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# DESIGN AND DEVELOPMENT OF BOSWELLIC ACID LOADED NANOSTRUCTURED LIPID CARRIER BASED ANTI PSORIATIC NANO GEL FOR DERMAL DELIVERY

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## ABSTRACT

Anti-inflammatory activity Boswellia serrata is mainly due to 11-keto-β-boswellic acid (KBA) which has the activity against the production of leukotrienes by inhibition of 5- lipooxygenase. However, most recent research shows that Boswellic acid could also play an important role in targeting the microsomal prostaglandin synthesis inhibition, which is the major inflammatory mediator. Boswellic acid acts by inhibiting excessive activation of nucleic acid transcription factors TNF-kappa-β in diseased skin and preventing TNF alpha from being excessively synthesized, this mechanistic account of Boswellic acid helps in curing psoriasis.

**KEYWORDS:** Anti-inflammatory activity, Boswellia Serrate, lipooxygenase.

Pharmacokinetic studies have evidenced that the systemic absorption of Boswellic acid is very low in animals and in humans owing to their low oral bioavailability. Boswellic acid also has a strong tendency to self-aggregate owing to their low stability and insolubility which limits its clinical application. The various approaches have been made to overcome the above problems. Among them, Nanostructured Lipid Carriers become the promising drug delivery. These novel carriers have been widely studied and have demonstrated an increment in the rate and extent of drug delivery and optimal therapeutic outcomes.

In this study we have formulated Nanostructured lipid carriers of Boswellic acid with the particle size 161.2 nm with a polydispersity index of 0.234 and zeta potential 14.7 Mv. The study showed the increased solubility and bioavailability of the NLCs as compared to the drug.

## MATERIALS AND METHODS

Boswellic acid was obtained from Bhoomi Neutraceuticals Pvt. Ltd. Basmat, Nanded. Stearic acid and oleic acid was obtained from from S.D. Fine Chemicals, Mumbai. Tween 80 was obtained from Hi Media Lab. Pvt. Ltd, Mumbai. Poloxamer 188 and Mannitol was obtained from Hi Media Lab. Pvt. Ltd, Mumbai. All other reagents used were of analytical grade.

### Preparation of BOS-NLCs

The Nanostructured Lipid Carriers are prepared by using hot homogenization technique. Stearic acid is used as solid lipid, oleic acid is used as liquid lipid. and poloxamer as a co-surfactant/emulsifier. The lipid Oleic acid was melted at 10-15$^0$c above the melting point, the drug Boswellic acid (5mg) and poloxamer was dispersed in the melted lipid and the dispersion was kept at the same temperature. An aqueous phase was prepared by dissolving Tween 80 (1.5%w/w of total weight of SLN dispersion) as a surfactant/ stabilizer was dissolved in double distilled water and heated to same temperature as that of melted lipid phase. The pre-heated aqueous phase was added to melted lipid phase and homogenized by hot high pressure homogenizer (Gea Niro, Soavi, Panda). The formulation was cooled down in an ice bath and diluted with deionised water upto 100ml. The prepared dispersion was lyophilized by using lyophilizer (Lark penguin classic plus) to obtained the off white cake of the drug which has the enhanced solubility and stability. Mannitol (5% w/v was added as cryoprotectant.

### Preparation of BOS-NLC nanogel

The gels were prepared by dispersing 0.50% w/w Carbapol 974P in the NLC formulation and subsequently neutralizing the Carbapol dispersion using triethanolamine. The final concentration of Boswellic acid in the gels is maintained as 0.5% w/w and named as Boswellic acid NLC GEL. The composition of boswellic acid loaded nanostructured lipid carriers based nanogel is showed in following table.

**Table 1: The composition of BOS-NLC nanogel.**

| Ingredient | BOS-NLC-1 | BOS-NLC-2 | BOS-NLC-3 |
|---|---|---|---|
| Boswellicacid (Drug)(%w/w) | 5%w/w | 5%w/w | 5%w/w |
| Carbapol 974P (%w/w) | 0.50% | 0.75% | 1% |
| Propylene glycol (%w/w) | 5 | 5 | 5 |
| Triethanolamine(ml) | 0.8 | 0.8 | 0.8 |
| Methyl paraben | 0.05 | 0.05 | 0.05 |
| Water (ml) | Q.s | Q.s | Q.s |

**Characterization of NLCs**

**Particle size and zeta potential**

The particle size is important parameter in process control and quality assurance because physical stability of vesicle dispersion depends on particle size. Zeta potential is very useful parameter for the assessment of the physical stability of colloidal dispersions. Dyanamic light scattering (ZetaSizer Nano-ZS: Malvern Instruments Ltd, United Kingdom) was used to measure the partilcle size, polydispersity index and zeta potential of the Nanostructured Lipid Carriers. Nanostructured Lipid Carriers were suspended in double distilled water for sample preparation for the test.

**Differential scanning calorimetry (DSC)**

The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion. The DSC thermo grams of the drug and lyophilised SLNs was recorded using instrument (Diamond DSC, Perkin)) at heating rate of 10°C/min from temperature 0-250°C under $N_2$ flow.

**X-ray Diffraction Studies (XRD)**

XRD study of drug Boswellic acid and Nanostructured Lipid Carriers. of drug were carried out to detect the changes in the crystallanity. XRD patterns were recorded using (Bruker, D8) with Cu-kα radiation. The scanning angle ranged from 10° to 50° of 2θ.

**Scanning electron microscopy (SEM)**

The shape and surface characteristics of Nanostructured Lipid Carriers were determined by SEM using gold sputter technique Nanostructured Lipid Carriers loaded with drug were fixed on stub using double-sided tape. The stubs containing the sample were coated with gold using JEOL fine coat (JFC-1100F ion sputtering device).

**In vitro dissolution studies**

In-vitro dissolution studies of Boswellic acid Nanostructured lipid carriers were carried out by USP type XXIV rotating basket type dissolution apparatus (Electro lab, Mumbai). Optimization of formulation batches was estimated on the basis of cumulative percentage drug release with respect to time. The dissolution carried out in two different media distilled water and phosphate buffer saline (pH 6.8) each of 900 ml. NLCs were placed in each vessel and the medium was allowed to maintain at 100 ppm at 370c± 0.50c. Samples of 10 ml were withdrawn at various time intervals up to 24 hr and sink condition was maintained. The

absorbance of the sample was measured by a UV double beam spectrophotometer (Shimadzu, Japan UV-1800) at 260nm and cumulative percentage drug releases were calculated.

**Evaluation of boswellic acid loaded nanogel nanogel**

**In-Vitro drug release**

In vitro, drug release studies of boswellic acid-NLC gels were performed using a dialysis bag technique. The activation of the dialysis membrane was carried out. The experiments were carried out under sink conditions. 5 g of each formulation i.e. NLCNG1, NLC NG2, NLCNG3 gel, and marketed formulation (Proxym gel) was loaded into a dialysis bag (Himedia molecular weight cut-off 13-14 kg) immersed in 100 ml of pH 6.8 phosphate buffer solution magnetically stirred at 320C at pH 6.8. Samples (5ml) were taken at predetermined intervals of 0 min, 30 min, 45min, 2, 4, 6, 8, 10, 12 hours from the receiver solution, replaced with equal volumes of fresh solvent, and spectrometric ally assayed for drug concentration at λmax260 nm. The correction for the cumulative dilution was calculated. The release studies were performed in triplicate. A graph of % cumulative release against Time in hours was plotted to describe the kinetics of drug release from the gel, mathematical models such as zero-order, first order, and Higuchi were used. The criterion for selecting the most appropriate model was based on a goodness-of-fit test.

**Skin irritation study**

All Procedures followed for this study were in accordance with the standard operating procedures at PRADO (Preclinical Research and Development Organization) and the Guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in the Gazette of India, December 15, 1998.

The irritation potential of Boswellic acid-NLCNG 1 (0.5%w/w) was evaluated by carrying out the Draize patch test on rat (Wistar rat) both Male and Female rat with age of 9-10 weeks was selected for the test. Animals were divided into three groups as Group 1: No application (Control); Group 2: Boswellic acid-NLC (0.5% w/w); Group 3: Market gel. The hair was removed from the around trunk between flank and shoulders exposing an area equivalent to approximately 10% of the total body surface. An amount of 2 g of the formulation was applied to the hair-free skin of rats by uniform spreading within hair free exposed area. The skin was observed for any visible change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations. The mean erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema i.e., No erythema = 0, Slight erythema

(barely perceptible – light pink) = 1, Moderate erythema (dark pink) = 2, Moderate to severe erythema (light red) = 3, Severe erythema (extreme redness) = 4.

**Anti-inflammatory activity**

For the carrying out of this in vivo studies, approval is taken from Institutional Animal Ethics Committee and their guidelines must follow in the complete study. For that carrageenan-induced hind paw, the method should be used which is developed by Winter et.al in Wistar rat. Three groups of animals were taken, in each group, 3 animals were included. young male Wistar of 180-250 gm weight was taken for study. These animals were housed in polypropylene cages, with free access to standard laboratory diet (Lipton food) and water ad libitum under standard laboratory conditions of temperature; 25±20C with relative humidity: 55±5%.

Paw edema was induced by injecting 0.1ml of the 1% homogeneous suspension of carrageenan in saline. A total of three group, that is group 1 treated with carrageenan only which is as a control group, group 2 is treated with carrageenan and topically applied Boswellic acid loaded nanostructured carrier based nanogel and Group 3rd any marketed formulation taken as standard. The volume of the paw is measured with a digital plethysmometer.

Edema rate can be calculated by formula

Edema Rate (E) = Vt -Vo / Vo

Where Vo the mean paw volume before carrageenan injection, Vt the mean paw volume after the carrageenan injection at time t, Ec is the edema rate of the control group and Et is edema rate of the treated group at time t.

**RESULT AND DISCUSSION**

**1. Particle size and zeta potential**

The optimized NLCs were in the nanometric size range (161.2 nm) with low polydispersity index 0.234 (fig 1). Surfactant greatly influences the particle size of formulation by causing stabilization. The nano size may be the reason for enhanced solubility of drug. Zeta potential of the optimized NLC is found 14.7 which shows the greater stability of the drug.

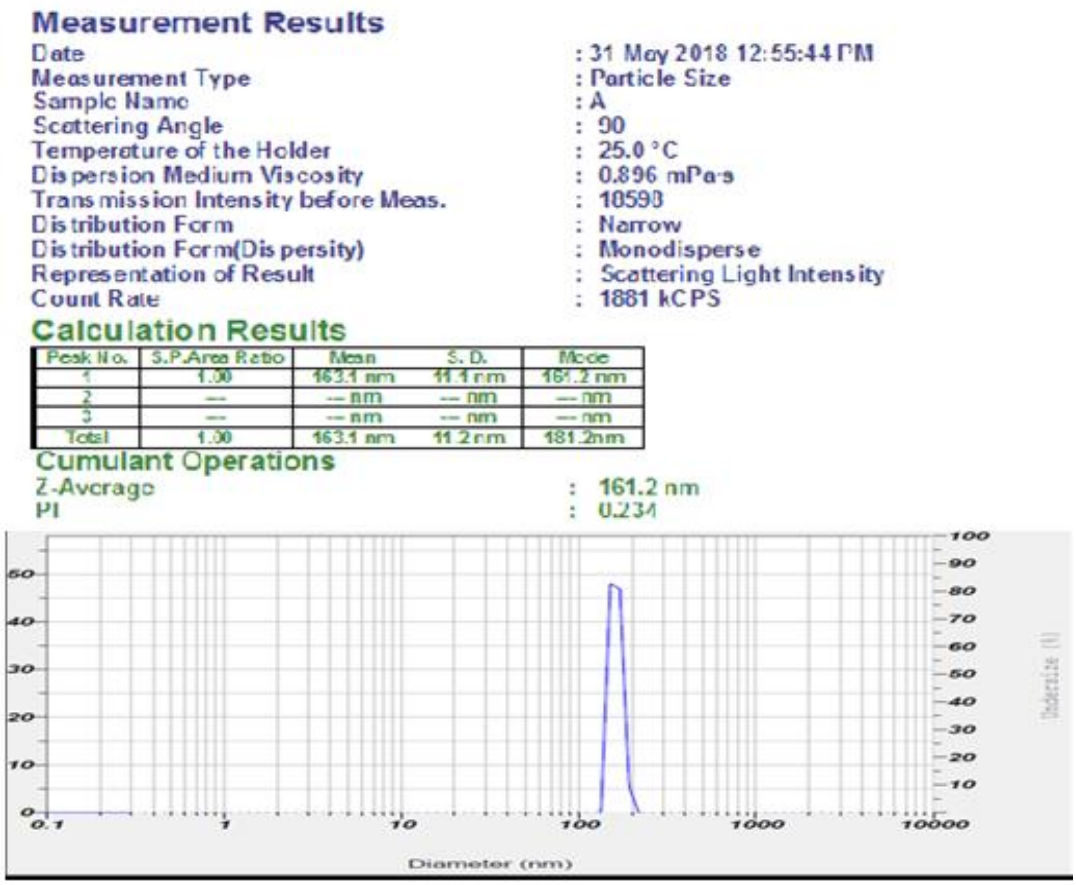


**Figure 1: Particle size of the NLC.**

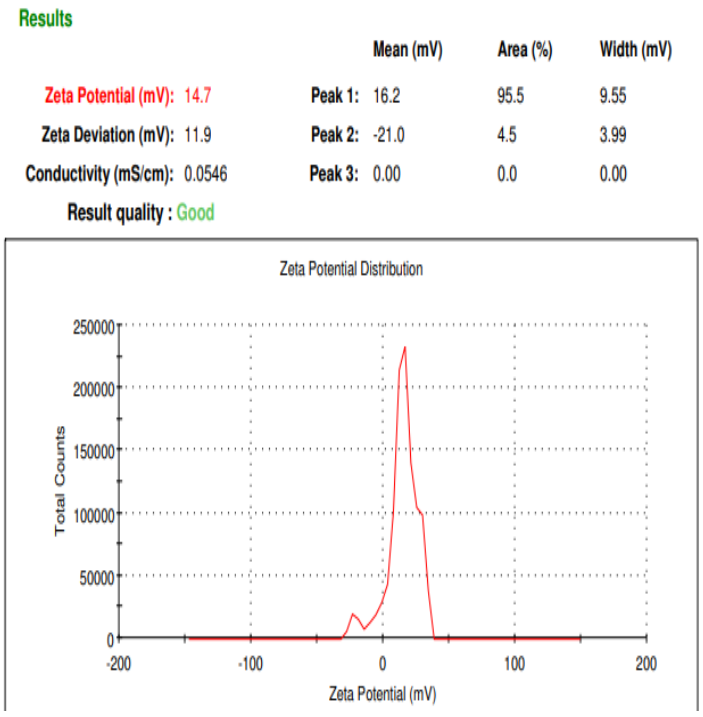


**Figure 1: Zeta potential of the optimized NLC 1.**

**Figure 2: Zeta potential of the NLC.**

## 2. Scanning Electron microscopy

The SEM images of optimized NLC is shown into the figure 3. It shows the circular shapes of the particles. Its shows the result of the encapsulation of the drug into the lipid matrix into the circular manner.

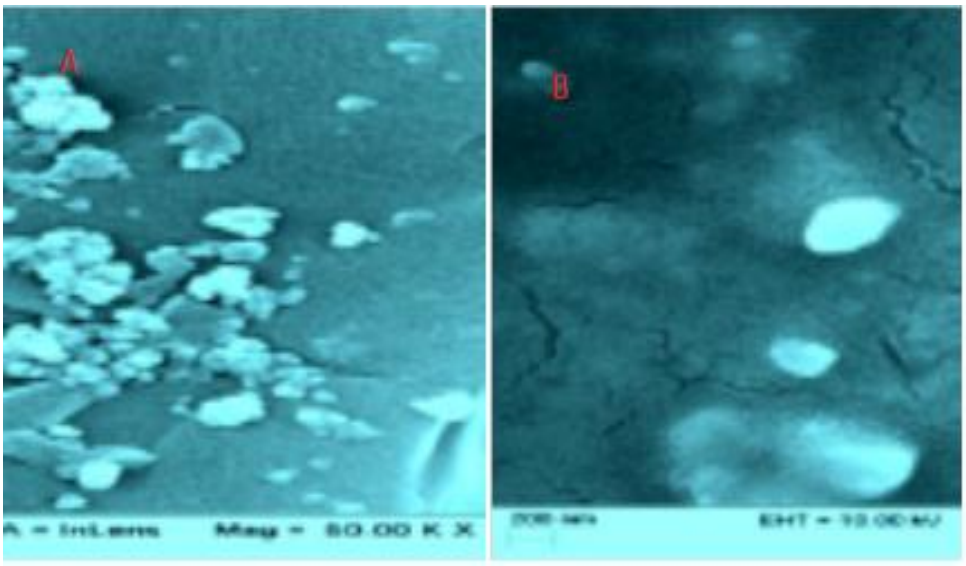


**Figure 3: SEM Image of the optimized NLC.**

## 3. XRD Study

The Boswellic acid powder was highly crystalline it gives sharp peaks seen at the 2θ value in the x-ray scan (fig 4). It also shows the XRD patterns of freeze-dried Boswellic acid loaded NLC showing that peak intensity is reduced indicating reduction in crystallinity. The absence of these peaks in optimized NLCs demonstrated the total solubilization of drug within the lipid phase.

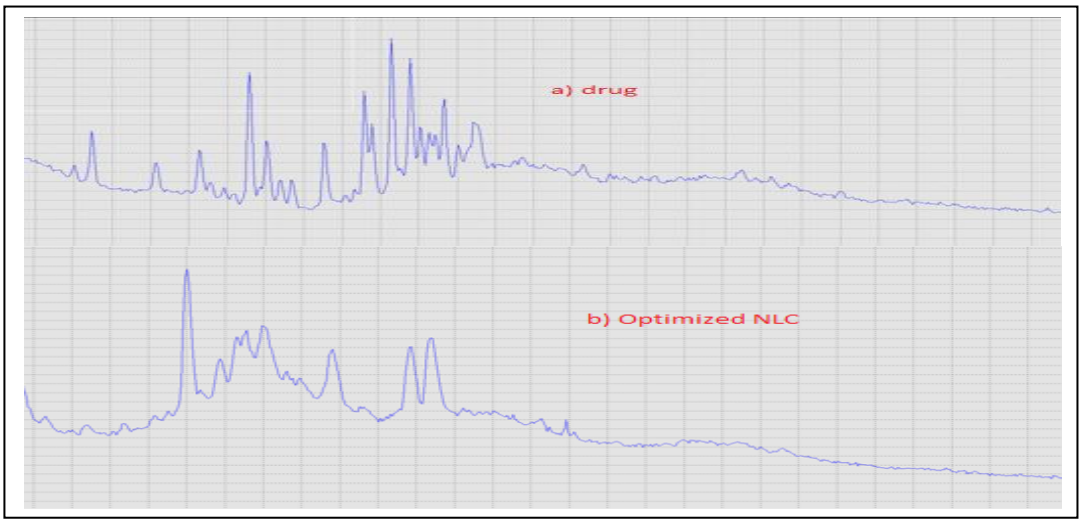


**Figure 4: XRD Study of the drug(a) and optimized NLC(b).**

**4. DSC Study**

DSC is usually used to get information about both the physical and the chemical properties of a compound or formulation. DSC measures the heat loss or gain as a result of physical or chemical changes within a sample as a function of the temperature**.** DSC curve of pure drug shows the peak at 196.54$^0$c corresponding to the melting point of the drug. DSC graph of optimized formulation NLC 1 shows two peaks sharp endothermic peak at 165.56$^0$c of drug in the formulation and another one at 277.51$^0$c with the disappearance of melting endothermic peak of drug (figure 5), indicating drug was encapsulated into the lipid with amorphous state. A shift in the peak was seen (fig 5) which may be due to the interaction of the drug with lipid matrix.

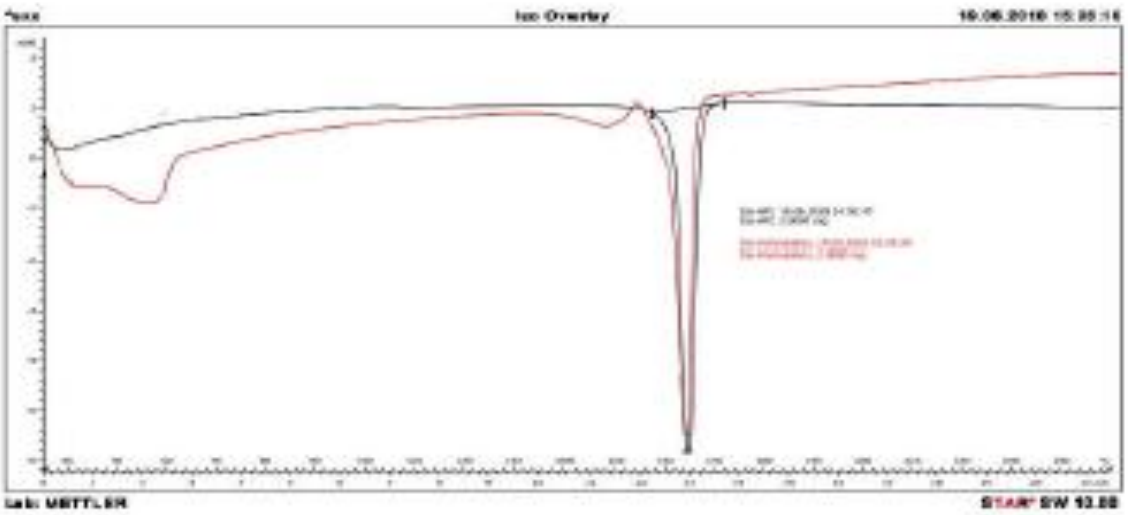

**Figure 5: DSC Thermogam of drug and optimized NLC.**

**5. In-vitro drug diffusion study of Boswellic acid-NLC GEL using dialysis bag**

In-vitro diffusion studies of NLC gel showed in (fig 6). from this result, we can be seen that 58.81±0.47% of the drug released within 8 hours from the formulation NLC 0.50 it was greater than other NLCG gel formulation (NLCG 1). So, NLCG 1 was optimized for the further evaluations. When in comparison to marketed gel the releases of NLCG1 gel is also greater and faster than marketed gel (34.46±0.32%). The released data from NLCG1 gel were fitted to the different models. The value for r2 was found to be highest for the Higuchi model (r2=0.929). This indicates that the test product follows matrix diffusion based release kinetics.

**Table 2: The % drug release of NLCs Nano gel and marketed gel.**

| Time in Hr | NLCG 1 (% CR) | NLCG 2 (% CR) | NLCG 3 (% CR) | Market gel (% CR) |
|---|---|---|---|---|
| 0.5 | 0.97±0.01 | 0.94±0.05 | 1±0.05 | 0.92±0.05 |
| 1 | 3.57±0.27 | 1.99±0.20 | 2.22±0.50 | 2.33±0.18 |
| 2 | 7.71±0.55 | 4.81±0.031 | 5.20±0.80 | 4.95±0.85 |
| 3 | 13.36±0.24 | 8.91±0.42 | 9.43±0.85 | 7.77±0.50 |
| 4 | 20.40±1.29 | 14.56±0.78 | 15.33±0.75 | 11.37±1.60 |
| 5 | 28.90±1.05 | 21.80±0.37 | 22.65±0.40 | 15.81±0.35 |
| 6 | 38.80±2.02 | 31.86±0.95 | 31.25±0.20 | 20.65±0.30 |
| 7 | 49.10±2.20 | 43.20±0.17 | 41.30±0.95 | 26.10±0.15 |
| 8 | 61.11±0.28 | 55.60±0.22 | 52.45±0.05 | 31.95±0.10 |

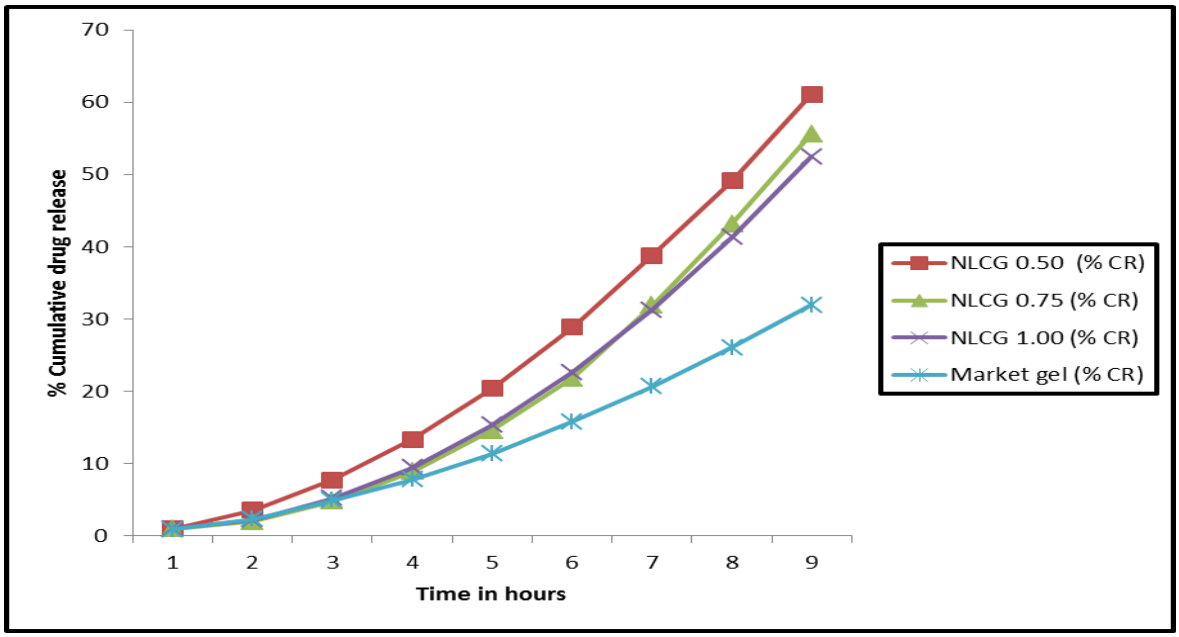


**Figure 6: In-vitro drug release of NLC gels and Marketed gel.**

## 6. Skin irritation study of the Nanogel

No abnormal changes were observed in clinical signs and no skin reaction for formation of Erythema and Oedema were seen in any animal from control, marketed or treatment group animals.

Based on the present study conditions, it can be concluded that NLC-gel sample when applied daily to Wistar Rats dermally for a period of 3 days, was well tolerated with no evidence of irritation potential in rats.

**Table 3: Primary irritation index values on the skin at the end of 24, 48 and 72 hrs.**

| Formulation | Irritation score | | |
|---|---|---|---|
| | Time of application | | |
| | 24 hr | 48 hr | 72 hr |
| CONTROL | A | A | A |
| MARKETED GEL | A | A | A |
| BOS-NLC GEL | A | A | A |

A-No reaction, B-Slight erythema, C-Moderate erythema, D-Severe erythema.


**Figure 7: Photographs of skin irritation study carried out on wistar rat.**

[A] Control (no application) [B] Marketed g[C] BOS-NLC GEL

### 7. In-Vivo anti-inflammatory study

In-Vivo anti-inflammatory activity was studied by using carrageenan-induced rat paw edema method. From the result, it is concluded that Boswellic acid has an anti-inflammatory activity. The activity of Boswellic acid was enhanced due to the increased permeation through the skin due to the nanometer size range.

**Table 4: Effect of Boswellic acid-NLC (0.5%w/w) on paw edema induced by carrageenan in rats.**

| | | Inflamation volume in ml | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **Dose** | **30 min** | **1 Hr** | **2 Hr** | **3 Hr** | **4 Hr** | **5 Hr** |
| Positive control | Carrageenan 1 % (0.1 ml) | 0.26 | 0.39 | 0.70 | 0.81 | 0.73 | 0.66 |
| BOS-NLC Nanogel | | 0.25 | 0.34 | 0.61 | 0.59 | 0.57 | 0.60 |
| Standard gel | | 0.22 | 0.25 | 0.34 | 0.36 | 0.35 | 0.32 |

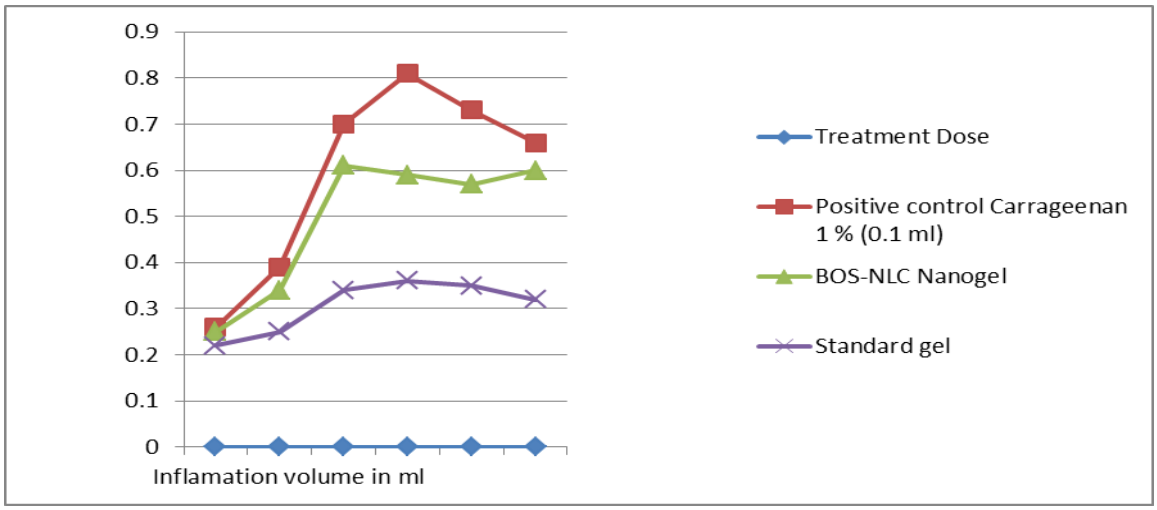


**Figure 8: Inhibition of edema due to BOS-NLC Nanogel.**

## CONCLUSION

After formulation of NLC the the solubility of poorly soluble drug boswellic acid was enhanced and it shows greater solubility as well as bioavailability which is responsible for the anti-psoriatic activity of the drug. In this study Nanostructured lipid carriers of Boswellic acid with the particle size 123.8 nm was formulated. Which shows entrapment efficiency 91.88± 12 and zeta potential -31.1 Mv. The study showed the increased solubility and bioavailability of the NLCs as compared to the drug.

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