ORIGINAL ARTICLE

Encapsulation of bio active compound ursolic acid as proniosomes and its evaluation

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In the present study, novel proniosomal gel of ursolic acid was prepared by encapsulation of the drug in a mixture of span 60, cholesterol and alcohol by coacervation phase separation method to overcome the bioavailability problem of this potential biologically active compound. The formulated system was characterized for size, drug entrapment, *in-vitro* drug release studies and *in-vivo* anti-inflammatory effect through animal model. *In-vitro* release data of ursolic acid from proniosomal gel revealed a time dependent near linear relationship. Entrapment efficiency of the formulated gel was found > 90% and total % cumulative release of ursolic acid satisfactory as 60.8% after 24 h. Release pattern was also found almost constant. *In-vivo* evaluation revealed that the developed formulation exhibited significant anti-inflammatory value ($P < 0.05$) in carrageenan induced rat paw edema model and was found quite comparable with the standard diclofenac gel (voveran gel) in terms of % inhibition of edema.

Key words: *Non-steroidal anti-inflammatory drugs, proniosomes, topical drug delivery, ursolic acid*

INTRODUCTION

Ursolic acid, 3 β-hydroxyurs-12-en-28-oic acid is a pentacyclic triterpenoid occurs in numerous plants and is a constituent of several herbal medicines marketed in Asia and World-wide for possessing potential anti-inflammatory properties.[1-3] It has also proved to have anti-tumor, anticancer,[4] anti-hyperlipidaemic,[5] antibacterial, antifungal,[6] hepatoprotective[7] and beneficial effect in inflammation-driven cancers[8] with a low toxicity profile as compared to non-steroidal anti-inflammatory drugs in various conditions of inflammatory etiology, degenerative conditions such as rheumatoid arthritis and osteoarthritis.[9-11] Studies reveal that ursolic acid play important role in inhibition of histamine release from mast cells, inhibition of cyclooxygenase and lipoxygenase, inhibition of elastase, which are responsible for rheumatic inflammation.[7] Apart from this, ursolic acid is also known to eliminate the expression of the metalloproteinase-MM in a dose dependent manner and able to reduce interleukin-1 beta or tumor necrosis factor-alpha induced activation of protein kinase C-zeta which are known to have role in arthritis.[12,13] Ursolic acid is also a significant COX-2 inhibitor, with an IC_{50} value of 130 μM and COX-2/COX-1 selectivity ratio of 0.6.[14] The molecular docking analysis reveal that ursolic acid inhibit COX-2 enzyme by hydrophobic and hydrogen bonding interactions.[15] Usolic acid is considered as a potential bioactive compound in the medicine world. However water solubility of this bioactive compound is limited, thus limiting its bioavailability in the body. To combat the problem of drug delivery of synthetic or herbal molecules, several novel drug delivery systems have been in the recent years employed with promising results. In this regard, encapsulation of the drug in vesicular structures is one such system, which has been reported to enhance permeability of drug through the stratum corneum barrier as in vesicular structures like liposomes, niosomes, ethosomes, transferosomes, proniosomes etc.

Proniosomes are liquid crystalline-compact niosomal hybrid, offer a versatile vesicle delivery concept with potential for drug delivery through transdermal route.[16] These are devoid of toxicity related to use of ionic surfactant, due to the use of non-ionic surfactants viz. glucosyldialkyl ethers, polyoxyethylene alkyl ether, ester-linked surfactants, polyglycerol alkyl ethers and series of spans and tweens. Moreover, proniosomes minimize the problem associated with

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the noisome stability and also provide convenience in transportation, distribution, storage and dosing.[17] These are the formulations, which on *in-situ* hydration from skin converted into niosomes, which then penetrate into the skin at a faster rate when compared with the free drug.[18] (Since both hydrophilic and hydrophobic substances can be embedded in niosomal vesicles[19] ensures the sparingly water soluble drugs be entrapped in vesicles.[20] Consequently, these effects of proniosomes will offer a special advantage for ursolic acid for its anti-inflammatory effect and).

Based on above, ursolic acid was felt to be an ideal candidate for encapsulation in the form of proniosomes since no attempt was made in this direction until date. Thus the present study was aimed to design an optimum proniosomal formulation for topical delivery of ursolic acid, having increased characteristics of safety and efficacy.

MATERIALS AND METHODS

Reagents and chemicals

Ursolic acid (98% pure) was obtained from Yucca Enterprises (Mumbai, India). Soya lecithin and dialysis membrane were purchased from Hi-Media Laboratories Ltd. (Mumbai, India). Cholesterol, span 60, glycerol and ethanol were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Potassium dihydrogen phosphate, disodium hydrogen phosphate was obtained from CDH Chemicals Pvt. Ltd. (Delhi, India). High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Spectrochem Pvt. Ltd., (Mumbai, India). Ursolic acid was analyzed for its purity before carrying out further studies.

Preparation of proniosomal gel

Proniosomal gel was prepared by validated coacervation phase separation method.[21] Precisely, hundred milligrams of surfactant mixture, surfactants: alcohol (1:1) and drug (1% w/w) were weighed in a clean and dry, wide mouth small glass tube. Surfactant ratio used was soya lecithin: span 60:cholesterol (9:9:2). After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent loss of solvent and then warmed on a water bath at 60-70°C for about 5 min, until the surfactants were dissolved completely. The aqueous phase (0.1% glycerol solution) was then added and warmed on a water bath until clear solution was formed. The mixture was allowed to cool to room temperature until the dispersion was converted to proniosomal gel. The final ratio of surfactant: alcohol: aqueous phase was 5:5:4 by weight.[22] The gel obtained was preserved in dark until characterization.

In-vitro evaluation

Particle size analysis

The average size of the prepared proniosomes was performed by laser diffraction particle size analysis using Microtrac S3500, Laser diffraction particle size analyzer by suspending a small amount of formulation in aqueous dispersing phase and stirring for suitable time on a vortex mixer at room temperature.

Scanning electron microscopy

The surface morphology (roundness, smoothness and formation of aggregates) of the prepared proniosomes formulation was determined by scanning electron microscopy (SEM) (JSM-6510 SEM at 15 KV). A very small amount of the gel was mounted on an aluminum stub with double sided adhesive carbon tape. The formulation then sputter coated with gold using a vacuum evaporator and examined with the SEM.

Entrapment studies

10 mg of proniosomes gel was weighed in a glass tube and 10 ml of aqueous phase (methanol) was added. The aqueous suspension was then sonicated and filtered through 0.2 µm syringe filter. The filtrate was then subjected for HPLC analysis. The percentage of entrapped drug was calculated by the following equation:

$$\% \text{ Entrapment} = \frac{\text{Total amount of drug-unentrapped drug}}{\text{total amount of drug}} \times 100 \quad (1)$$

HPLC analysis

The HPLC system used was Hitachi Elite Lachrom L2130 series module equipped with a L2455 diode array detector. The control system and data acquiring system was installed with EZ chromlite data station for LC system. The injection volume was 10 µL and the column temperature was maintained at 27°C. A number of trials for chromatographic separation were performed using a Reliasil reversed phase C18, 5 µm, 250 × 4.6 mm column with a mobile phase consisting of acetonitrile/water (85:15, v/v) as mobile phase in an isocratic run, with a flow rate of 0.6 ml/min for a single run of 6 min. The eluent was monitored for ursolic acid peak at 210 nm.

Drug diffusion studies

Franz diffusion cell method was applied using phosphate buffer (pH 5.8) at room temperature for *in-vitro* drug release studies. A cellophane membrane (dialysis membrane) was used to carry out the study and soaked overnight in phosphate buffer at room temperature to be prepared. The membrane was then placed between donor and receptor compartment of diffusion cell with an exposed membrane surface area of 2.97 cm^2 to the receptor compartment. The receptor compartment was filled with 16.4 ml of freshly prepared ethanolic phosphate buffer (pH 7.4) maintained at 35 ± 0.5°C with constant stirring using a teflon coated magnetic stir bead. One g of proniosomal gel formulation was placed on the membrane and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h), 2 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to

maintain sink conditions. The amount of drug released from was determined by HPLC as mentioned above.

In-vivo evaluation

Male albino Wistar rats were procured from Animal house, M. D. University and acclimatized before starting the experiment. Standard food and water *ad libitum* was administered to the animals during the whole study. The experimental protocol was duly approved by Institutional Animal Ethics Committee (Reference no. Pharma Sc./91-101). The animals were divided into three groups each containing 6 rats. One group was kept as control, second treated with marketed diclofenac gel (Voveran® Emulgel) (standard group) and the remaining third group was treated with ursolic acid loaded transdermal formulation. Localized inflammation was induced by sub-plantar injection of 0.1 ml carrageenan suspension (1% w/v in distilled water) into right hind paw, 1 h before drug administration where maximum edema was reached.

The initial paw size was then determined using plethysmometer. Each group was assessed for edema volume at different time intervals using plethysmometer. Readings were taken every hour for 5 h and percentage inhibition of inflammation was calculated using the following equation:

$$\% \text{ Inhibition} = ([C\text{-}T]/C) \times 100\%, \quad (2)$$

where C = Control paw edema,
T = Test paw edema.

Statistical analysis

Data were collected and coded prior to analysis. All data were expressed as means ± SD For all continuous data that were normally distributed, one-way analysis of variance test followed by a Tukey's *post-hoc* test; $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

In-vitro evaluation of proniosomes

Particle size analysis

The formulated proniosomal gel was found to have particle size ranging between 4 and 13 µm which is suitable for topical drug delivery and was uniform throughout the formulation [Figure 1]. These results correlate well with the particle size of proniosomes of ibuprofen.[23]

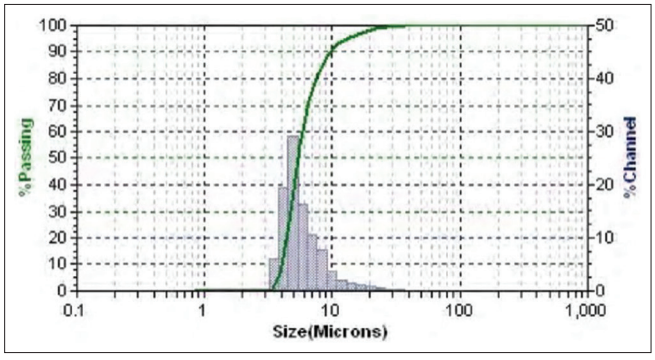


Figure 1: Particle size range and distribution pattern in proniosomal gel loaded with ursolic acid

SEM

Surface morphology by SEM imaging confirmed the coating of surfactant onto the vesicles [Figure 2]. Most of the vesicles were well-identified and were spherical. Some unevenness and aggregation of vesicles that observed under the study may be due to drying process under normal environment condition.

Entrapment study

The entrapment efficiency of the proniosomal gel of ursolic acid showed a higher value, i.e., 93.4 ± 2.4% (mean value ± SD). This high entrapment efficiency may be attributed to the high octanol/water partition coefficient log Kow = 7.92[24] that is expected to be partitioned almost completely within the lipid bilayers of niosomes. Hence in proniosomal formulation, the entire drug was intercalated within the bilayers as opposed to the aqueous spaces within the gel. This result was consistent with the entrapment efficiency of celecoxib, levonorgestrel, ketorolac, oestradiol in proniosomes.[25,26] It may also be attributed to the fact that span 60 results in higher entrapment efficiency.[27,28]

Drug release study

Total percentage cumulative drug release after 24 h from the proniosomal gel was found to be 60.8%. When cumulative percentage of drug released was plotted against time, the release profile showed a linear relationship with time for 12 h after which release becomes constant. Lag phase couldn't be detected because of the minimum sampling time of 1 h. *In-vitro* drug release study of ursolic acid revealed that the pattern of drug release was faster for first 12 h followed by almost constant and slow for next 12 h in 24 h study [Figure 3]. This may be attributed to higher entrapment of the drug into the vesicles.

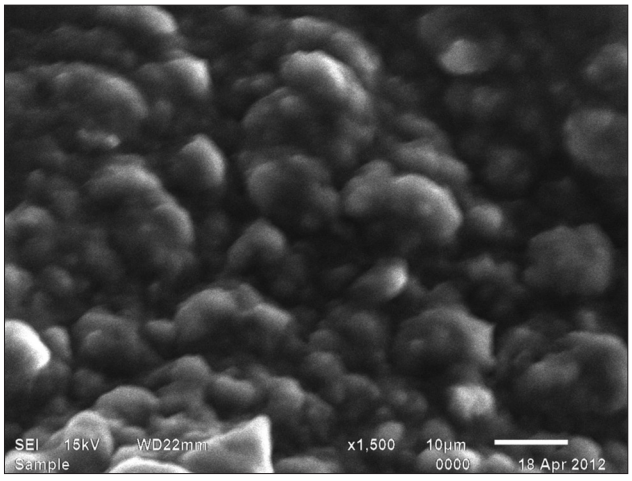


Figure 2: Scanning electron microscopy image of proniosomal gel loaded with ursolic acid

Table 1: Paw edema and % inhibition in different groups at various time intervals

Group	Paw edema (mm) (% inhibition)				
	1 h	2 h	3 h	4 h	5 h
Control	5.73±0.16	6.68±0.15	6.78±0.14	6.63±0.16	6.46±0.15
Standard (voveran gel) (%)	5.08±0.15* (11.34)	5.35±0.19* (19.9)	5.05±0.18* (25.5)	4.87±0.22* (26.5)	4.76±0.22* (26.3)
Test (proniosomal gel) (%)	5.23±0.16* (8.73)	5.8±0.14* (13.17)	5.15±0.19* (24.04)	4.75±0.27* (28.3)	4.35±0.27* (32.67)

Values are the mean±SD (n=6). Significantly different from control at: *P<0.05. SD: Standard deviation

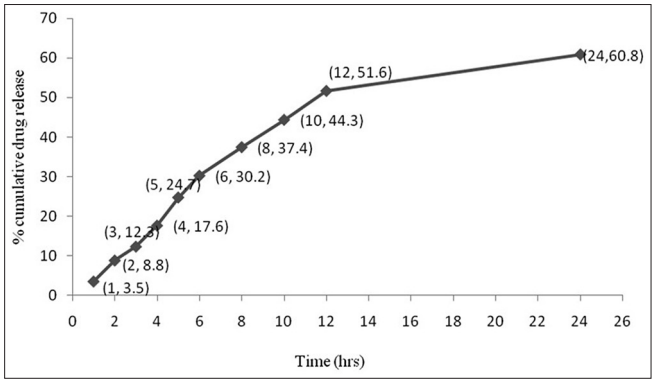
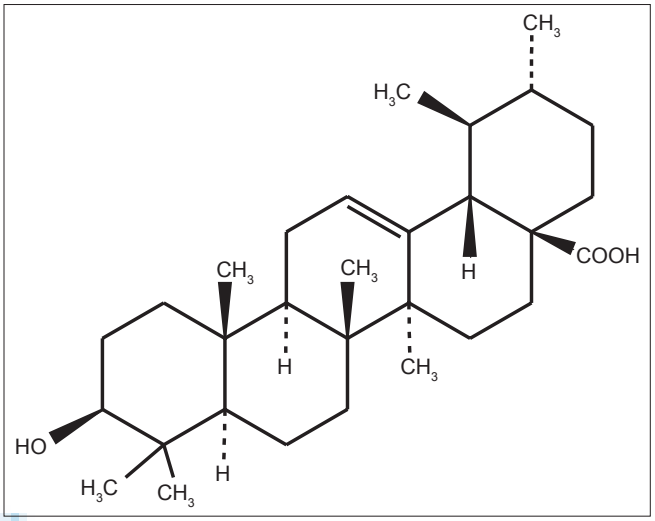


Figure 3: *In-vitro* release profile of ursolic acid from proniosomal gel



Chemical structure of ursolic acid

In-vivo anti-inflammatory effect

Pharmacological evaluation as an anti-inflammatory agent revealed that proniosomal gel of ursolic acid exhibit a significant higher value of $P < 0.05$ in carrageenan induced rat paw edema with comparison to control [Table 1]. The activity was quite comparable to the standard gel of diclofenac, i.e., voveran gel in terms of % inhibition of edema which in turn indicated the higher efficacy of the proniosomal gel loaded with ursolic acid. At the 5th h the percentage inhibition of edema by ursolic acid loaded proniosomes was more comparative to voveran gel which concluded that the proniosomes has enhanced the ursolic acid delivery through the skin, thereby the anti-inflammation effect. The drug release pattern showed that single topical application could be beneficial for 12 h which can be quite helpful in chronic inflammatory conditions.

CONCLUSION

From the present study, it has made clear that proniosomal gel loaded with ursolic acid has shown promising results during *in-vitro* as well as *in-vivo* evaluation with desirable physicochemical parameters thus holds a good future potential.

REFERENCES

1. Kim KA, Lee JS, Park HJ, Kim JW, Kim CJ, Shim IS, *et al*. Inhibition of cytochrome P450 activities by oleanolic acid and ursolic acid in human liver microsomes. Life Sci 2004;74:2769-79.
2. Baricevic D, Sosa S, Della Loggia R, Tubaro A, Simonovska B, Krasna A, *et al*. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: The relevance of ursolic acid. J Ethnopharmacol 2001;75:125-32.
3. Vasconcelos MA, Royo VA, Ferreira DS, Crotti AE, Andrade e Silva ML, Carvalho JC, *et al*. *In vivo* analgesic and anti-inflammatory activities of ursolic acid and oleanoic acid from *Miconia albicans* (Melastomataceae). Z Naturforsch C 2006;61:477-82.
4. Pandey G. Some important anticancer herbs: a review. Int Res J Pharm 2011;2:45-52.
5. Parfent'eva EP. Effect of ursolic acid and its derivatives on lipid metabolism in experimental atherosclerosis. Chem Abstr 1979;9:9279.
6. Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN. Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. leaves. J Ethnopharmacol 2008;119:238-44.
7. Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49:57-68.
8. Laszczyk MN. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. Planta Med 2009;75:1549-60.
9. Nair B, Taylor-Gjevre R. A review of topical diclofenac use in musculoskeleton disease. Pharmaceuticals 2010;3:1892-908.
10. Crofford LJ. Use of NSAIDs in treating patients with arthritis. Arthritis Res Ther 2013;15 Suppl 3:S2.
11. Li Y, Xing D, Chen Q, Chen WR. Enhancement of chemotherapeutic agent-induced apoptosis by inhibition of NF-kappaB using ursolic acid. Int J Cancer 2010;127:462-73.
12. Huang HC, Huang CY, Lin-Shiau SY, Lin JK. Ursolic acid inhibits IL-1beta or TNF-alpha-induced C6 glioma invasion through suppressing the association ZIP/p62 with PKC-zeta and downregulating the MMP-9 expression. Mol Carcinog 2009;48:517-31.
13. Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, *et al*. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 2007;7:344-51.
14. Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L. Ursolic acid from *Plantago major*, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. J Nat Prod 1998;61:1212-5.
15. Manikrao AM, Khatale PN, Jawarkar RD, Vyas JV, Mahajan DT, Masand VH, *et al*. Presuming the probable anti-inflammatory mechanism of ursolic acid: A plant derived pentacyclic triterpenoid, using molecular docking. Scholar Res Libr 2011;1:9-13.
16. Blazek-Welsh AI, Rhodes DG. Maltodextrin-based proniosomes. AAPS Pharm Sci 2001;3:E1.

17. Bairwa NK, Choudhary D. Proniosome: A review. Asian J Biochem Pharm Res 2011;2:690-4.
18. Goyal C, Ahuja M, Sharma SK. Preparation and evaluation of anti-inflammatory activity of gugulipid-loaded proniosomal gel. Acta Pol Pharm 2011;68:147-50.
19. Niemiec SM, Ramachandran C, Weiner N. Influence of nonionic liposomal composition on topical delivery of peptide drugs into pilosebaceous units: An *in vivo* study using the hamster ear model. Pharm Res 1995;12:1184-8.
20. Arunothayanun P, Bernard MS, Craig DQ, Uchegbu IF, Florence AT. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether. Int J Pharm 2000;201:7-14.
21. Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release 1998;54:149-65.
22. Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH. *In vitro* skin permeation of estradiol from various proniosome formulations. Int J Pharm 2001;215:91-9.
23. Hu C, Rhodes DG. Proniosomes: A novel drug carrier preparation. Int J Pharm 1999;185:23-35.
24. Shanmugam MK, Dai X, Kumar AP, Tan BK, Sethi G, Bishayee A. Ursolic acid in cancer prevention and treatment: Molecular targets, pharmacokinetics and clinical studies. Biochem Pharmacol 2013;85:1579-87.
25. Alam MI, Baboota S, Kohli K, Ali J, Ahuja A. Pharmacodynamic evaluation of proniosomal transdermal therapeutic gel containing celecoxib. Sci Asia 2010;36:305-11.
26. Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. Eur J Pharm Biopharm 2005;59:485-90.
27. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 1994;105:1-6.
28. Bhaskaran S, Panigarhi L. Formulation and evaluation of niosomes using different non-ionic surfactants. Indian J Pharm Sci 2002;64:63-5.

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