—Regular Articles—

Preparation and *in Vitro*, *in Vivo* Characterization of Elastic Liposomes Encapsulating Cyclodextrin-Colchicine Complexes for Topical Delivery of Colchicine

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In the present study, an attempt was made to develop a cyclodextrin-colchicine complex and to study its effect on skin permeation and deposition of colchicine. Colchicine β cyclodextrin (BCD) complex was prepared by freeze-drying method and complex formation was confirmed by NMR and *in vitro* drug release study. Formulation containing cyclodextrin-drug complex showed 6-fold increase in transdermal flux in comparison with drug solution. Skin retention studies were carried out with the objective of determining the depot effect of elastic liposomes in skin. The amount of drug deposited was 12.4-fold higher in case of elastic liposomes of colchicine-cyclodextrin complex ($567 \pm 1.5 \mu g$) than drug solution ($46 \pm 1.1 \mu g$). The biological evaluation of various vesicular formulations and drug solution was carried out using monosodium urate-induced air pouch model. The results of anti-gout activity in rats showed better and sustained biological effects over 24 h measured in terms of exudate volume, reduction in leukocyte count, decrease in inflammatory cell accumulation, and collagen deposition with colchicine-BCD elastic liposomal formulation than drug solution. Hence the present study reveals that colchicine-cyclodextrin-elastic liposomes approach possesses good potential to enhance skin accumulation, prolong drug release, and improve the site-specificity of colchicine.

Key words——cyclodextrin-colchicine complex; topical administration; sustained delivery; skin deposition; anti-gout activity

INTRODUCTION

Colchicine is an ancient drug, widely used in the treatment of gout. This medication has also found a place in the treatment of several cutaneous diseases such as psoriasis[1] and actinic keratosis.[2] However, the risk/benefit ratio of colchicine use is high. Gastrointestinal side-effects such as nausea, diarrhea, vomiting, and stomach upset occur in 80% of patients with therapeutic doses of colchicine.[3] Oral colchicine cannot be given for >1 week because its accumulation in the body leads to risk of bone marrow suppression.[4] Accumulation of colchicine in patients with renal dysfunction is a risk factor for neuromyopathy.[5] Colchicine can be administered intravenously; however, this route of administration is associated with potentially serious adverse effects such as tissue necrosis, cytopenias, disseminated intravascular coagulation, and death.[4] Another limitation of colchicine use is its very narrow therapeutic index. Colchicine is effective at a dose of 0.015 mg/kg, toxic in doses >0.1 mg/kg, and lethal when given at 0.8 mg/kg. Therefore the narrow therapeutic margin of colchicine is a source of concern for prescribing physicians.[6]

Oral administration of colchicine has several dose-dependent side effects that suggest the need for developing an alternative dosage form that selectively delivers colchicine topically to affected joints. Colchicine has been shown to have beneficial effects on different dermatological conditions including leukocytoclastic vasculitis, psoriasis, and Sweet's syndrome on topical administration. Topical colchicine has also been reported an effective treatment for actinic keratosis. The main limitation is poor skin permeation therefore effective concentration has not been fully developed.[7] Colchicine medication is less expensive than most available immunosuppressive agents; however, current therapy and its associated toxic effects limit its use as first-line therapy in different dermatological conditions. It has been suggested that topical administration of colchicine could be beneficial in the treatment of gout and other dermatological conditions along with increase in its site-specificity. In our previous study, we prepared a colchicine elastic liposomal formulation and found 8-fold enhancement in skin deposition and 4-fold increase in biological anti-gout activity as evaluated by Mono sodium urate (MSU)-induced arthritis model[8] in comparison with colchicine solution in PBS. In this

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study an attempt was made to prepare β cyclodextrin-colchicine complex and to study its effect on *in vitro* skin permeation and deposition and biological anti-gout activity.

Elastic liposomes have been widely studied as vehicles for dermal and transdermal drug delivery,[9] and their benefit in enhancing skin permeation is well established. Poor encapsulation of water-soluble drug in aqueous compartment of these vesicles, poor dermal deposition, and drug leaking of these vesicles on storage was observed.[10] Recently, encapsulation of drug in the form of cyclodextrin (CD)-drug complex in vesicular formulation has been investigated as a new strategy for overcoming the above problems and merging the relative advantages of the two types of carrier into a single system,[11] by obtaining drug-in-cyclodextrin-in-elastic liposome formulations. The present work was carried out to explore the advantage of using CDs as drug carriers and encapsulation of this complex in elastic vesicles and its effects on skin deposition of colchicine. Elastic vesicles are known to increase skin permeation of colchicine and further use of drug-BCD complex is expected to increase skin deposition due to complexation property and facilitating skin permeation due to penetration-enhancement effect of BCD. In particular, the focus of the present investigation was to see whether colchicine-BCD complexation would help in increasing the formation of skin depot and sustaining the release of drug.

MATERIALS AND METHODS

Materials Colchicine was received as a gift sample from Cepham Pharmaceuticals Pvt. Ltd., India. Soya phosphatidylcholine (PC), Sephadex G-50, picrosirius red, and 6-carboxyfluorescein (6-CF) were purchased from Sigma Chemicals USA. Span 60, β-Cyclodextrin, Span 80, and cholesterol were purchased from Hi Media Ltd., India. Ethanol, acetonitrile, acetic acid, hematoxylin, eosin, and phosphomolybidic acid were procured from E. Merck, India. All reagents used in this study were of analytical grade.

Preparation of Cyclodextrin-colchicine Complex Preparation of complex of colchicine and cyclodextrin was performed by freeze-drying method.[12] Complex of colchicine and cyclodextrin (1 : 2 M) was made by adding a required quantity of colchicine (1 M) to aqueous cyclodextrin solution (2 M) while

mixing by magnetic stirrer at slow speed (100 rpm). After 24 h agitation, the resulting solution was frozen at −60℃ and lyophilized in a freeze-dryer (HETO, Allerod, Denmark) for 24 h. Formation of colchicine-cyclodextrin complex was confirmed by NMR studies.

Preparation of Formulations The elastic liposomal formulation was prepared by conventional rotary evaporation sonication method.[13] Briefly, phospholipid and surfactant were taken in a clean, dry, round-bottom flask. This lipid mixture was dissolved in chloroform : methanol mixture (2 : 1). The organic solvent was removed by rotary evaporation above the lipid transition temperature (Rotary Evaporator, Superfit, India). Final traces of solvent were removed under vacuum. The deposited lipid film was hydrated for 1 h with drug, drug-cyclodextrin complex, or fluorescence marker solution in ethanol (7% v/v) by rotating at 60 rev min^{-1} at 40±1.0℃. The resulting vesicles were swollen at room temperature for 2 h to obtain large multilamellar vesicles (LMLVs). To prepare smaller vesicles, LMLVs were probe-sonicated (40 w) at 4℃ for 20 min (Probe Ultrasonicator, Imeco Ultrasonics, India). The sonicated vesicles were extruded through a sandwich of 100 and 200 nm polycarbonate membranes (Millipore, USA). The final lipid and drug concentration in vesicular formulations was 5% w/v and 0.2% w/v, respectively.

Characterization of Elastic Liposomal Formulations The vesicle size and distribution were determined by dynamic light scattering (DLS) method (CILAS, 1064L, France). For morphological characterization, transmission electron microscopic (TEM) studies using phosphotungastic acid as negative stain were performed (Moragagni 268D FEI, Netherlands). A drop of the sample was placed on a carbon-coated copper grid to leave a thin film on the grid. Before the film dried on the grid, it was negatively stained with 1% phosphotungastic acid (PTA). A drop of the staining solution was added to the film and excess solution drained off with filter paper. The grid was allowed thoroughly to dry in air and samples were viewed under a transmission electron microscope. Vesicles without sonication were also visualized by optical microscope (Olympus, DX31, Japan).

The entrapment efficiency was determined after separating unentrapped drug by Sephadex G-50 column. The eluted vesicles were lysed by Triton-X 100 (0.1% v/v) and subsequently analyzed for drug

content. Elastic liposomal formulation (without sonication) was diluted 5 times with 0.9% NaCl solution and elastic liposomes/mm³ was counted by optical microscopy using haemocytometer. Elasticity of vesicle membrane was determined by extrusion method.

Skin Permeation and Deposition Study   The *in vitro* skin permeation of colchicine from different formulations was studied using Franz glass diffusion cell maintained at $37\pm1^{\circ}C$ under nonocclusive conditions. The effective permeation area of the diffusion cell was 2.303 cm². The receptor compartment contained 22.5 ml phosphate buffer saline (PBS pH 6.4) and was constantly stirred at 100 rpm. Excised albino abdomen rat skin was mounted between the donor and the receptor compartment. Elastic liposomal formulation (2.0 ml equivalent to 4.0 mg of colchicine) was applied to the skin epidermal surface. The samples (0.5 ml) were withdrawn through the sampling port of the diffusion cell at 1, 2, 4, 6, 12, 16, 20, and 24 h time intervals and analyzed. An equal volume of fresh phosphate buffer maintained at $37\pm1^{\circ}C$ was replaced into the receptor compartment after each sampling. An *in vitro* drug release study of different formulations was also performed with cellophane membrane (molecular weight cutoff 12000–14000, HIMEDIA, Ltd. Mumbai, India) using the same method as described above.

At the end of the permeation experiments (24 h), the surface of the skin was washed 5 times with 50% ethanol then with water to remove excess drug from the surface. The washing protocol was verified and found to remove >95% of the applied dose at zero time. The skin was then cut into small pieces. The tissue was further homogenized with 50% ethanol (10 ml) and left at room temperature for 24 h. After shaking for 5 min and centrifugation for 5 min at 3000 rpm, the colchicine content in the upper phase was determined.[14,15]

Vesicle-Skin Interaction Study

Scanning Electron Microscopy   Wistar rats weighing 150–200 g were divided into 4 groups each comprising 3 rats. The first group served as control and received topical application of drug solution (1.0 mg/ml) prepared in PBS pH 6.4. The second, third, and fourth groups received 1.0 ml of drug-cyclodextrin solution in PBS, colchicine elastic liposomal formulation, and colchicine-BCD elastic liposomal formulation, respectively. The formulations were applied non-occlusively to the abdomen side of the rat

over an area of 1 cm². The treated rats were caged and sacrificed after 6 h of treatment. The skin was removed immediately and fixed at $4^{\circ}C$ in Karnovsky's fixative overnight followed by 1% w/v osmium tetraoxide for 2 h and finally in ruthenium tetraoxide 0.2% w/v and $K_3Fe(CN)_6$ 0.25% w/v for 1 h. After fixation, the samples were dehydrated in a range of ethanolic solutions 70, 90, 95, and 100% v/v and coated with gold coater.[16,17] The coated samples were visualized under scanning electron microscope (SEM, LEO43 SVP, Cambridge). All investigations were performed after approval of the Institutional Animal Ethics Committee of the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala and in accordance with the disciplinary principles and guidelines of CPCSEA.

Confocal Laser Scanning Microscopy (CLSM)
Wistar rats weighing 150–200 g were divided into 4 groups each consisting of 3 animals. First group served as control and received topical application of 1.0 ml of 0.16% w/v solution of marker 6CF in PBS (pH 6.4). The second, third, and fourth groups received topical application of 1.0 ml of 6CF-BCD solution, 6CF loaded elastic liposomes, and 6CF-BCD loaded elastic liposomes, respectively. The formulations were applied topically on the abdomen of rats at a marked area of 1 cm². The animals were caged individually after application of formulation and sacrificed after 6 h of application. The skin was removed immediately, cut into pieces, and washed with PBS. The skin was blotted and fixed in Carny's fluid (absolute alcohol : chloroform, 3 : 1) for 3 h and sectioned into the pieces of 1 mm² size and evaluated for depth of penetration of 6-CF. The full skin thickness was optically scanned at different increments through the z-axis of a CLS microscope (Leica, DMIRE2, Germany). Optical excitations were carried out with a 489 nm Argon laser beam; fluorescence emission was detected above 515 nm for 6-CF.

In Vivo **Evaluation**

Evaluation of Anti-gout Activity   To perform biological evaluation of anti-gout activity of elastic liposomal formulation of colchicine, the monosodium urate crystals were synthesized by an established procedure.[18] The crystals were suspended in PBS at a concentration of 10 mg/ml. The crystals were sonicated to obtain rod-shaped crystals of uniform length (5–25 μm) and sterilized by autoclaving at $121^{\circ}C$ for 30 min. The subcutaneous air pouch model was used

for evaluation of anti-gout activity.[19] Briefly, the rats were anesthetized, the dorsal area was shaved, and 10 ml sterile air was injected subcutaneously. Sterile air was reinjected in air pouch every 2 days to maintain pseudo-gout conditions. After 6 days the rats were randomly divided into 2 groups. Group 1 [GP1 (sham control)] comprised 9 animals that received 10 ml normal saline solution into subcutaneous air pouch. Group 2 (GP2) comprised 45 animals that received 10 ml MSU (1 mg/ml) into subcutaneous air pouch. Furthermore; GP2 was again subdivided into 5 subgroups each containing 9 rats. After 24 h of MSU administration, rats in the first subgroup [GP2a (control group)] received no treatment whereas the second subgroup (GP2b) rats were treated with drug solution in PBS on the air pouch. Rats in the third, fourth, and fifth subgroups were treated by applying drug-cyclodextrin solution in PBS, colchicine elastic liposomes formulation, and colchicine-cyclodextrin elastic liposomes formulation on the air pouch. Rats ($n=3$) from GP1 and subgroups of GP2 were sacrificed at 6, 12, and 24 h, respectively, by spinal dislocation method and various parameters were employed to check the extent of anti-gout activity.

Measurement of Exudate Volume of Air Pouch and Leukocytes Count   The pouch exudate was collected from all groups by glass syringe just before sacrificing animals. The exudate volume was measured immediately after collection by graduated centrifuge tubes. Inflammatory exudate harvested from each animal was placed into heparinized saline. An aliquot of the diluted exudate was used to count leukocytes.

Histopathological Studies   The skin from air pouch was excised and immediately immersed in 10% buffered formalin, dehydrated in graded concentrations of ethanol, immersed in xylene, and embedded in paraffin. The 5-μm thick sections of skin were cut by microtome and mounted on slides using commercial Baker's mounting fluid. The paraffin wax was removed by warming the slide gently until the wax melted and then was washed with xylene followed by washings with absolute alcohol and water. The sections were stained with hematoxylin-eosin and picrosirius red stains to determine gross histopathology and collagen deposition, respectively. The slides were analyzed at 100-fold magnification by optical microscope.

Statistical Analysis   Data are expressed as mean ± standard deviation (S.D.) of obtained results. Statistical analysis of the data was performed by analysis of variance (ANOVA) (Graphpad, Version 2.01, San Diego, CA). A p value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Colchicine is a highly hydrophilic drug and has poor skin permeation due to its hydrophilic nature.[20] Colchicine is recommended in different skin disease conditions along with primary use in gout.[1,2] In our previous study we encapsulated colchicine in elastic liposomes and found that skin deposition was increased 10 fold in comparison with drug solution while biological activity was found to increase 4 fold.[8] In this study colchicine-β cyclodextrin complex (BCD) was encapsulated in elastic liposomes and its skin permeation and deposition potential studied.

Table 1 shows the composition of different formulations of colchicine elastic liposomes and colchicine-BCD elastic liposomes. Colchicine elastic liposomal formulation was prepared using the formula PC: Span 80 (85 : 15) and colchicine (0.2% w/w) as optimized in previous study.[8] BCD was selected as complexing agent among the CDs due to its low water solubility (1.88 % w/w) and ability to form a complex with hydrophilic drug.[21] BCD complex was reported for increasing the skin deposition of hydrophilic drug. Martins et al.[22] reported 2-fold enhancement in skin deposition of meglumine antimoniate hydrophilic drug by complexation with BCD. Colchicine is a highly hydrophilic drug and a major rate-limiting step for the transport of colchicine is its permeation through the stratum corneum (SC).[2,23] Once permeated through the SC, colchicine is rapidly absorbed into the systemic circulation. As a result, hydrophilic drugs elicit poor local pharmacological response due to low retentivity in the skin layers. Encapsulation of colchicine in elastic liposomes was found to increase its skin deposition 8.0 fold and use of colchicine-BCD complex is further expected to increase the skin deposition of colchicine by retarding its rapid release and absorption in the systemic circulation.

In Vitro Characterizations   Colchicine-BCD complex was prepared by freeze drying method. ^{1}H NMR spectral analysis was carried out to check the formation of colchicine-BCD complex (Fig. 1). Triplet observed at δ 7.3 and doublet at δ 7.1 shows

Table 1. Characterization of Colchicine and Colchicine-BCD Elastic Liposomal Formulations

Characterization parameters	*Colchicine elastic liposomal formulation	**Colchicine-BCD elastic liposomal formulation
Shape	Spherical vesicular	Spherical vesicular
Vesicle size	135 ± 11	152 ± 14
Entrapment efficiency	66.3 ± 2.2	79.5 ± 1.6
No. of vesicles per cubic mm % Drug release across the cellophane membrane	51 ± 2.0	50 ± 1.0
After 12 h	50.2 ± 1.9	44.1 ± 2.9
After 24 h	79.2 ± 2.3	65.9 ± 4.2
Elasticity	56.9 ± 2.8	58.5 ± 4.2

Values represented as mean $\pm$ S.D. $(n=3)$. * Composition: Lipid 5% w/v [PC+Span 80 (85 : 15)]+Drug 0.2% w/v. ** Composition: Lipid 5% w/v [PC+Span 80 (85 : 15)]+Drug-BCD complex 0.2% w/v.
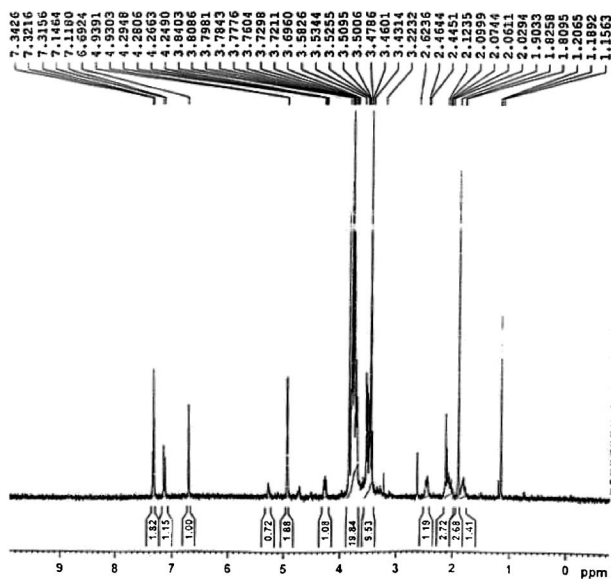

Fig. 1. NMR Spectra of Colchicine-BCD Complex

Fig. 2. *In Vitro* Drug Release of Different Formulations of Colchicine across the Cellophane Membrane
All values shown as mean $\pm$ standard deviation $(n=3)$.

the presence of aromatic nucleus of colchicine. Doublet observed at δ 4.9 accounts for BCD. Shifts in peaks at δ 2.24, 2.26, and 2.6 are due to formation of complex of colchicine with BCD.[24]

Formation of complex was further confirmed by *in vitro* drug release study across the cellophane membrane in comparison with plain drug. Figure 2 compares the percent drug release of colchicine from solution in PBS, colchicine-BCD complex solution in PBS, and colchicine and colchicine-BCD loaded elastic liposomal formulation across the cellophane membrane. The release rate was slower in the case of colchicine-BCD complexation in comparison with colchicine probably due to formation of complex. The release rate of colchicine from colchicine-BCD complex was lower than plain drug. After 2 h, 93.2%
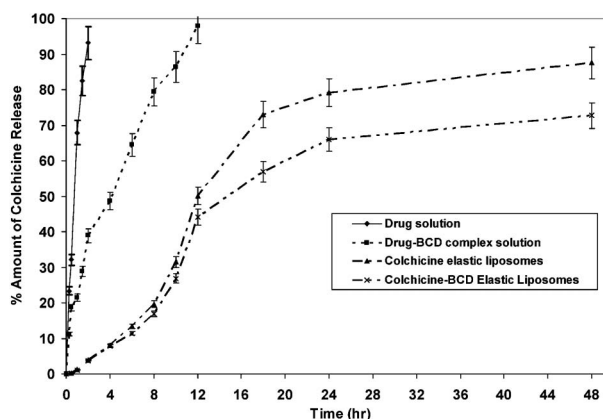
drug was released from drug solution. In comparison at 2 h, 38.9% and at 8 h 79.4% of colchicine was released from the colchicine-BCD complex. Further decrease in release rate was observed with elastic liposomal formulation prepared with drug-BCD complex (65.9% at 24 h and 72.7% at 48 h). The release experiments clearly indicate sustained release of colchicine from elastic liposomal formulations. This decrease in the release rate is due to the formation of additional barrier due to complexation for releasing the drug from vesicle membrane.

Different characteristic parameters of vesicular formulations are summarized in Table 1. Vesicle size of colchicine elastic liposome and colchicine-BCD elastic liposomes was found 135 ± 11 and 152 ± 14 nm, respectively. No significant difference was found in no. vesicles/mm³ of colchicine elastic liposomes and colchicine-BCD elastic liposomes formulation. The entrapment efficiency of colchicine elastic liposomes

and colchicine-BCD formulation was found 66.3± 2.2% and 79.5±1.6%, respectively. The significantly higher entrapment efficiency of colchicine-BCD elastic liposomes formulation is due to better retention of colchicine-BCD complex in elastic liposomes interior due to the hydrophobic nature of BCD. Colchicine is a hydrophilic drug and has poor entrapment efficiency in vesicular bilayer due to poor partition.[25]

Skin Permeation and Deposition Study The results of *in vitro* skin permeation studies of different formulations of colchicine across excised rat abdominal skin conducted using Franz-diffusion cell are summarized in Table 2. The value of transdermal flux for colchicine and colchicine-BCD complex loaded elastic liposomes was found 44.4±1.9 and 26.4±1.2 $\mu g/cm^2/$ h, respectively. On the other hand, colchicine-BCD complex solution in PBS showed a 3.4-fold increase in skin permeation in comparison with colchicine drug solution.

Skin retention studies were carried out with the objective of determining the depot effect of elastic liposomes in skin. For effective management of inflammation in gout, and therapeutic effect in other dermatological conditions such as psoriasis and leukocytoclastic vasculitis, the dosage form should accumulate drug mainly into the skin. The amount of drug deposited in skin was found 8.4-fold higher after 24-h administration of elastic liposomes and 3.4-fold higher after administration of colchicine-BCD solution as compared with drug solution. Therefore it was hypothesized that encapsulation of colchicine-BCD complex in elastic liposomes could lead to potentiation of colchicine deposition. The skin deposition of colchicine-BCD formulation of elastic liposomes was found 12.4 times higher after 24-h administration (Table 2).

The observed better skin accumulation of colchicine-BCD elastic liposomes could be attributed to difference in the rate of drug diffusion in skin. Cyclodextrin is mainly used as permeation enhancer in pharmaceutical preparations. The feasibility of using cyclodextrin to increase the skin accumulation of colchicine was investigated. Skin permeation and deposition study showed that complexation of colchicine with cyclodextrin significantly increased its skin deposition. Colchicine is a hydrophilic drug and after reaching in deeper layers of skin, that is its site of action in different dermatological conditions, it gets rapidly absorbed in systemic circulation and shows poor local pharmacological response.[2,23] In comparison, when encapsulated as BCD complex the drug is released slowly as evidenced by *in vitro* drug release study (Fig. 2) and acts as local depot. The decrease in permeation rate may be attributed to the formation of stable complex. Ammar *et al.*[24] reported the formation of stable β-cyclodextrin-colchicine complex with marked protection from photo-degradation of colchicine. It is reported that BCD complexation decreases the permeation of topically applied drug and increases its skin accumulation by formation of stable complex.[26] The hydrophobic nature of β-cyclodextrin retards the dissolution of water-soluble drugs to produce sustained release effect.[27] Results of skin permeation study also indicate the depot forming ability of colchicine-BCD formulation of elastic liposomes. The value of transdermal flux for colchicine and colchicine-BCD-loaded elastic liposomal formulation was found 44.4±1.9 and 26.4±1.2 $\mu g/cm^2/h$, respectively. The significant ($p<0.05$) decrease in flux value of BCD-colchicine formulation suggests depot-forming ability of formulation. Similarly, Tenjarla *et al.*[28] and Simeoni *et al.*[29] reported reduced skin permeation and increased skin accumulation of miconazole and oxybenzone by cyclodextrin complexation.

Vesicle Skin Interaction Study The outermost

Table 2. Transdermal Permeation Parameters of Colchicine Formulations across Rat Skin

S. No.	Formulation code	Jss[1] ($\mu g/cm^2/h$)	Amount of drug deposited (μg)	ER[2]	ER[3]
1	Colchicine elastic liposomes	44.4±1.9	383±2.4	8.4	10.2
2	Colchicine-BCD elastic liposomes	26.4±1.2	567±1.5	12.4	6.1
3	Colchicine-BCD solution	12.6±0.8	156±1.4	3.4	2.9
4	Drug solution	4.3±0.6	46±1.1	—	—

Value represented as mean ±S.D. ($n=3$). Jss[1] =Transdermal flux, calculated from the slop of Cartestan plot of cumulative amount of drug present in receptor compartment *versus* time. ER[2] =Enhancement ratio; It is ratio of amount of drug deposited from formulation to drug solution. ER[3] =Enhancement ratio; It is ratio of transdermal flux from formulation to drug solution.

layer of the skin, SC, is believed to constitute the major barrier for drug permeation and is regarded as a heterogeneous 2 compartment system composed of keratin-filled corneocytes, embedded in an intercellular lipid matrix. SEM studies using rat skin were conducted to investigate the effect of BCD-colchicine complexation on surface morphology of skin measured in terms of lipid perturbation and pore formation on the skin surface. Figure 3 (a)−(d) shows SEM photomicrographs of rat skin treated with PBS (pH 6.4) containing drug considered as control, colchicines-BCD solution in PBS, and colchicine and colchicines-BCD complex-loaded elastic liposomal formulation. In rat skin incubated with PBS containing drug, there was no lipid perturbation and surface irregularities showing that drug itself does not act as permeation enhancer. In comparison in rat skin treated with colchicines-BCD complex lipid perturbation and surface disordering were observed (Fig. 3 (b)) due to penetration-enhancement effect of BCD. BCD has been reported to act as a skin penetration enhancer by many authors.[23—26] Skin treated with elastic liposomes encapsulated with cyclodextrin-drug complex showed the greatest surface disordering due to lipid perturbation along with pore formation (Fig. 3 (d)). SC surface disordering is probably due to the lipid perturbation effect of cyclodextrin. In compari-

son the lipid perturbation effect was significantly less in skin treated with colchicine elastic liposomal formulation (Fig. 3 (c)), showing better skin permeation potential of drug-cyclodextrin elastic liposomal formulation. The observed order of disordering and pore formation of SC layer was colchicine-BCD elastic liposomes>colchicine elastic liposomes>colchicine-BCD complex solution>drug solution.

The skin deposition potential of BCD-colchicine complex was further confirmed by CLSM studies. Untreated rat skin did not show any fluorescence. Figure 4 (a)−(d) shows CLSm photomicrographs of rat skin treated with 6-CF solution in PBS (pH 6.4), 6CF-BCD complex solution in PBS (pH 6.4), and 6-CF and 6-CF-BCD-loaded elastic liposomal formulation. CLSM photomicrographs show significantly deeper (250 μm) and higher penetration of fluorescence probe 6-CF when applied as complex encapsulated in elastic liposomal formulation (Fig. 4 (d)) in comparison with plain 6-CF-loaded elastic liposomal formulation (Fig. 4 (c)) (200 μm). Application of 6-CF-BCD complex as PBS solution (80 μm) showed 2.5-fold less skin deposition (Fig. 4 (b)) in comparison with that encapsulated in elastic liposomes. These results suggest good skin deposition potential of BCD complex-loaded elastic liposomal formulation probably due to synergistic effect of combination of com-
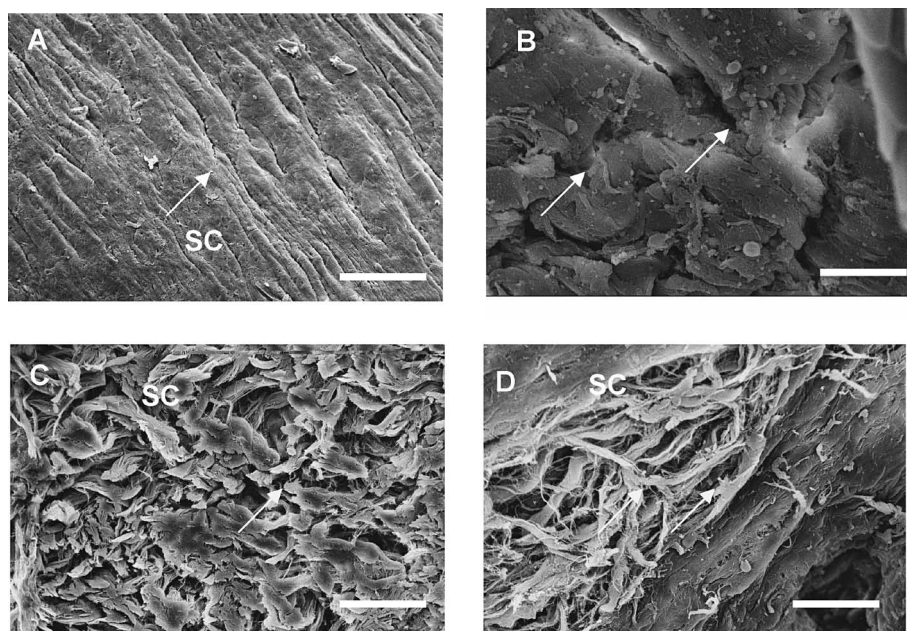

Fig. 3. Scanning Electron Microscopy Micrograph of Viable Rat Skin
    Treated with Phosphate buffer solution of drug (A), solution of drug-BCD (B), colchicine elastic liposomes (C) and colchicine-BCD elastic liposomes (D) after 6 h of treatment. SC= Stratum corneum. Arrow indicates the effect of treatment on surface morphology of skin. IL=Inter lamellar space. Scale bar=100 nm. Magnification×15000.

plexation and vesicular approach. Elastic liposomes have better skin permeation potential in comparison with conventional liposomes formulation due to elasticity of vesicle membrane and permeation effect of phospholipids and surfactant as constituent. Different scientific groups reported better skin permeation of elastic liposomes.[8–10,13,30,31)] BCD is a well-known skin permeation enhancer and increases permeation of drug molecules by intercellular lipid perturbation as evidenced by SEM study, the same pathway that elastic liposomes follow for skin penetration. The observed better skin deposition of BCD-elastic liposmes formulation is due to the lipid perturbation effects of BCD that facilitated the permeation of elastic liposomes and retarded release of colchicine.

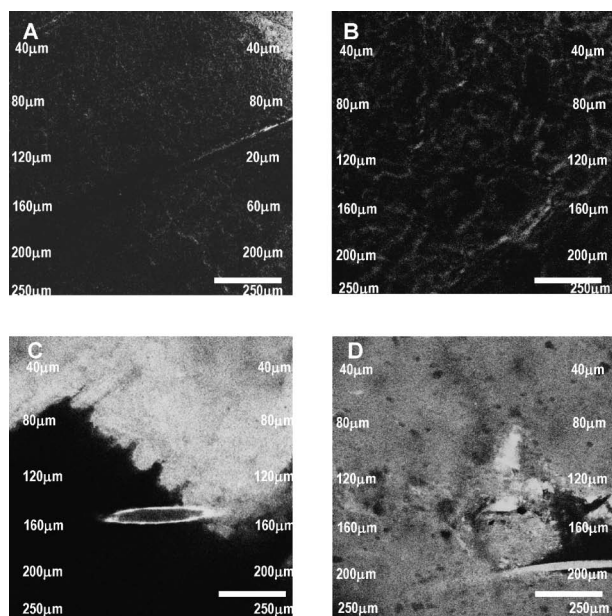**Evaluation of Biological Anti-gout Activity**   *In*

Fig. 4.   CLSm Photomicrograph of Viable Rat Skin
    Treated with 6-CF marker solution in PBS (A), 6-CF-BCD solution in PBS (B), colchicine elastic liposomes (C) and colchicine-BCD elastic liposomes (D) after 6 h of treatment. Scale bar＝100 nm. Magnification× 10000.

vivo performance of different colchicine formulations was evaluated by MSU-induced rat air pouch model.[32)] Edema is a characteristic feature of inflammation that was measured in terms of exudate volume in the present study. Results of exudate volume and leukocyte count are indicators of inflammation in joint spaces and their increase is proportional to the severity of inflammatory condition. The exudate collected was measured before rats were sacrified at 6, 12, and 24 h in various groups employed in the present study (Table 4) and leukocytes quantification was done as summarized in Table 4. There was a continuous increase in exudate volume in MSU-treated control group (GP2a; 3.0 ± 0.2, 5.1 ± 0.6, and 6.5 ± 0.9 ml measured at 6, 12, and 24 h) than saline-treated sham group (GP1; 2.0 ± 0.1, 2.1 ± 0.2, and 2.0 ± 0.2 ml). Moreover, exudate collected in the control group (GP2a) was more turbid than that from the sham control group (GP1), indicating enhanced inflammatory cell infiltration. Colchicine formulations applied after 24-h administration of MSU as pseudo-gout conditions were fully developed at this time as evidenced by 3.0-fold higher exudate volume and leukocyte count of MSU-treated group in comparison with the sham control group. The colchicine and colchicine-BCD-complex loaded elastic liposome-treated group showed 6.6 and 8.1-fold higher reduction in exudate volume as compared with drug solution after 24 h of treatment (Table 4). Similar better reduction in exudates volume was also observed at 6 and 12 h of treatment. Similarly, significantly higher reduction in leucocytes count was observed after 6, 12, and 24 h of treatment with colchicine-BCD encapsulated elastic liposomal formulation in comparison with colchicine-encapsulated elastic liposomes, colchicine-BCD complex solution, and drug solution in PBS groups. The better *in vivo* performance of BCD-colchicine complex is possibly due to increased depot-forming ability

Table 3.   *In Vivo* Study Frame for Rat Air Pouch Model for Evaluation of Anti-Gout Activity

S. No.	Group	Normal saline	MSU (1 mg/ml) solution	Treatment
1	GP1 (SHAM GROUP)	10 ml	NO	NO
2	GP2a (CONTROL GROUP)	NO	10 ml	NO
3	GP2b (TREATED GROUP)	NO	10 ml	Drug solution
4	GP2c (TREATED GROUP)	NO	10 ml	Colchicine-BCD solution
5	GP2d (TREATED GROUP)	NO	10 ml	Colchicine elastic liposomes
6	GP2e (TREATED GROUP)	NO	10 ml	Colchicine-BCD elastic liposomes

Table 4. Evaluation of Anti-gout Activity

Group	% Reduction in volume of exudate*			% Reduction in leukocyte count*		
	6 h	12 h	24 h	6 h	12 h	24 h
GP2a (Control Group)	—	—	—	—	—	—
GP2b (Drug solution Treated Group)	7.1±0.4	12.1±0.8	9.6±0.5	6.9±0.3	14.1±1.8	5.9±0.3
GP2c (Colchicine-BCD solution Treated Group)	17.9±0.6 (2.5)**	28.2±2.1 (2.3)	13.2±1.4 (1.4)	15.2±1.0 (2.2)**	35.8±2.1 (2.5)	11.2±1.1 (1.9)
GP2d (Colchicine elastic liposomes Treated Group)	30.0±1.5 (4.2)	41.2±3.6 (3.4)	63.1±5.7 (6.6)	29.1±2.2 (4.2)	51.8±3.2 (3.7)	65.2±6.0 (11.1)
GP2e (Colchicine-BCD elastic liposomes treated group)	29.8±1.1 (4.2)	48.3±4.2 (4.0)	78.2±6.2 (8.1)	28.7±1.8 (4.2)	63.2±4.0 (4.5)	78.6±6.7 (13.3)

Value represented as mean±S.D. ($n=3$). * Values indicate the percentage reduction of volume of exudates and leukocyte count compared to control group ($(V_{control}-V_{treated})/V_{control}*100$). ** Enhancement ratio: Ratio of biological effect compared to drug solution treated group.

along with sustained release and increase amount of colchicine available at the site of action.

Better anti-gout activity of developed formulation was further confirmed by staining of air pouch membrane with eosin-hematoxylin for checking the extent of infiltration of inflammatory cells and picrosirius red stain for determination of collagen deposition.[33] Figure 5(a)−(d) shows eosin-hematoxylin-stained air pouch membrane photomicrographs of colchicine solution, colchicines-BCD complex solution, colchicine, and colchicine-BCD complex-loaded elastic liposomal formulation, respectively. The level of infiltrating inflammatory cells was minimum in colchicine-BCD elastic liposomes-treated group (Fig. 5 (d)) whereas the maximum was in colchicine solution-treated group. The extent of treatment on air pouch membrane of different colchicine formulations was found colchicine-BCD elastic liposomes>colchicines elastic liposomes>drug-BCD complex solution >drug solution. In addition to this the reduction of inflammatory response was sustained to 24 h in colchicine-BCD elastic liposomes-treated group in comparison with the drug solution-treated group which did not show any effect after 6 h of administration.

Figure 5(e)−(g) shows collagen-specific picrosirius red stained air pouch membrane photomicrographs of saline-treated sham control group, control group, drug solution, BCD-drug complex solution, and drug and drug-BCD complex-loaded elastic liposomal formulation. Collagen deposition is also an important indicator for development of fibrosis associated with gout.[34] An increase in collagen deposition reflects the severity of disease. MSU-treated control group showed significantly higher collagen deposition in comparison with the sham control group, indicating

the development of pseudo gout conditions.[8] Collagen deposition was found minimum in colchicine-BCD elastic liposomes-treated group (Fig. 5(g)) whereas the maximum was in drug solution-treated group (Fig. 5(e)). The extent of treatment on collagen deposition of different colchicine formulations was found drug-BCD elastic liposomes>colchicines elastic liposomes>drug-BCD complex solution> drug solution. This study further confirms good *in vivo* performance of colchicine-BCD elastic liposomal formulation and sustained and better effect for management of gout.

CONCLUSION

Colchicine is an ancient drug, widely used in the treatment of gout. This medication has also found a place in the treatment of several dermatological conditions due to its immunosuppressive and anti-inflammatory effects. Colchicine is a useful, inexpensive immunosuppressive agent that has application in a wide variety of dermatological diseases along with its primary use in gout. The current conventional therapy however is associated with a number of side effects. Drug release study *in vitro* shows that developed colchicine-BCD elastic liposomal formulation can sustain the release of colchicine effectively as compared with free drug. Skin permeation and deposition studies also have shown enhanced skin permeation and deposition of colchicine-BCD elastic liposomal formulation. These findings indicate synergistic effects of combination of drug in cyclodextrin in elastic liposomes. The *in vivo* anti-gout activity also showed better and sustained biological effects. Therefore the toxic side effects of colchicine can be expected to be reduced. We can speculate that the colchicine-BCD
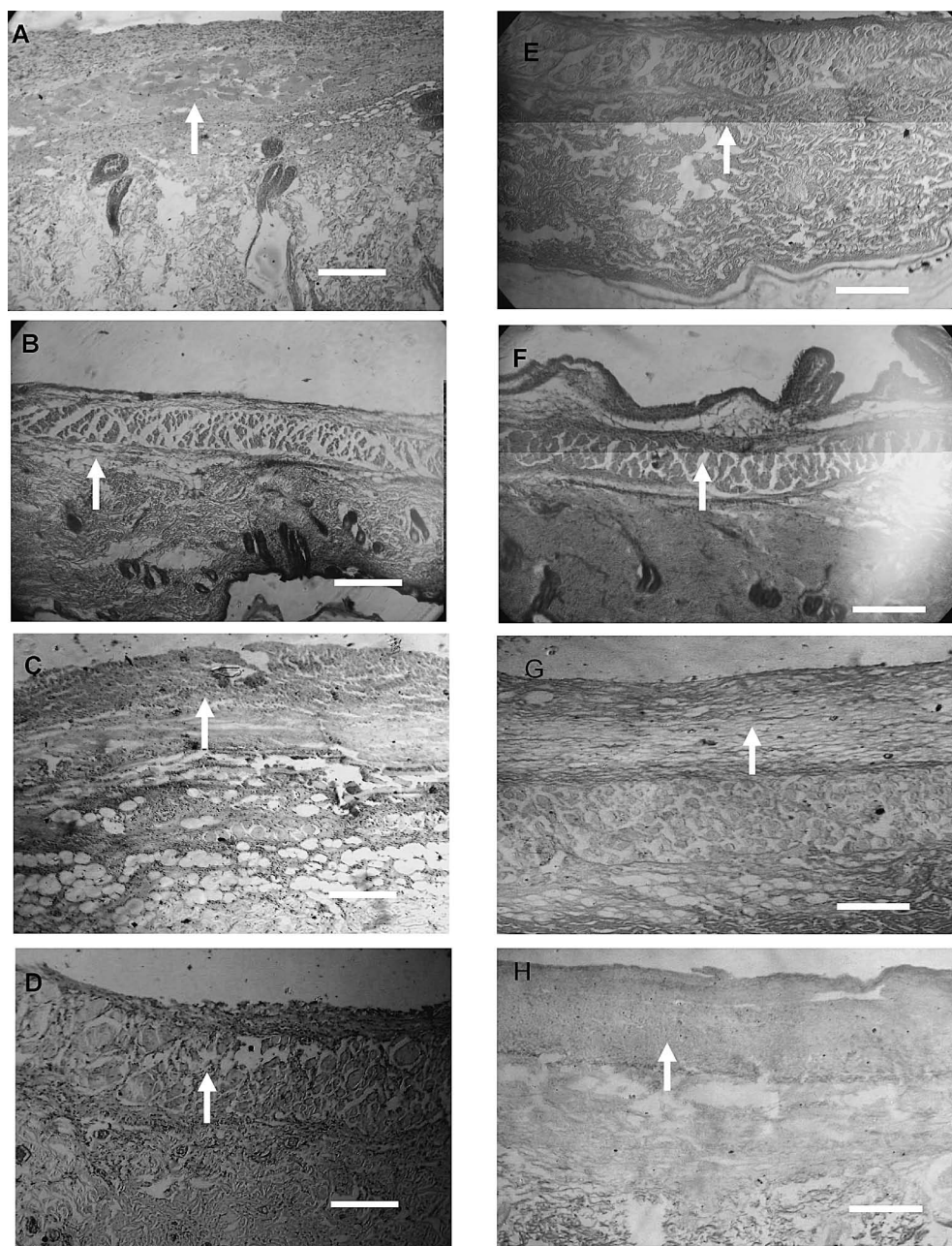
Fig. 5.   Eosin-hematoxylin and Picrosirius Red Stained Photomicrograph of Skin of Rat Pouch Model

Drug solution treated group (A, E), drug-BCD solution treated group (B, F), colchicine elastic liposomes group (C, G) and colchicine-BCD elastic liposomes treated group (D, H) after 6 h of treatment. Arrow indicates presence of inflammatory cells in eosin-hematoxylin stain and collagen deposition in picrosirius stain. Scale bar=500 μm. Magnification×100.

elastic liposomes formulation is a good candidate for localized delivery of colchicine.

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