
20	20	16.66±0.88
	50	22.33±1.81
	100	34.74±0.71
	150	48.60±1.39
22	20	14.36±1.74
	50	19.07±1.66
	100	32.25±1.51
	150	46.65±2.50
24	20	12.88±0.75
	50	14.22±1.26
	100	27.99±0.73
	150	40.52±1.51

Texture analysis gives structural properties of gel formulations. The topical gel formulations must have adequate cohesiveness, hardness and bio adhesiveness. These are vital parameters for the development of semi-solid dosage forms because the acceptability of the product depends on organoleptic properties. Hardness defines the force required for deformation i.e. spreading of gel on the skin surface, while adhesiveness is the indicator of the work required for overcoming attractive forces between the finger and the skin. It also reflects how the gel adheres to the skin. The formulations having more hardness are not preferred because of their strenuous application on the surface of the skin, whereas the formulations having very low mechanical properties are also not accepted as they may drip out from the surface of skin. Cohesiveness defines the structural recovery of the formulated gel upon compression and indicates the strength of internal bonds of the gel and how easily the gel holds its bonds together after deformation and adherence to the skin (Martinovic *et al.,* 2022). The results of texture analysis are expressed in Table 2.

The overlay FTIR spectra of berberine chloride, Poloxamer 407, berberine loaded Poloxamer gel (lyophilized) was plotted in the Fig. 1. The distinctive peaks of berberine chloride were observed at 3049/cm indicating aromatic C-H stretching, 2915/cm, 2846/cm because of aliphatic C-H stretching, 1634/cm attributed to iminium ion, 1568/cm corresponding to aromatic C=C, 1272/cm, 1142/cm due to asymmetric and symmetric C-O-C stretching, respectively and 1064/cm owning to C-O stretching. The FTIR spectra of Poloxamer 407

Table 2. Texture profile of Poloxamer gel

Formulation	Concentration (%)	Hardness (N)	Cohesiveness	Adhesiveness (g/sec)
Poloxamer gel	18	1.30±0.04	0.62±0.01	-0.47±0.04
	20	1.39±0.01	0.85±0.03	-0.62±0.06
	22	1.44±0.05	0.87±0.06	-0.68±0.04
	24	1.51±0.03	0.91±0.07	-1.01±0.88

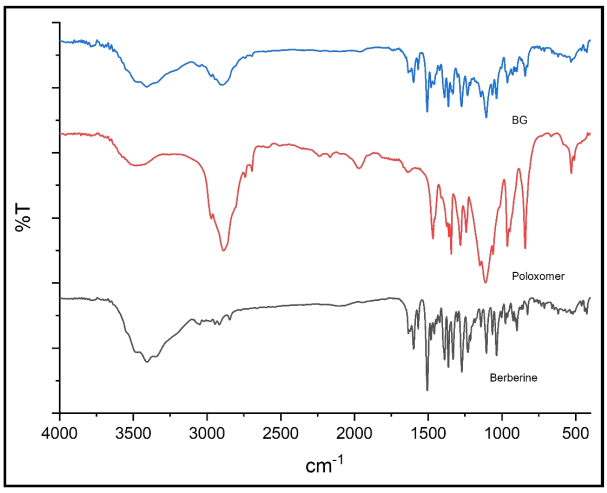

Fig. 1. FTIR spectra of berberine chloride, Poloxamer, BG.

showed peaks at 3473/cm because of OH stretching, 2971 and 2887/cm confirmed the presence of asymmetric and symmetric stretching, respectively, 1343/cm because of in plane O-H bend and 1111/cm confirmed the presence of C-O stretching. Notably, the FTIR spectra of the (BG) gel showed similar characteristic peaks as those of berberine chloride and Poloxamer 407. Importantly, no new characteristic peaks were observed, and there was absence of any significant changes in the spectra. This finding indicated the compatibility between the formulation excipients and berberine chloride.

Scanning electron microscopy was used to determine the surface morphology of bovine hooves. Fig. 2 displays the micrograph of control and gel treated hooves. In gel micrograph, small pores were identified. These pores may be attributed to the breakage of the disulfide bond of keratin due to mercaptoethanol which made the hoof membrane permeable for the diffusion of drug. The XRD observations of the berberine chloride, Poloxamer and lyophilized gel are shown in Fig. 3. Berberine showed diffraction peaks at 15.72, 21.54 and 24.0. Poloxamer 407 showed peaks at 14.39, 18.42, 30.72 and (BG) gel showed peaks at 21.33, 23.57. The intensity of the berberine chloride peaks in the gel was decreased due to the dilution effect.

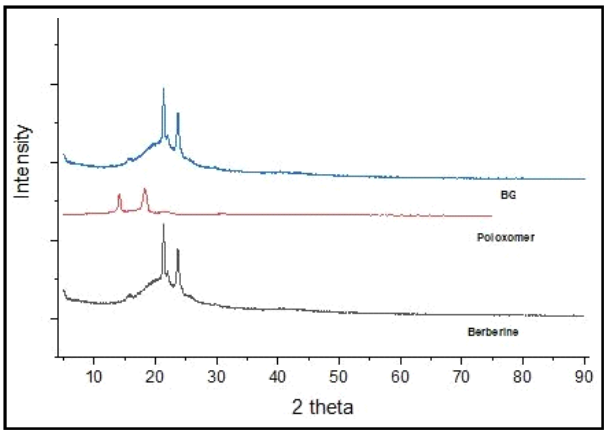

Fig. 3. XRD spectra of berberine chloride, Poloxamer 407 and BG.

In vitro antifungal activity of berberine gel was assessed by determining the zone of inhibition of the formulations against *C. albicans* (MTCC 277). Fig. 4 shows the zone of inhibition produced by gel formulation against *C. albicans*. To confirm that antifungal activity was not due to the presence of other components of gel, vehicle control was also taken. The control formulation did not show any zone of inhibition,

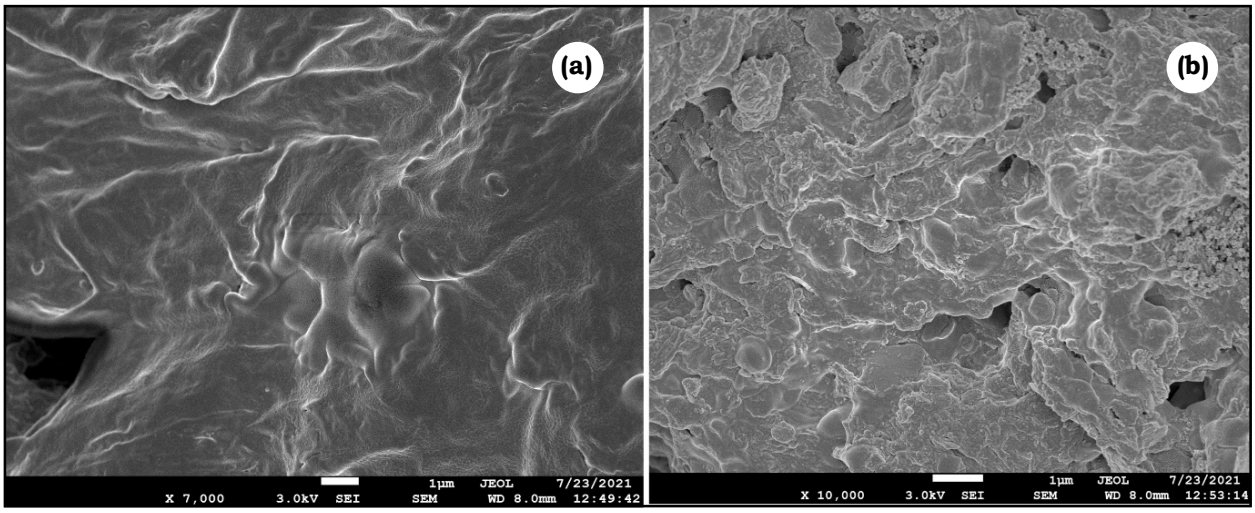

Fig. 2. SEM micrograph of (a) control and (b) mercaptoethanol treated hoof.

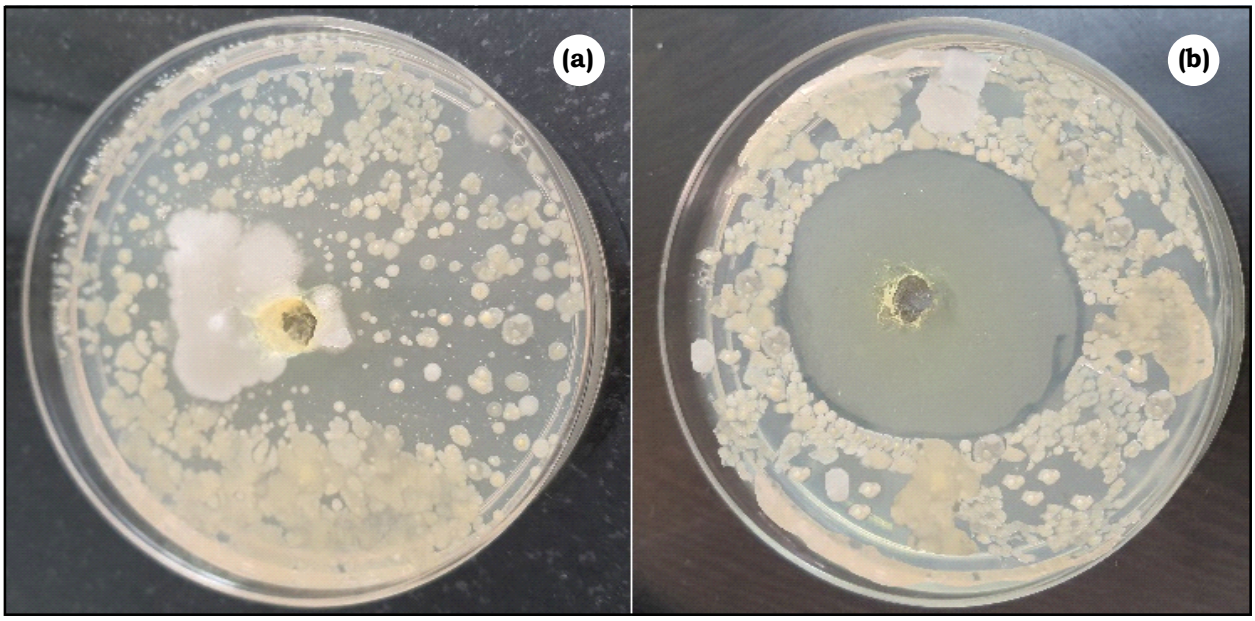

Fig. 4. Zone of inhibition of vehicle control, gel.

while gel had zone of inhibition of 12 mm. Therefore, gel possessed potent antifungal activity against *C. albicans*.

The overlay Raman spectra of berberine chloride and Poloxamer 407 (Fig. 5a) showed the characteristic peaks of berberine chloride at 1064, 1204, 1278 and 1399/cm (Fig. 5b). The characteristic peaks of Poloxamer 407 appeared at 363, 846 and 1281/cm (Fig. 5b). Figure 4b represents the 2D image showing uniform drug distribution in the lyophilized berberine gel (Melian *et al.*, 2018).

Fig. 6 shows the results of histological studies of the bovine hooves treated with control, berberine loaded gel formulations gel. The hoof treated with blank formulation had no damage in the tissue, while the hooves treated with the gel formulation showed presence of pores. This may be due to the rupture of disulphide linkage of the keratin layers.

The cummulative release (%) of berberine chloride from the Poloxomer gel of 18 to 24% is shown in Fig. 7. The 18% gel showed highest release (68.94±1.10%) in 24 h, while the 24% showed minimum release (49.60± 0.67%). The formulation having 18% showed highest *ex vivo* permeation in 24 h, but increasing the proportion of Poloxamer in gel formulation resulted in the decrease in the % permeation. It may be due to high viscosity and formation of a dense matrix around nail plate which retards the drug release from the formulation.

The stability study was performed to determine the changes in the formulations over a period of 90 days at room temperature (25°C). The results of stability studies showed that the gel

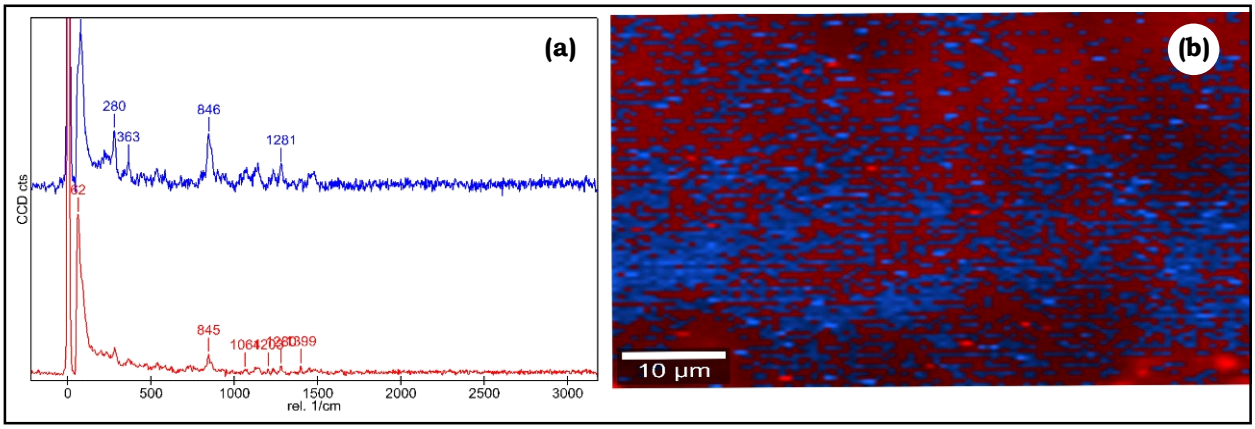

Fig. 5. (a) Confocal Raman spectra and (b) Drug distribution of gel.

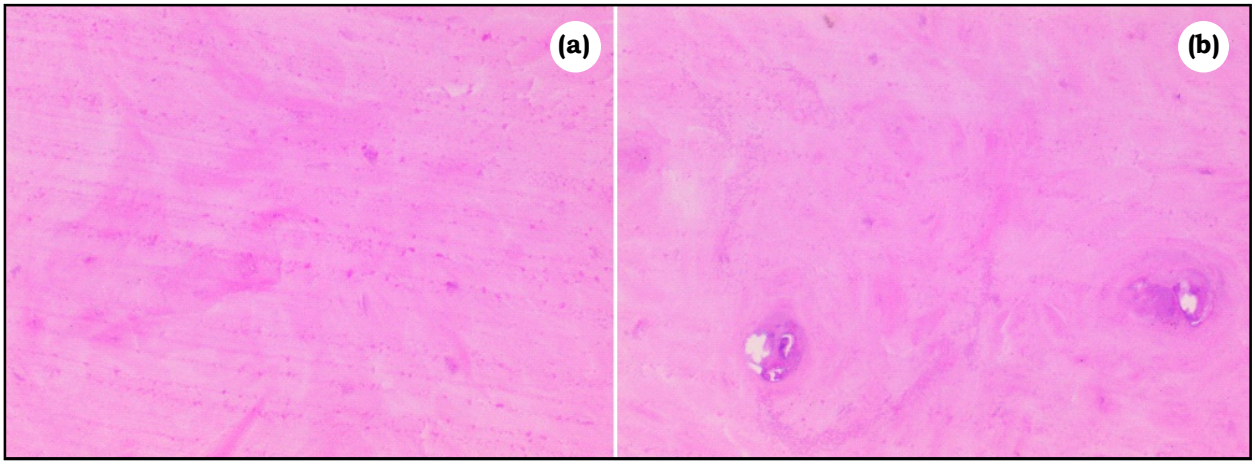

Fig. 6. Histopathogy studies of (a) control and (b) gel.

Table 3. Stability studies profile of gel

Type of formulation	Storage conditions	Period (days)	pH	Homogeneity	Spreadability
Gel	25°C	0	5.54±0.12	Pass	16.66±0.88
		30	5.56±0.01	Pass	15.41±0.42
		60	5.56±0.01	Pass	14.40±0.04
		90	5.58±0.03	Pass	14.43±0.02

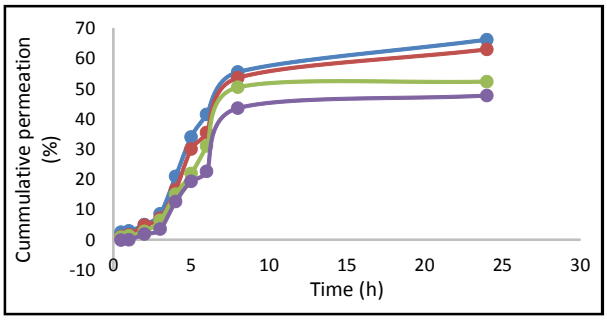

Fig. 7. Cumulative permeation of formulated gel (18, 20, 22 and 24%).

did not have any eloquent changes in pH, homogeneity and spreadability. Table 3 expresses the results of stability studies. It can be concluded that gel formulations were stable and exhibited promising properties over a period of 90 days.

CONCLUSION

The antifungal properties of berberine chloride for the treatment of onychomycosis were explored through the development of gel formulations. The results of confocal Raman microscopy confirmed uniform distribution of drug in the gel formulation. The formulation exhibited effective antifungal activity against *Candida albicans*. Histopathological studies further confirmed that formulations containing penetration enhancers facilitated the permeation of berberine chloride by disrupting the disulphide bonds of keratin. Additionally, the stability studies conducted over a six-month period indicated no significant changes in the physico-chemical characteristics of the gel. Based on the findings of this study, it can be concluded that the Poloxamer gel formulation of berberine chloride holds potential in the treatment of onychomycosis.

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