Annals of Biology **40** (1) : 81-87, 2024

Impact Score : 0.32 (Scopus)

Fabrication and Characterization of Poloxomer Based Gel Formulations for Drug Delivery

HIMANSHI TANWAR[1,*], MANU SHARMA AND MUNISH AHUJA[1]

Department of Pharmacy, Banasthali Vidyapith, Banasthali-304 022 (Rajasthan), India
**(e-mail: tanwarhimanshi@gmail.com; Mobile: 99961 50219)*

(Received: July 1, 2023; Accepted: August 7, 2023)

ABSTRACT

Oral antifungals are the most common treatment to cure fungal disease. These are effective but with some severe side effects. To overcome these side effects, now transungual delivery systems have been adopted. The objective of present study was to develop berberine chloride loaded poloxomer gel. The gel formulations were prepared in varying concentrations using thioglycolic acid as permeation enhancer. Formulations were evaluated with different parameters such as spreadability, rheology, FTIR, SEM, XRD, antifungal activity against *Candida albicans* and histopathology. On physiological evaluation gel showed 5.5 pH, adequate spreadability, optimum adhesion and cohesiveness as per specifications. The *ex vivo* permeation studies showed incorporation of berberine chloride in the gel formulations. The gel formulation components were compatible as shown in FTIR. The gel showed highest zone of inhibition against *C. albicans*. XRD studies showed the uniform distribution of berberine chloride in gel formulation. Gel formulations were stable and exhibited promising properties over a period of 90 days.

Key words: Transungual, poloxomer, thioglycolic acid, berberine chloride

INTRODUCTION

The regular use of oral conventional therapies shows severe gastrointestinal side effects, loss of taste, hepatotoxicity and drug interactions (Sveikauskaite and Briedis, 2017). Transdermal and transungual delivery systems are preferred options to overcome the side effects associated with oral therapies. However, the major challenge of transungual delivery is poor drug permeability across the nail plate, composed of dense and impermeable keratin structure (Agrawal *et al.,* 2023). The rate of drug diffusion across the nail plate is affected by the thickness of keratin layer, hydration of the nail plate, physico-chemical characteristics of the drug, and nature of excipients present in the formulation. Keratolytic agents like urea, salicylic acid, etc. have been found effective in enhancing drug permeation across nail plate. These agents can disturb the arrangement of keratin in the nail plate which reduces barrier properties of nail plate (Nair *et al.,* 2023). Transungual gels are widely endorsed by the patients as they protect the transonychial water loss that enhances nail hydration and imparts sustained drug diffusion across the nail (Bhairy *et al.,* 2023). Nail is composed of dense layers of keratin protein and disulphide hydrogen bonds (Kesharwani *et al.,* 2022). To increase the permeation of drug across the nail thiol group-based permeation enhancers are used which cleave the disulphide bonds and make it convenient for drugs to diffuse across the nail plate. Nail lacquers are in trend due to their easy application, and the glossy and coloured look they impart to the nails (Patel and Vora, 2016). These formulations are widely accepted by the patients as they protect the transonychial water loss which increases the water retention that hydrates the nail plate, and it imparts sustained drug diffusion across the nail. This makes the nail lacquer a suitable formulation for the treatment of nail infections.

Berberine, an isoquinoline alkaloid, is obtained from *Berberis aristata* (Family: Berberidaceae; Wang *et al.,* 2020). It is available in the form of quaternary ammonium salts such as berberine chloride, hydrochloride and sulphate (Gaba *et al.,* 2021). It possesses broad-spectrum antimicrobial properties, and has been reported to be very effective to cure many microbial

[1]Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125 001 (Haryana), India.

diseases caused by bacteria, fungi, protozoa and helminths (Jamshaid *et al.,* 2020). Berberine chloride was reported to exhibit anti-*candida* activity binding with its cell membrane. Berberine chloride has been derivatized to improve its oral bioavailability (Zoric *et al.,* 2017). In the present study, berberine chloride has been investigated for the transungual drug delivery. A novel berberine chloride loaded Poloxomer 407 with an optimal amount of permeation enhancers to enhance drug diffusion across the nail bed has been formulated. The permeation enhancers alter the structure of keratin layer and affect drug uptake. The optimized batch of gel formulations was further evaluated for uniform distribution of drug in the gel by confocal Raman microscopy. *In vitro* antifungal activity to justify the potential of transungual gel formulation against *C. albicans* and histopathological studies were carried out.

MATERIALS AND METHODS

Poloxomer 407, berberine chloride, thioglycolic acid was purchased from Thomas Baker, Mumbai, India. Absolute ethanol was obtained from Research Lab Fine Chem Industries, Mumbai, India.

The Poloxomer 407 gel was prepared by cold method on weight basis to yield different concentrations (18 to 24% w/w). A weighed amount of poloxomer 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, thioglycolic acid (5% v/v) was dissolved in the above prepared solution. The solution was warmed at room temperature until a smooth gel was produced.

The pH profile of formulated 1% aqueous solution Poloxomer 407 gel was determined using digital pH meter. Gel formulations were analyzed in triplicate. 0.5 g of formulated gel was placed on glass Petri dish. In the centre of Petri plate the gel was poured in the area of 1 cm. The spreadability was determined with varying the weight from 20 to 150 g. The weight was added on the upper surface of the Petri plate, and allowed to rest.

All prepared gels were assessed visually to test homogeneity. The gels were kept in glass container. After sometime when the gels have been set in the container, formulations were tested for their morphological appearance or presence of any aggregates. Texture analysis was performed to gain information about nature of gel like hardness, cohesiveness and adhesiveness of formulated gel. It was determined by process of penetration of a probe of texture analyzer on the gel sample at a predefined rate of force, velocity and depth. All the results were recorded in triplicate at room temperature.

Rheological evaluation of gel formulation was performed using Anton Paar MCR Rheometer using parallel plate geometry with 50 mm diameter and the gap of 0.1 mm between the two plates. The sample was placed between the two plates for 5 min to maintain equilibrium in temperature and for relaxation of stress. The spindle was PP 50 with 10 rpm speed and shear rate varing from 10^{-3} to 10^{3}/s.

The washed and cleaned bovine hooves were hydrated in phosphate buffer saline (pH 7.4) for 24 h. A sharp scalpel was used to remove the dead soft tissue of hooves; these were cut into small pieces according to the area of the diffusion cell. Hydrated hooves were placed in between the donor and receptor compartment of the Franz diffusion cell. The diffusion cell had a cross-sectional area of 1.1304 cm^2 with a volume of 10 ml. One mg of the formulation was applied to the membrane of the bovine hoof. The receptor compartment of the diffusion cell was filled with 10 ml phosphate buffer saline (pH 7.4). The Franz diffusion cell assembly was placed on the magnetic stirrer with the continuous revolution of 400 rpm/ min for 24 h at 37°C. One mg of sample was withdrawn via sampling port at a defined different time interval up to 24 h and replaced with an equal amount of fresh buffer solution into the receptor compartment. The aliquot samples were analyzed for the contents of drug present in the sample spectrophotometrically at 420 nm and the cumulative amount of drug permeated was calculated.

Scanning electron microscopy was carried out to identify the surface morphology of optimized formulation of nanoparticles. The nanoparticles were placed in the sample holder, attached on 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 compatibility studies between drug and

excipients were determined by Perkin Elmer Spectrum, BXII FTIR spectrophotometer. IR Absorption spectra were recorded in 4000 to 400/cm for berberine chloride, Poloxomer 407 using KBr pellet.

XRD were obtained using the Mini Flex2 Goniometer on powder diffraction system. The X-ray generator was operated at 30 kV and 15 mA, using the CuKα line at 1.54056 Å as the radiation source. The powdered sample was placed in a glass specimen holder. To prepare a sample for analysis a glass slide was mounted on the top face of the sample holder. Each sample was filled into the sample holder and tapped smoothly. The samples were scanned from 5 to 80° (2θ) count time of 2.00 s, using an automatic divergence slit assembly and a proportional detector. The samples were scanned at 25°C. Spectra obtained were evaluated for the physical state of the formulation.

The cylinder plate method was used to perform antifungal studies against *C. albicans*. The potato dextrose agar was used as culture media for the growth of *C. albicans (*MTCC227). One ml of the *C. albicans* strain was inoculated into 25 ml of sterile potato dextrose agar at 40°C and 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. One ml of the test formulation was added into the well and the plates were incubated at 37°C for 48 h. After the incubation period, the plates were evaluated for the zone of inhibition (Yang *et al.,* 2019).

Bovine hooves were dipped in distilled water for 24 h. The dust particles and soft tissues were removed and dipped in optimized gel-formulation for 48 h. Histological determination of hooves was performed in comparison with control. The control hooves were treated with permeation enhancer free gel formulation. For 24 h, the hooves were fixed in 10% formalin solution. The hooves were cut by edge surface and vertically, the flat portion was used for study. Selected part of hoof was dehydrated using absolute ethanol and then these were 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 under 100X magnification under microscope. The gel formulation was analyzed for stability studies at room temperature (25°C). The prepared formulation was evaluated for different parameters such as pH, spreadability and homogeneity.

RESULTS AND DISCUSSION

The prepared Poloxomer 407 gel containing thioglycolic acid (BGT; 18, 20, 22 and 24%) was found to possess approx. 5.5 pH as shown in Table 1. Hence, the prepared gel formulations may not cause irritation.

Table 1. pH profile of BGT

S. No.	Concentration of poloxomer 407 (%)	pH
1.	18	5.53±0.52
2.	20	5.78±0.40
3.	22	5.81±0.61
4.	24	5.80±0.50

Spreadability played an immense role in determination of the ease of application of gel formulations on the skin surface. It was a crucial parameter for the semisolid formulations to analyze the consistency of preparation. To achieve the adequate dose of topical formulations it should spread evenly over the surface. Ideally, viscous nature of semisolid preparations defined the spreadability of the formulations (Table 2), the more viscous semisolid preparations gave lower spreadability so, application over the skin was difficult.

Table 2. Spreadability profile of BGT

Type of formulation	Weight (g)	Concentration (%)			
		18	20	22	24
BGT	20	18.25±0.46	17.53±0.78	15.93±0.82	12.15±0.72
	50	22.59±0.84	20.96±0.83	19.96±1.06	16.25±0.64
	100	40.22±1.54	36.20±0.95	34.56±1.05	31.41±1.15
	150	52.55±1.99	50.38±1.40	47.73±1.94	40.79±2.52

Table 3. Texture profile of BGT

Formulation type	Concentration (%)	Hardness (N)	Cohesiveness	Adhesiveness	Springiness
BGT	18	1.47±0.04	0.68±0.04	-0.51±0.02	0.59±0.03
	20	1.54±0.12	0.91±0.02	-0.63±0.06	0.66±0.04
	22	1.69±0.09	0.96±0.02	-0.71±0.02	0.70±0.02
	24	1.70±0.07	0.99±0.02	-1.16±0.24	0.86±0.05

The topical gel formulations will be acceptable with appropriate cohesiveness, hardness and bio adhesiveness. The formulations having high cohesiveness and hardness are unacceptable due to strenuous in their application on the surface of skin. Whereas the formulations having low mechanical properties are not acceptable as they may drip out from the surface of skin. So, it can be concluded that the adhesiveness, cohesiveness and hardness (Table 3) were crucial parameters for the development of semisolid preparations.

18% BGT showed highest release (66.94±1.10%) in 24 h, while the 24% showed minimum release (50.85±0.67%). Fig. 1 displays the cumulative release profile of BGT (18, 20, 22, and 24%).

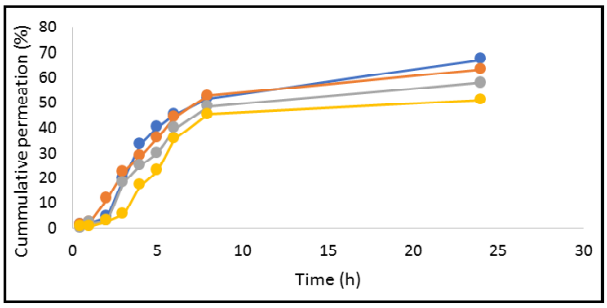

Fig. 1. Cumulative permeation profile of BGT.

The apparent viscosity (η) of the BGT reduced when applied at shear rate (s^{-1}) increases. The results revealed that the gel behaved as a pseudo plastic behaviour (non-Newtonian). Fig. 2 portrays the apparent viscosity of BGT to the applied shear rate.

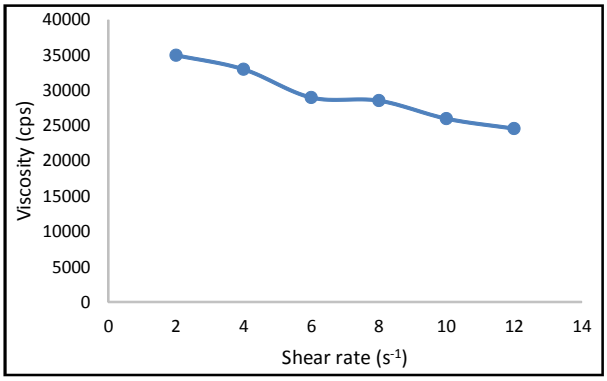

Fig. 2. Rheology profile of BGT.

Scanning electron microscopy was used to determine the surface morphology of bovine hooves. Fig. 3 displays the micrograph of control and BGT treated hooves.

The over lay of FTIR spectra berberine chloride, Poloxomer 407, berberine gel containing thioglycolic acid (BG) is plotted in Fig. 4. The characteristic peaks of berberine chloride were observed at 3049.63 owning to aromatic C-H

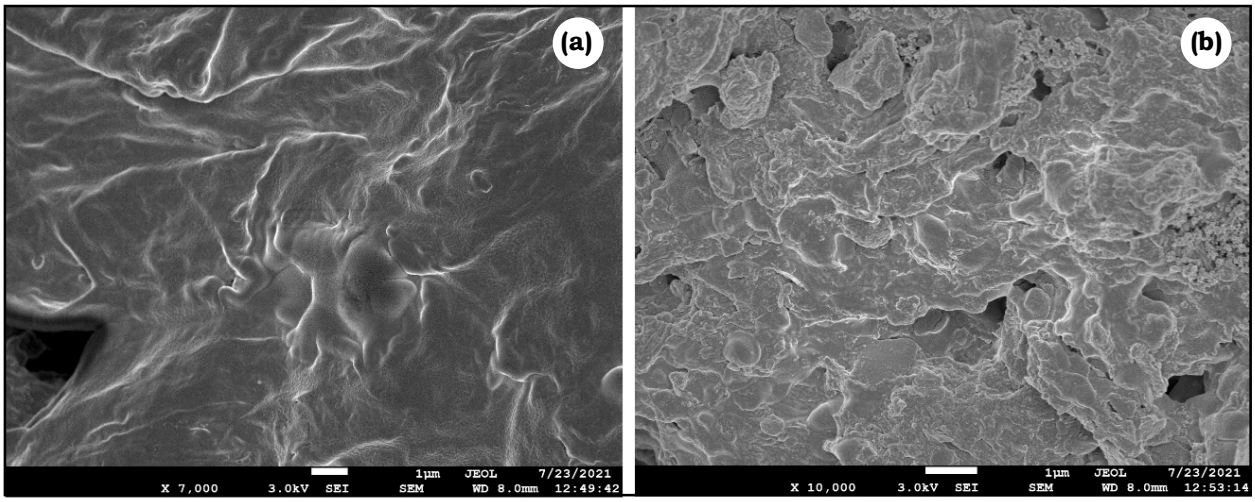

Fig. 3. (a) Control hoof; without permeation enhancer and (b) Impact of thioglycolic acid on the surface morphology of bovine hoof.

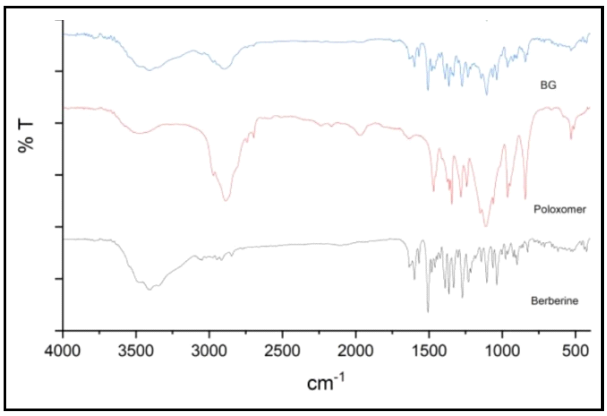

Fig. 4. FTIR spectra of berberine chloride, Poloxomer 407, BG.

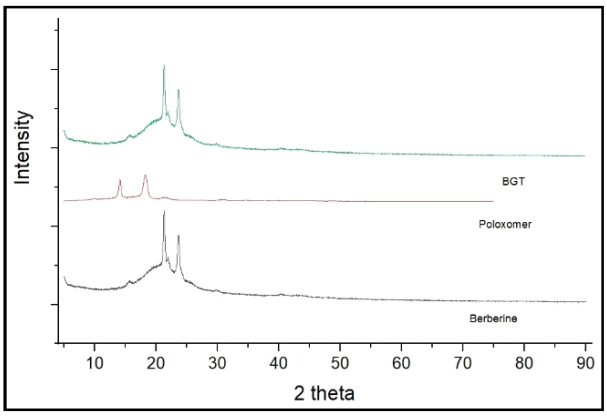

Fig. 5. XRD spectra of berberine chloride, poloxomer 407 and BGT.

stretching, 2915.51, 2846.65 because of aliphatic C-H stretching, 1568.19 corresponding to aromatic C=C, 1272.03, 1142.81 due to asymmetric and symmetric C-O-C stretching, respectively, 1064.95 owning to C-O stretching. The FTIR spectra of Poloxomer 407 showed peaks at 3473.34 due to OH stretching, 2971.08 and 2887.58 confirmed the presence of asymmetric and symmetric stretching, respectively, 1343.69 because of in plane O-H bend and 1111.50 confirmed the presence of C-O stretching. Further, the similar characteristic peaks of berberine chloride, Poloxomer 407 were exhibited in FTIR spectra of BGT. Further, neither absence of any characteristic peak nor formation of new peaks was seen in the spectra. This represented the compatibility between the formulation excipients and berberine chloride.

The XRD observations of the berberine chloride, Poloxomer, BGT, showed significant differences between the diffraction patterns of the drug, excipients and prepared formulations (Fig. 5). Berberine showed diffraction peaks at 13.29, 14.84, 16.69, 20.31, 21.55, 25.78 and 26.61. Poloxomer 407 displayed peaks at 15.54, 21.35, 21.88 and 23.76 and BGT showed peaks at 13.94 and 18.23. The intensity of the berberine chloride peaks in the BGT was decreased due to the dilution effect. Thus, it can be concluded that the drug was uniformly dispersed in the gel formulations.

The *in vitro* antifungal activity of berberine gel was assessed by determining the zone of inhibition of the formulations against *C. albicans* (MTCC 277). The zone of inhibitions produced by BGT formulation showed ZOI against *C. albicans* (Fig. 6). To confirm that the antifungal activity was not due to the presence

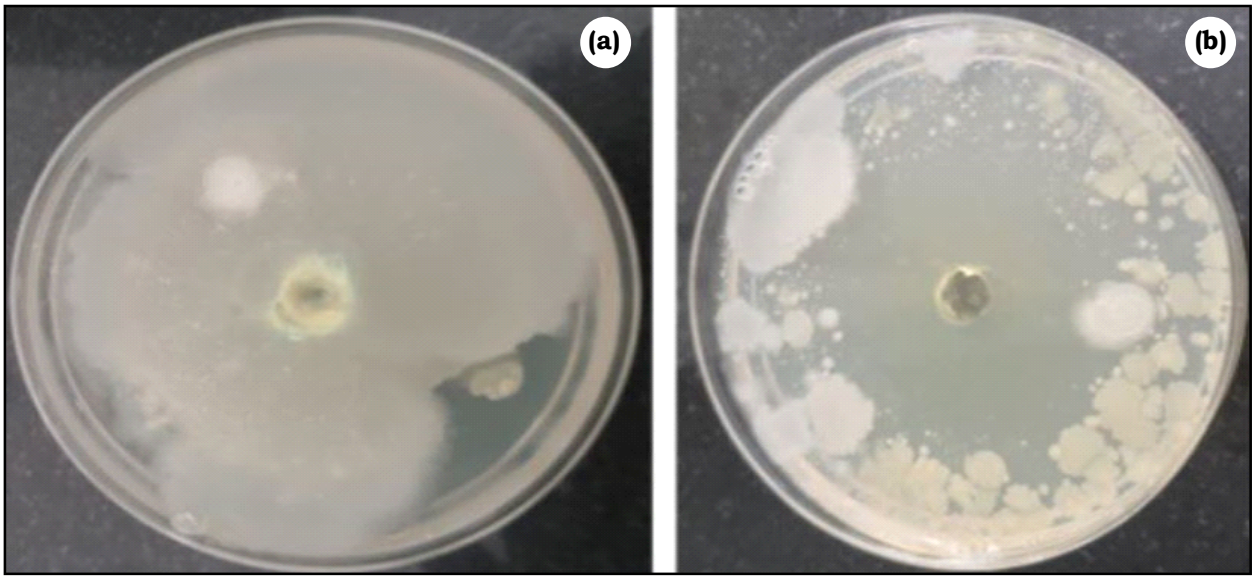

Fig. 6. Zone of inhibition of vehicle control, BGT.

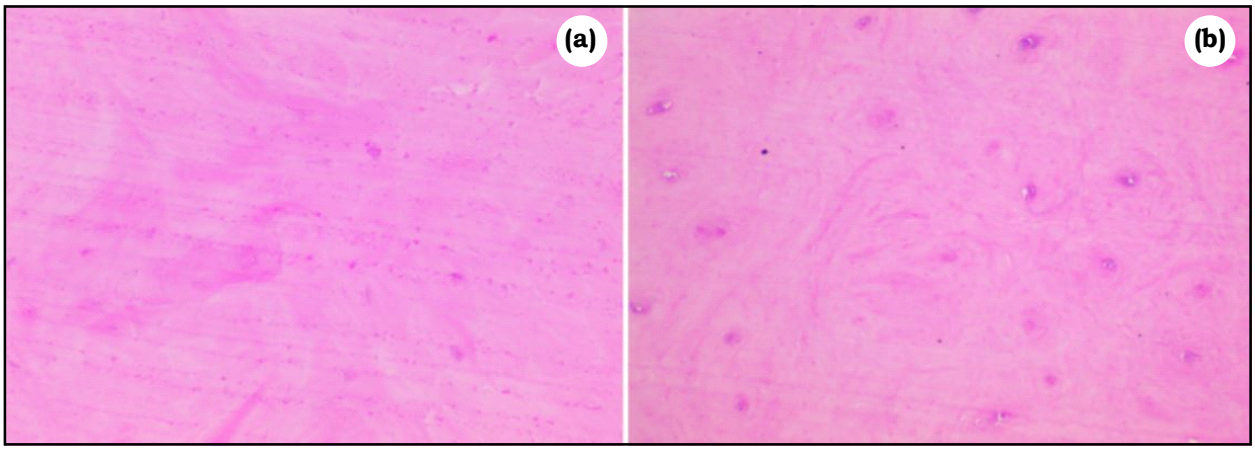

Fig. 7. Histopathology studies of (a) Control and (b) BGT.

Table 4. Stability studies profile of BGT

Type of formulation	Storage conditions	Period (days)	pH	Homogeneity	Spreadability
BGT	25°C	0	5.53±0.52	Pass	17.53±0.78
		30	5.55±0.21	Pass	17.49±0.01
		60	5.62±0.29	Pass	17.30±0.06
		90	5.83±0.25	Pass	17.39±0.13

of other formulation components of gel, vehicle control was also conducted. The results of zone of inhibition of the control formulations were found to be much smaller which confirmed the antifungal activity of BGT. The results showed that the BGT possessed potent antifungal activity against *C. albicans*.

The hoof treated with blank formulation showed no damage in the tissue, while the hooves treated with the BGT batch of gel formulations showed presence of pores which may be attributed to the rupture of disulphide linkage of the keratin layers (Fig. 7).

The stability study was performed to determine the quantitative changes in the formulations over a period of 90 days at room temperature (25°C). The results of stability studies showed that the BGT did not have any eloquent changes in pH, homogeneity and spreadability (Table 4). It can be concluded that BGT formulations were stable and exhibited promising properties over a period of 90 days.

CONCLUSION

In the present study, the antifungal study of berberine chloride was explored for the treatment of onychomycosis by preparing gel formulations. The formulation showed adequate antifungal activity. The results of histopathological studies established that penetration enhancers based formulations promote permeation of berberine chloride by disrupting the disulphide bond of keratin. Further, the six months stability studies data indicated no significant changes in the physico-chemical properties. On the basis of results of the present study, it can be concluded that poloxomer gel formulation of berberine chloride is suitable for the treatment of onychomycosis.

REFERENCES

Agrawal, V., Patel, R. and Patel, M. (2023). Design, characterization and evaluation of efinaconazole loaded poly (D, L-lactide-co-glycolide) nanocapsules for targeted treatment of onychomycosis. *J. Drug Delivery Sci. Tech.* **80**: 01-11.

Bhairy, S., Momin, A., Pednekar, M., Madan, D., Shaikh, A., Bankar, R. and Pitchika, S. (2023). A note on the current status of the transungual drug delivery system at a glance. *Drug Sci. Pharmacy* **3**: 01-21.

Gaba, S., Saini, A., Singh, G. and Monga, V. (2021). An insight into the medicinal attributes of berberine derivatives: A review. *Bioorganic and Med. Chem.* **38**: 01-20.

Jamshaid, F., Dai, J. and Yang, L. X. (2020). New development of novel berberine derivatives against bacteria. *Mini Rev. Med. Chem.* **20**: 716-724.

Kesharwani, P., Fatima, M., Singh, V., Sheikh, A.,

Almalki, W. H., Gajbhiyem V. and Sahebkar, A. (2022). Itraconazole and difluorinated-curcumin containing chitosan nanoparticle loaded hydrogel for amelioration of onychomycosis. *Biom.* **7**: 01-17.

Nair, A. B., Aldhubiab, B., Shah, J., Jacob, S., Attimarad, M., Sreeharsha, N., Venugopala, K. N., Joseph, A. and Morsy, M. A. (2023). Design, development, and evaluation of constant voltage iontophoresis for the transungual delivery of efinaconazole. *Pharmaceutics* **15**: 01-22.

Patel, M. M. and Vora, Z. (2016). Formulation development and optimization of transungual drug delivery system of terbinafine hydrochloride for the treatment of onychomycosis. *Drug Delivery Transl. Res.* **6**: 263-275.

Sveikauskaite, I. and Briedis, V. (2017). Effect of film-forming polymers on release of naftifine hydrochloride from nail lacquers. *Int. J. Polymer Sci.* **2017**: 1-7.

Wang, Y., Liu, Y., Du, X., Ma, H. and Yao, J. (2020). The anti-cancer mechanisms of berberine: A review. *Cancer Manag. Res.* **12**: 695-702.

Yang, Q., Liu, S., Gu, Y., Tang, X., Wang, T., Lui, J. and Jianhua (2019). Development of sulconazole-loaded nanoemulsions for enhancement of transdermal permeation and antifungal activity. *Int. J. Nanomed.* **14**: 55-66.

Zoric, N., Kosalec, I., Tomic ,S., Bobnjaric, I., Jug, M., Vlainic, T. and Vlainic, J. (2017). Membrane of *Candida albicans* as a target of berberine. *BMC Comp. Alt. Med.* **1**: 01-10.