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Research Article

Biocompatible Phospholipid-Based Mixed Micelles for Tamoxifen Delivery: Promising Evidences from *In - Vitro* Anticancer Activity and Dermatokinetic Studies

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Abstract. Tamoxifen (TAM) is frequently prescribed for the management breast cancer, but is associated with the challenges like compromised aqueous solubility and poor bioavailability to the target site. It was envisioned to develop phospholipid-based mixed micelles to explore the promises offered by the biocompatible carriers. Various compositions were prepared, employing soya lecithin, polysorbate 80, sodium chloride/dextrose, and water, by self-assembled technique. The formulations were characterized for micromeritics and evaluated for *in vitro* drug release, hemolysis study, dermatokinetic studies on rodents, and cytotoxicity on MCF-7 cell lines. Cellular uptake of the system was also studied using confocal laser scanning microscopy. The selected composition was of sub-micron range (28.81 ± 2.1 nm), with spherical morphology. During *in-vitro* studies, the mixed micelles offered controlled drug release than that of conventional gel. Cytotoxicity was significantly enhanced and IC_{50} value was reduced that of the naïve drug. The bioavailability in epidermis and dermis skin layers was enhanced approx. fivefold and threefold, respectively. The developed nanosystem not only enhanced the efficacy of the drug but also maintained the integrity of skin, as revealed by histological studies. The developed TAM-nanocarrier possesses potential promises for safe and better delivery of TAM.

KEY WORDS: bioavailability; dermal kinetics; MCF-7 cell lines; MTT assay; percutaneous delivery; skin compatibility; topical delivery.

INTRODUCTION

Various new drug molecules with potential biological efficacy are being explored, but most of them pose delivery challenges like compromised solubility, lower bioavailability, and poor pharmacokinetic profile (1,2). Tamoxifen (TAM) is one such drugs, which finds immense applications in the management of various cancers, esp. breast cancer. Generally, TAM is by oral and parenteral route for the management of cancer. It is listed as a BCS class II drug and many-a-times its citrate salt is used in the pharmaceutical products owing to the poor solubility issues. To circumvent the delivery challenges associated with TAM, variety of drug delivery carriers including dendrimers nanosponges (3), graphene (4), lipid nano capsules (5), nanostructured lipid carriers (6), polymeric nanoparticles (7), hollow manganese ferrite nanocarriers (8), chitosan/lecithin nanoparticles (9), self-nanoemulsifying drug delivery systems (10), flexible membrane vesicles, liposomes (11), lipoplex (12), vesicles (13), lipid vesicles (14), lecithin organogels (15), nanoemulsions (16), gold nanoparticles (17), and cyclodextrin nanoparticles (18) have been developed. In our laboratory, recently, a few more attempts have been made to deliver TAM employing pluronic lecithin organogels (19), flexible membrane vesicles, and polymeric micelles (20,21). Though all these attempts are novel, but they either require tedious synthetic approaches or employ non-economic and bio-incompatible excipients.

A close scrutiny by this research group revealed that phospholipid-based mixed micelles have not yet been explored for topical delivery of TAM. The main ingredient, phospholipid, is a well-established biomaterial with immense biocompatibility and delivery promises. On the other hand, Tween 80 is non-ionic, biocompatible, and non-immunogenic

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surfactant, approved by various federal agencies for i.v., oral, and topical routes. Henceforth, it was envisioned to develop phospholipid-based mixed micelles loaded with TAM and explore the same for the delivery of TAM by topical route that too employing simple dispersion technique.

MATERIAL AND METHODS

Materials

Phospholipid 90G was provided *ex gratis* by M/s Phospholipid GmBH, Nattermannalle, Germany. Sodium chloride, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were purchased from M/s Central Drug House (P) Ltd., New Delhi, India. Dextrose and ethanol were procured from M/s RFCL Limited, New Delhi, India and M/s Jai Chemical Pharma Works, Jaipur, resp. HPLC column and membrane filters were provided by M/s Merck Specialties Pvt. Ltd., Mumbai, India. HPLC grade water, methanol, acetonitrile (ACN), and sodium lauryl sulphate were bought from M/s Spectrochem Pvt. Ltd., Mumbai, India. Dialysis membrane (average flat width 22.54 mm, average diameter 14.3 mm, capacity 1.61 mL/cm, molecular cut off 12,000 to 14,000 Da, and pore size of 2.4 nm) was procured from the M/s Himedia Laboratories Private Limited, New Delhi, India. Tamoxifen (TAM) and dimethyl sulphoxide (DMSO) were procured from M/s Sigma-Aldrich, New Delhi, India. MCF-7 cancer cell lines were acquired from European Collection of Cell Cultures (ECACC), a Culture Collection of Public Health, England. All other chemicals were of analytical grade and used without additional purification. Distilled water was employed throughout the study.

Animals

Wistar rats (6–8 weeks old, 200 ± 20 g) and female Laca mice (4–6 weeks, 15 ± 5 g) were obtained from Central Animal House, Panjab University, Chandigarh, India. All the pathogen-free animals were kept at a temperature of 25 ± 2°C and a relative humidity of 70 ± 5% under natural light/dark conditions for at least 48 h before dosing. The experiments were performed according to the animal ethical guidelines. All the animal protocols were duly approved by *Institutional Animal Ethics Committee* (IAEC/S/14/79-2014-15), Panjab University, Chandigarh, India.

Methods

Preparation of TAM-Loaded Phospholipid-Based Mixed Micelles

Mixed micelles of TAM were prepared by spontaneous micelles formation, as described by Song *et al.*, 2011 (22). Briefly, a total of 27 batches were prepared for the final selection of the formulation ($n = 3$), as per the compositions shown in Table I. The 09 formulations were designed in a strategic manner and coded from F_1–F_9. Initial three formulations (F_1–F_3) were prepared without NaCl/dextrose, with NaCl (0.9% *w*/*v*) and dextrose (5% *w*/*v*), respectively. In next three formulations, *i.e.*, F_4–F_6, the amount of phospholipid was doubled and the formulations without NaCl/dextrose, with NaCl (0.9% *w*/*v*) and dextrose (5% *w*/*v*) were prepared. In the last set of F_7–F_9, the level of Tween 80 was increased from 532 g to 1064 mg and the formulations without NaCl/dextrose, with NaCl (0.9% *w*/*v*) and dextrose (5% *w*/*v*) were prepared. For selection of ethanol amount, preliminary studies ranging from 1 to 10% *w*/*w* of ethanol were performed and 6% *w*/*w* was selected on the basis of drug and lipid solubility. In brief, for any coded formulation, TAM, Tween 80, and PL were dissolved in dehydrated ethanol with continuous stirring. Subsequent to that, this homogeneous phase was added in a streamlined manner in 60 s to 5% (*w*/*v*) dextrose solution or 0.9% (*w*/*v*) NaCl solution or plain water to give clear micellar dispersion. The resulting mixed micellar dispersions were filtered from the membrane filter (0.22 μm) and stored in refrigerator till use.

Particle Size, Zeta Potential, and PDI

Particle size, PDI, and zeta potential were determined by means of Malvern Zetasizer (S. No. MAL1040152, Software version v6.01, M/s Malvern Instruments Limited, Worcestershire, UK) installed at the Department of Pharmacy, Birla Institute of Technology, Pilani, Rajasthan, India. The average value of three measurements for each sample was reported as the final result.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) analysis was performed using Hitachi H-7500 (M/s Hitachi High-Technologies Europe GmbH, Krefeld, Germany) installed at the Central Instrumentation Laboratory, Panjab University, Chandigarh, India. For analysis, 20 μL of the sample were deposited on carbon/membrane-coated copper grids and stained with 1% phosphotungstic acid. Finally, the grid was air dried and observed under electron microscope and micro photographed.

Entrapment Efficiency

Entrapment efficiency (EE) of the drug in the mixed micelles was determined by dialysis bag method. Mixed micelles equivalent to 1 mg of TAM were packed in dialysis bag and dipped in 30 mL of ethanol in a beaker for 2 h with continuous stirring. After complete diffusion of un-entrapped drug, the contents of the dialysis bag were analyzed for entrapped drug.

In Vitro Drug Release Studies

Phospholipid-based mixed micelles equivalent to 1 mg of TAM were placed in a pouch made up of dialysis membrane and the sealed pouch was dispersed in 30.0 mL diffusion medium, comprised of a solution of ethanol and phosphate buffer saline, pH 5.6 in 1:9 *v*/*v* ratio. The system was maintained at 37 ± 1°C with gentle magnetic stirring at 50 rpm. Samples of 0.5 mL were withdrawn at the time intervals of 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360, 720, and 1440 min, and the receptor compartment was replaced by equal volume of fresh diffusion medium, every time. The amount of TAM released was determined by double-beam

Table I. Composition of Various Mixed Micelle Formulations

S. No.	Formula code	TAM (mg)	PL (mg)	T 80 (mg)	EtOH (g)	NaCl (mg)	Dextrose (mg)	Water (g)
1.	F1	20	60	532	1.2	–	–	18.2
2.	F2	20	60	532	1.2	180	–	18.0
3.	F3	20	60	532	1.2	–	1000	17.2
4.	F4	20	120	532	1.2	–	–	18.1
5.	F5	20	120	532	1.2	180	–	17.9
6.	F6	20	120	532	1.2	–	1000	17.1
7.	F7	20	120	1064	1.2	–	–	17.6
8.	F8	20	120	1064	1.2	180	–	17.4
9.	F9	20	120	1064	1.2	–	1000	16.6

UV-visible spectrophotometer at the λ_{max} of 301 nm. Blank micelles (without drug) were subjected to the same protocol and the respective samples from these blank formulations served as the blank for the UV-visible spectrophotometry to nullify the interference, resulting from the possible leaching of PL and Tween 80 (Cary 100 UV–vis, M/s Agilent Technologies, Manesar, Haryana, India).

Ex Vivo Hemolysis Studies. Healthy Wistar rats were employed for collection of the blood samples. Sampling of 1 mL of blood was done from retro-orbital plexus of the animal and immediately collected in 124 mM sodium citrate (1:9 *v/v* ratio of sodium citrate solution and blood). Erythrocytes were immediately harvested by centrifuging the blood and washed thrice with normal saline. The collected RBCs were re-suspended in normal saline. To this, RBC dispersion of the test sample (TAM and TAM-loaded phospholipid-based mixed micelles) was added. The resulting two test tubes with the test samples and the third one with RBCs dispersed in double-distilled water were incubated for 1 h at 37°C with the aid of gentle shaking. Centrifugation at 2000×*g* for 5 min. was performed, after incubation and the supernatant was analyzed spectrophotometrically at the λ_{max} of 415 nm. Hemolysis induced by double-distilled water was treated as reference, *i.e.*, 100% hemolysis (22).

MTT Assay. Human breast cancer cells, *i.e.*, MCF-7 cells, were grown in 96-well tissue-culture plates. Two different concentrations (equivalent to 1 and 10 μg/mL of TAM) of test samples (TAM and TAM micelles) were added to cells. Plates were incubated at 37°C for 48 h using CO_2 incubator. To the incubated plates, MTT solution of 20 μL (2.5 mg/mL) was added and the set-up was gently stirred. Plates were again incubated and centrifuged for 15 min at 400×*g*. The resulting MTT-formazan crystals were dissolved in 150 μL of DMSO, which were previously collected by discarding the supernatant. Plates were again stirred and measured for optical density at 570 nm, and 620 nm as reference wavelength with the help of microplate reader. Treated cells were washed and pictured under inverted microscope for morphological changes. The microphotographs were taken under resolution of ×30 ($n = 3$).

Confocal Laser Scanning Microscopy. MCF-7 cells were grown in culture medium and transferred into 96 plates at the density of 15,000 cells/cm^2. Coumarin 6 dye was loaded into the mixed micelles in the concentration of 50 μg/mL. Tamoxifen-loaded mixed micelles were transferred into the 96-well plates containing cultured cells and incubated at 37°C for 24 h. Cells were washed thrice employing PBS 7.4. Ice cold methanol was added to fix the cells and again it was washed with PBS 7.4. To stain cell nuclei, 300 nM DAPI solution was added into cell culture containing mixed micelles for 05 min. Cells were again washed employing PBS 7.4 to remove excess amount of DAPI. The cells were scanned and observed under confocal laser scanning microscope (Nikon C2 Plus, with NIS Elements Version 4.3 Software, M/s NIKON Instruments INC., Melville, NY, USA), installed at UGC-Centre of Excellence in Applications of Nanomaterials, Nanoparticles & Nanocomposites, Panjab University, Chandigarh, India. Coumarin-6 dye was excited at 488 nm and emitted at 500–550 nm. DAPI was excited and emitted at 405 nm and 417–477 nm, respectively. The photographs of fluorescent nuclei and the cells were clicked at ×60 magnification. All the experiments were performed in the triplicate ($n = 3$).

Ex Vivo Dermatokinetic Studies. Wistar rat skin was used for the studies on Franz diffusion cells (M/s Permegear, Inc., PA, USA), as per the previous reported method (23). After sacrificing the animals, the hair on the dorsal side of animals were removed. The skin was harvested, freed of adhering fat layers, and mounted on Franz diffusion cells having a cross-sectional area of 3.142 cm^2 and receptor volume of 30.0 mL. Methylene blue dye test was performed to test the integrity of the skin (24). The diffusion medium in the receptor compartment was composed of ethanol–phosphate-buffered saline mixture, pH 5.6 in 1:9 *v/v* ratio. The assembly was maintained at 37 ± 1°C with the help of thermo-regulated outer water jacket, while the diffusion medium was stirred continuously at 50 rpm using a magnetic stirrer. Diffusion cells were covered with aluminum foil to avoid contact of the donor as well as receptor components with the light. Formulation (conventional gel and TAM-loaded mixed micelles; equivalent to 1 mg of TAM) were applied on the skin with the help of micro-spatula and the drug amount was determined by mass difference. However, the mixed micellar dispersion was spread on rat merely by means of a pre-calibrated dropper. The formulations stayed fix throughout the study, as the donor was sealed with parafilm, and the study was continued. Permeation study was performed for duration of 6 h. The whole skin was removed from the Franz cell at the respective

sampling time and washed thrice to remove any adhering formulation. The clean skin sample was soaked in hot water (60°C) for 30 s to facilitate the detachment of epidermis from dermis. The resulting wedge of the rat skin, exposing the cleavage of epidermis and dermis, was pulled out with the help of a forceps. Both of these separated sections were cut into pieces of small size in separate containers, and kept in methanol (5 mL) for 1 day for complete drug extraction, in refrigerator. After filtering the solution through a membrane (0.45 μm), the filtrate was analyzed using the validated RP-HPLC technique (LC-2010C HT, M/s Schimadzu Co., Ltd., Chiyoda-ku, Tokyo, Japan) with the conditions as follows: Merck HPLC Column: Oyster BDS C18 (250 × 4.6 mm, 5 μm); mobile phase acetonitrile: 50 mM potassium phosphate pH 3.0 (45:55%, *v*/*v*); column temperature: 30°C; detection wavelength 254 nm; flow rate: 1.0 mL/min; injection volume: 5 μL; run time: 30 min.; and detector used: PDA (SPD-M20A) (15). The various dermatokinetic parameters were calculated on MS-office Excel using of one compartment open body model (21).

Skin Compliance Studies. Pathogen-free female Laca mice were employed for the skin compliance studies and were allocated into three groups of four animals each. Hair was removed by use of depilatory cream (Veet, M/s Reckitt Benckiser, Gurgaon, India). Hair-removed skin was wiped three to four times with saline-presoaked cotton to remove the adhere materials. Each group from 1 to 3 was treated with conventional hydrogel, mixed micelles formulation, and saline solution (treated as control), respectively (equivalent to 2 mg of TAM/dose), once a day. Each animal was kept in separate cage under dark/light standard laboratory conditions (temperature 25 ± 2°C and relative humidity 70 ± 5%). The respective application was continued for 2 weeks. The animals were sacrificed by cervical dislocation, and skin was harvested in 10% formalin solution. Skin histopathology was performed, after staining the skin with hematoxylin and eosin (24).

RESULTS AND DISCUSSION

Particle Size, PDI, and Zeta Potential

Figure 1 shows the micromeritic as well as surface charge profiles of the developed nanocarriers. The size range of the micelles varied from 10.08 to 222.60 nm, whereas the PDI values were consistently below 0.280. Lower values of PDI assured homogeneity in the particle size of the dispersed system. However, slightly negative values of zeta potentials may be ascribed to the presence of alcohol, whereas phospholipid and Tween 80 tend to bring the zeta potential values close to neutral. As a general observation, the particle size was slightly enhanced when phospholipid levels were increased, whereas increased Tween 80 levels resulted in smaller-sized micelles systems. On the other hand, the dextrose seems to increase the size of the micelles; however, in conjugation with Tween 80, the size enhancement was negligible. To proceed further, the formulation-coded F_1 was selected, as it offered maximum sustenance (>90 days).

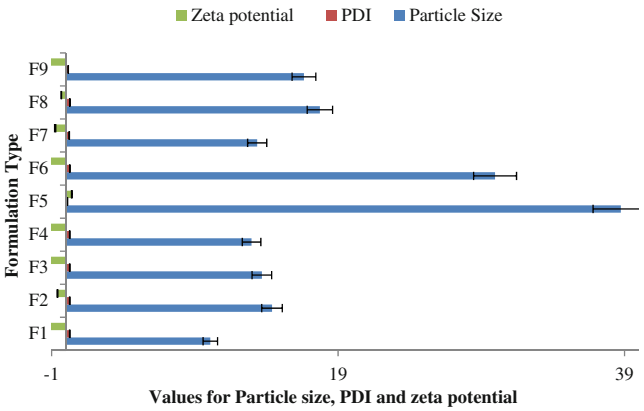

Fig. 1. Average size, PDI, and zeta potential values of various developed systems ($n = 3$)

Transmission Electron Microscopy

The transmission electron microscopy (TEM) microphotograph of selected mixed micelles is portrayed in Fig. 2. The microphotograph has been captured at ×400,000 magnification. The photograph depicts the presence of turgid micellar vesicles with near spherical geometry. The micellar dispersion was composed of non-aggregated spherical nanoconstructs without significant aggromelation.

Entrapment Efficiency

EE of the mixed micelles was found to be 86.56 ± 2.60%. Content of TAM in 1 mL was 0.86 mg. This higher drug entrapment assured adequate loading of the substantial amount of the chemotherapeutic agent for sustained release.

In Vitro Drug Release

The drug release profile from the studied system has been shown in Fig. 3. The amount of naïve drug diffused

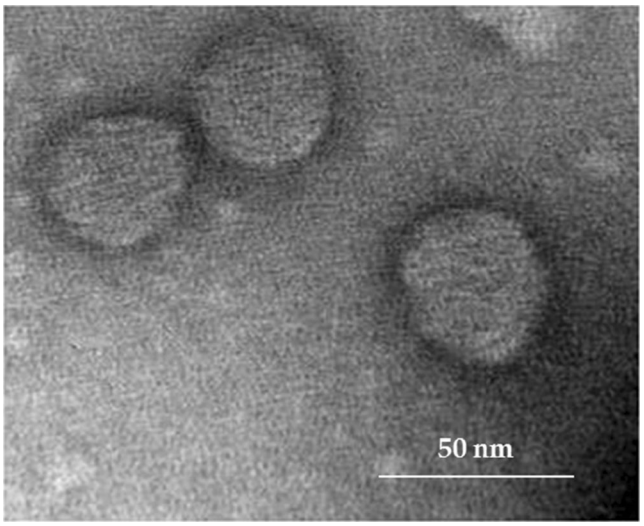

Fig. 2. TEM microphotograph of mixed micelles at ×400,000

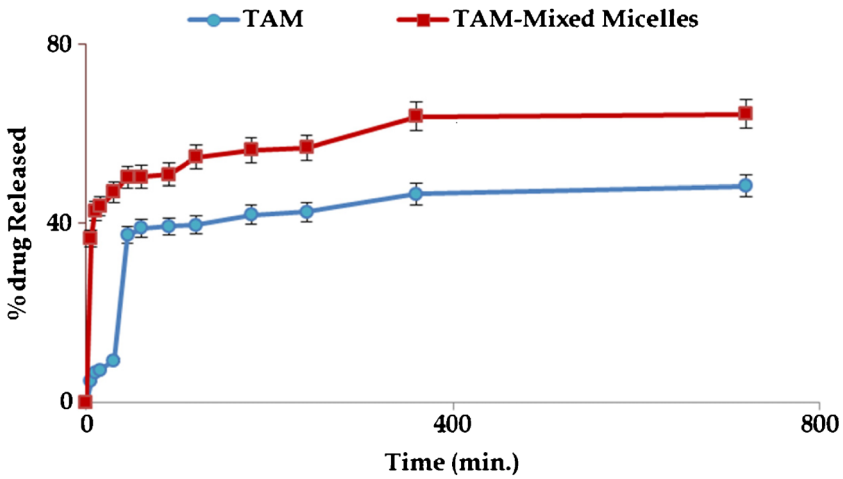

Fig. 3. *In vitro* release pattern of TAM from naïve drug and mixed micelles ($n = 3$)

across the semipermeable membrane was about 75% to that of the amount of drug diffused from the mixed micelles in the studied period of 24 h. The average drug diffusion flux for the naïve drug was 7.35 μg cm^{-2} h^{-1}, whereas for the micelle-loaded TAM, the obtained value was 9.94 μg cm^{-2} h^{-1}. The enhanced diffusion of a poorly soluble drug can be ascribed to the solubility enhanced by the strategically formulated micellar composition.

Biological Evaluation of Phospholipid-Based Mixed Micelles

Ex Vivo Hemolysis Studies

Percentage hemolysis (2.1%) was decreased to its half value (1.12%) to that of TAM-encapsulated phospholipid-based micelles. On the other hand, the blank micelles were well-tolerated by the erythrocytes (0.11% hemolysis), owing to biocompatible and immunoneutral components. The obtained results assured that encasement in mixed micelles can significantly enhance the blood-compatibility of the anticancer agents, indicating a potential surfactant-free anticancer product. Though the developed system is for topical application, but hemolysis study is important as there are reports that drug-loaded nanocarriers can reach the systemic circulation. Henceforth, the findings ensured substantial hemo-compatibility, if the drug-loaded nano-carriers reach the systemic circulation.

MTT Assay

The results obtained from MTT assay in MCF-7 breast cancer cell lines are portrayed in Fig. 4a, b. Cytotoxicity of blank phospholipid-based mixed micelles was found negligible at the studied concentration of 1 and 10 μg/mL. However, pure TAM offered cytotoxicity in the range of 16 to 35% at these concentrations. On the other hand, incorporation in mixed micelles substantially enhanced the cytotoxicity by approx. two times. This can be

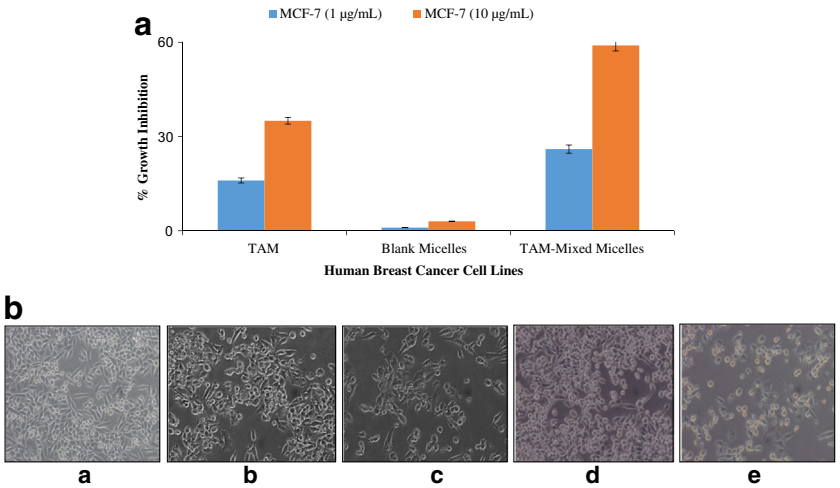

Fig. 4. **a** Graphical presentation of the cancer cell cytotoxicity on MCF-7 cells offered by various treatments. **b** Microphotographs of MCF-7 cell lines: (*a*) control; (*b*) TAM, 1 μg/mL; (*c*) TAM, 10 μg/mL; (*d*) TAM-loaded mixed micelles, 1 μg/mL; (*e*) TAM-loaded mixed micelles, 10 μg/mL

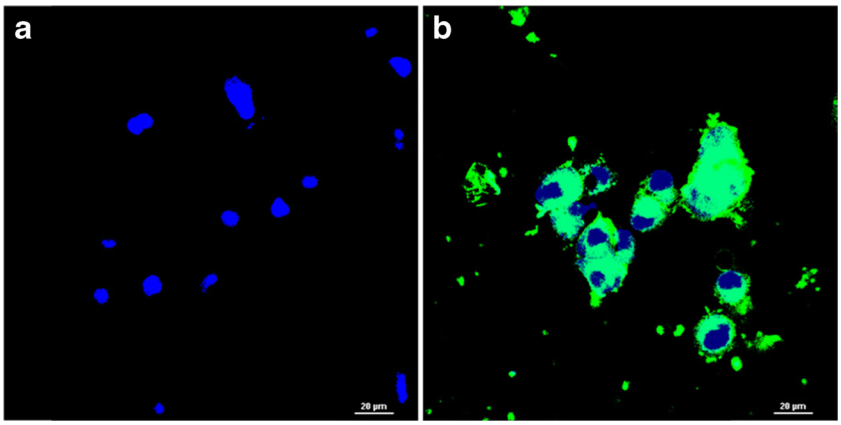

Fig. 5. Confocal laser scanning microphotographs of (×60): **a** Control cells with stained nuclei. **b** Coumarin-6 tagged TAM-loaded mixed micelles

ascribed to the better adhesion and penetration of drug encased in phospholipid-based carriers. The resemblance in the biological membranes of the cells and the mixed micelles, *i.e.*, phospholipid bilayer might have significantly contributed to the better penetration of the drug-loaded carriers. On the other hand, enhanced solubilization of this highly permeable, but solubility compromised drug can also be one of the major contributing factors.

Confocal Laser Scanning Microscopy

It was observed in Fig. 5 that coumarin-6 tagged TAM-loaded mixed micelles got an easy access to the cytoplasm as well as cell nuclei of the cancer cells. The blue stain represents the DAPI-stained nuclei, whereas the green color represented the invasion by coumarin-6 tagged drug-loaded nanocarriers. Interestingly, it was also seen that plain dye was unable to penetrate the cells. Significant portion of nucleoplasm was also observed to be penetrated by the nanocarriers, indicating enhanced cellular uptake. Data derived from the confocal laser scanning microscopy correlates the enhanced cytotoxic efficacy of the mixed micelles, and justifies the result of MTT assay as well as dermatokinetic studies.

Ex Vivo Dermatokinetic Studies

Dermatokinetic studies confirmed substantial deposition of TAM in epidermis and dermis of Wistar rat skin, as shown in Fig. 6a, b. It was observed that the mixed micelles significantly enhanced the epidermal and dermal bioavailability of TAM in substantial amounts *vis-à-vis* the conventional gel, as shown in Table II. The bioavailability enhancement in epidermis was about five times, whereas in dermis, it was around three times to that of the conventional gel. Mixed micelles offered enhanced permeation to epidermis (approx. three times) and dermis (about two times) in comparison to the conventional gel. The results are in consonance with the drug diffusion studies employing semipermeable membrane. The elimination (K_e) rate constant for both the layers were found to be significantly lower for mixed micellar system ($p < 0.01$). The drug clearance from epidermis was retarded by approx. eight times and from dermis, this retardation was of the order of 21 times to that of the conventional hydrogel. As per the applied dose per 3.14 cm^2, the maximum concentration achieved could have been 318.47 µg/cm^2; this amount was leached to various layer in various time points, depending on the characteristics of carriers, drug, skin, and sink on the contrary; no peak of 4-hydroxy tamoxifen or *n*-

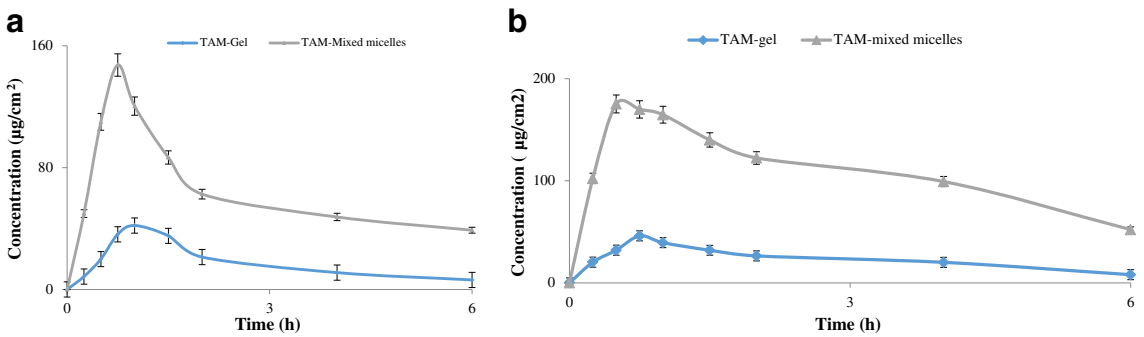

Fig. 6. **a** Graphical representation of the amount of drug present in the epidermis of Wistar rats at various time intervals. **b** Graphical representation of the amount of drug present in the dermis of Wistar rats at various time intervals

Table II. *Ex Vivo* Dermatokinetic Modeling of Conventional Hydrogel and Mixed Micelles in Dermis and Epidermis

Dermatokinetic parameters	Conventional hydrogel		Mixed micelles	
	Epidermis	Dermis	Epidermis	Dermis
$AUC_{0-\infty}$ (μg cm^{-2} h)	163.30 ± 9.8	127.70 ± 8.91	895.85 ± 71.67	434.96 ± 30.44
C_{max}^{Skin} (μg cm^{-2})	37.92 ± 2.41	26.97 ± 2.07	261.14 ± 17.76	123.33 ± 8.01
T_{max}^{Skin} (h)	0.91 ± 0.06	1.20 ± 0.08	0.47 ± 0.03	0.68 ± 0.05
K_p (h^{-1})	2.70 ± 0.20	1.58 ± 0.09	8.00 ± 0.054	2.72 ± 0.22
K_e (h^{-1})	0.30 ± 0.01	0.37 ± 0.02	0.21 ± 0.01	0.68 ± 0.04

desmethyl tamoxifen was observed in whole study, indicating no degradation of drug. This indicates that the developed system possesses better skin delivery potential as well as forms a drug depot in skin layers. This depot formation will help to sustain the drug release to the target site for longer periods. This desired pharmacokinetic change in skin offers huge potential in cancer chemotherapy, especially in breast cancers.

Skin Compliance Studies

Histopathological microphotographs of animal skin treated with normal saline, conventional formulation, and mixed micelles have been depicted in Fig. 7. The studies confirmed the biocompatibility of the developed system on the skin, as no marked changes in skin histopathology were observed, after the treatment. However, signs of skin inflammation and acute damage were observed in the skin sections of the animals receiving once-a-day application of convention gel of TAM.

CONCLUSIONS

The developed TAM-nanocarrier possesses potential promises for safe and better delivery of TAM to the cancer cells with substantial blood and skin compatibility. The enhancement in the skin bioavailability and sustained release of drug from skin layers by means of biocompatible nanocarriers are the unique desired outcomes. Such biocompatible, simple, and scalable nanocarriers provide a hope to enhance the outcomes from chemotherapy in early stages of cancer. The same can be further explored to *in-vivo* studies and such simple nanocarrier systems can emerge as better alternative carriers for the delivery of various anticancer agents.

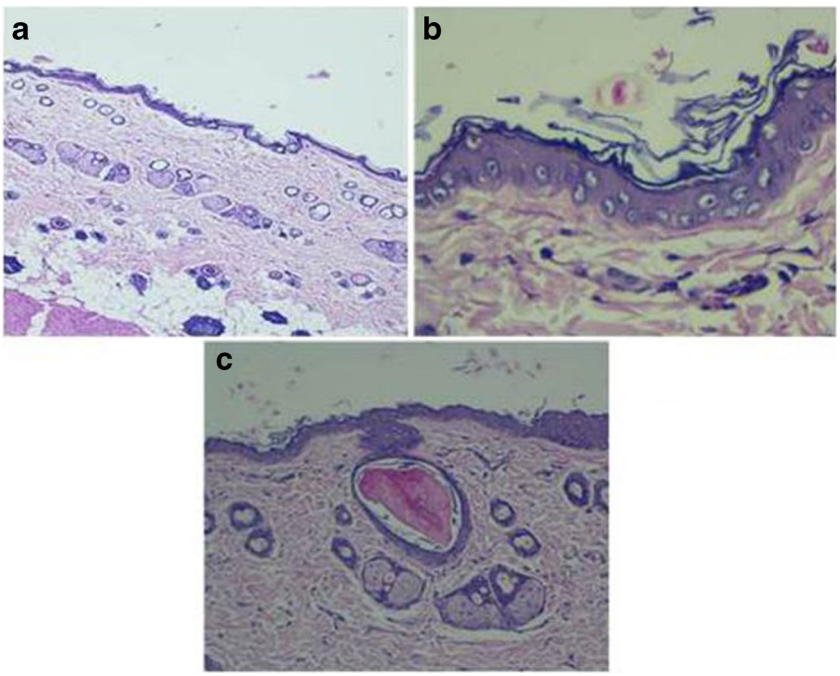

Fig. 7. Photomicrograph of skin sections of mice treated with: **a** saline (control), **b** conventional hydrogel, and **c** mixed micelles

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

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