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DEVELOPMENT OF A NOVEL LIPOSOMAL HERBAL NANOCARRIER OF BOSWELLIA SERRATA: IN VITRO AND IN VIVO EVALUATION FOR PSORIASIS MANAGEMENT

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ABSTRACT
Psoriasis is a chronic immune-mediated inflammatory skin disorder characterised by keratinocyte hyperproliferation and excessive production of inflammatory cytokines. Although several conventional therapies are available, their long-term use is associated with adverse effects and limited therapeutic efficacy. The present study aimed to develop and evaluate a novel liposomal herbal nanocarrier containing Boswellia serrata extract for improved anti-psoriatic activity. Liposomes were prepared using ethanol injection and reverse-phase evaporation techniques employing different lipid compositions. Preformulation studies, including physicochemical characterization, GC-MS analysis, and RP- method development and validation for quantification of 3-acetyl-11-keto-β-boswellic acid (AKBA) were performed according to ICH guidelines. The formulated liposomes were evaluated for drug loading, entrapment efficiency, particle size, zeta potential, and stability characteristics. Among the developed formulations, optimized liposomes exhibited high entrapment efficiency (89.31%), desirable drug loading, nanosized particle distribution (69.4–203.1 nm), and good zeta potential indicating enhanced stability. In vivo anti-psoriatic activity was assessed using an oxazolone-induced psoriasis model, where Boswellia serrata liposomes significantly reduced ear thickness, inflammatory cell infiltration, and epidermal hyperplasia compared with the extract alone. Furthermore, in vitro cytokine studies using RAW 264.7 cells demonstrated suppression of pro-inflammatory IL-23 and enhancement of anti-inflammatory IL-10 levels. The findings suggest that the developed Boswellia serrata liposomal nanocarrier possesses promising anti-inflammatory and anti-psoriatic potential with improved therapeutic efficacy and may serve as an effective herbal nanomedicine for psoriasis management.

KEYWORDS: Boswellia serrata; Liposomes; Psoriasis; Nanocarrier; AKBA; Anti-inflammatory activity; Cytokines; IL-23; IL-10; Herbal drug delivery system

INTRODUCTION
Dermatitis is a superficial skin irritation with weakly demarcated erythema and irritating crusts and scales 1. Eczema and psoriasis are the most common dermatological conditions 2.
Psoriasis is a prevalent, long-lasting inflammatory illness that mostly impacts the skin and joints. The prevalence of psoriasis in North India, varies from 0.44 to 2.8%[3]. It is defined by distinct, red, thicker patches with a silver-white layer on top [4]. Polymorphic neutrophils in the skin support inflammation. Neutrophils are activated by keratinocyte and T-lymphocyte chemokines and lymphokines, respectively, which in turn activate lymphocytes and keratinocytes, creating a vicious cycle limited to acute lesions but linked to chronic inflammation [5]. The immunosuppressive medications methotrexate, cyclosporine, and fumaric acid esters treat psoriasis [6]. However, breakthroughs in mechanistic understanding of psoriasis signaling networks have led to biological therapy testing. Anticytokine therapy (adalimumab, etanercept, infliximab, ustekinumab) and immune suppressive medicines (eg, alefacept) are examples [7]. Despite the wide availability of pharmaceutical medications and biologics in the market, their influence on psoriatic patients is limited due to several constraints and limitations. Hence, *Boswellia serrata* Roxb, a form of complementary and alternative medicine (CAM), has been extensively studied and proven to be both safe and effective in treating mild to moderate psoriasis.
Boswellia serrata is a moderate sized deciduous tree that is native to India, the Middle East and Northern Africa. *Boswellia serrata* are used in traditional ayurvedic medicine to treat inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, osteoarthritis, and asthma [8–11]. Also used as antiseptic, astringent and gastrointestinal complaints. Boswellia extract is also used as a perfume and aroma (frankincense). Boswellia extract contains essential oils, terpenoids, sugars and volatile oils and prominently several pentacyclic triterpene acids such

as beta boswellic acid. In vitro boswellic acid inhibits the synthesis of 5-lipooxygenase and decreases production of downstream pro-inflammatory mediators.
Therefore, the purpose of this study was to develop, characterize, and evaluate a nanoparticulate-based drug delivery system. The study was designed to improve the oral bioavailability and efficacy of *Boswellia serrata*.

MATERIAL AND METHODS

Material:

3-Acetyl-11-keto-β-boswellic acid is purchased from Yucca enterprises, Mumbai.

Method:

Preformulation study

Physical properties of Frankincense oil (FO): The oil was checked for its color, odour, density, and solubility.
Determination of purity of oil by GC analysis: The purity was determined by GC- MS (Trace1300 G.C coupled withThermoTSQ8000TripleQuadrupoleMSConditions), using column 25m fused capillary column with CPSil 5CB.The scan was done between 50- 600. Solid dried sample was dissolved in methanol and injected. The sample volume 1µl was run for 20mins and then the GC graph was obtained. The list of components present were obtained from GC-MS library.

HPLC method Development and Validation

Frankincense oil mainly contains different derivatives of Boswellic acid like 11-keto β-boswellic acid (11-KBA) and 3-acetyl 11-keto β-boswellic acid (AKBA). And thus, we have developed HPLC method for the quantification of 3-acetyl 11-keto β-boswellic acid (AKBA) in formulations.

Development of HPLC method

Reverse-phase high-performance liquid chromatography was used to determine the amount of the drug in the sample. Using the Agilent Tech. (1100) equipment, the determination was completed. The separation of the chromatogram was carried out on a Fortis C18 column (100 x 4.6 mm id with 2.5 mm particle size) using HPLC grade acetonitrile, and distilled water with 0.1 percent Ortho phosphoric acid (OPA) in the ratio of 92:8 as the mobile phase, and the stationary phase was distilled water. Detection was carried out at a predetermined wavelength. The flow rate was maintained at two millilitre per minute. The drug's quantitative value was calculated by calculating peaks with the help of the CHEMSTATION 10.1 programme.

Preparation of mobile phase

Mobile phase was prepared by mixing HPLC grade acetonitrile, and distilled water with 0.10% Ortho phosphoric acid (OPA) in the ratio of 92:8 v/v. The pH of the solvent system was maintained at 3.5. The content was sonicated for 15 min and filtered through a 0.45 µm membrane filter. Mixed solvents were degassed and used as a mobile phase.

HPLC method Development

The optimization of the RP-HPLC chromatographic parameters was accomplished using various mobile phase compositions. Using the mobile phases mentioned above, the resolution and peak symmetry obtained were excellent, as was the symmetry of the peaks. The quantification of peak area was performed at the determined wavelength based on the peak area. The proposed method's suitability for the proposed system was assessed. The system suitability test was performed on a freshly prepared standard stock solution of AKBA to ensure that it was as effective as possible. To determine the suitability of the system, various parameters such as resolution, peak tailing, and HETP were investigated.

Method validation parameters

The validation of the developed HPLC method was carried out in accordance with ICH guidelines. The linearity was analysed for concentration ranging from 20 to 100 micro g/ml of AKBA by using Least-square regression analysis where, peak areas were plotted against the corresponding concentrations. The repeatability of the device was evaluated by repeatedly injecting a solution of a drug with a concentration of 60 micro g/ml. The intra- day and inter- day precision was evaluated by triplicates of three different concentrations of each AKBA was spotted and analyzed on same day for intra-day study and two different days for inter-day study with respective chromatographic conditions.
Recovery study method was employed to evaluate accuracy. The samples were spiked with 80, 100 and 120 % of median concentrations of standards.

$$Accuracy = \frac{\text{spiked concentration} - \text{mean concentration}}{\text{spiked concentration}} \times 100$$

Robustness was carried out by making deliberate changes in the detection wavelength (less than one nanometer), flow rate (less than 0.1 millilitres per minute) and mobile phase and quantitative analysis was determined. The estimation of LOD and LOQ were done by standard deviation method. Detection limit =3.3σ /S and quantitation limit=10 σ /S (σ is residual standard deviation of a regression line and S is the slope of the calibration curve).

DRUG-EXCIPIENT COMPATIBILTY STUDIES:

The oil and the common excipients used in the formulation were mixed in 1:1 ratio and stored in an air tight container and the physical appearance and drug content was observed for 15 days.

Physical appearance: The oil and excipient mixture were checked for its physical appearance i.e. colour and odour.

Drug content: 10mg of the sample containing the oil and excipient mixture was weighed, added to 10ml of volumetric flask. Small amount of methanol was added to dissolve the mixture and volume was made up with methanol to 10ml. The absorbance was determined at 264.7nm and the drug content was calculated from the regression equation.

Formulation Of Liposomes

Ethanol Injection Method

Three different lipids were experimented namely. DPPC, sunflower lecithin and soya lecithin with two different concentrations of each lipid.

Table No.1: Formulations of liposomes by Ethanol Injection Method

Content		EI1	EI2	EI3	EI4	EI5	EI6
FO		10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Lipid	**DPPC**	5 mg	10 mg	-	-	-	-
	Sunflower lecithin	-	-	5 mg	10 mg	-	-
	Soy lecithin	-	-	-	-	5 mg	10 mg
	Cholesterol	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg	2.5 mg
Organic Solvents	**Ethanol**	2 ml	2 ml	1 ml	1 ml	1 ml	1 ml
	Chloroform	-	-	1 ml	1 ml	1 ml	1 ml
Water		10 ml	10 ml	10 ml	10 ml	10 ml	10 ml

Accurately weighed quantities of drug and water were taken in a beaker. This mixture was subjected to stirring on magnetic stirrer at 500rpm until a clear solution was obtained. The solution level was marked with a marker on the beaker. In another beaker, the above-mentioned lipids were accurately weighed, the organic solvent was added and this organic mixture was added to the aqueous mixture dropwise, with the help of a syringe. The mixture was stirred at 500rpm at room temperature for an hour or until the solution level had been reduced to a level of the mark. The obtained liposomal mixture was refrigerated for 24 hrs and the further tests were carried out.

Reverse phase technique using Rota-vapor

The drug, lipids and cholesterol were added to a 100 ml round-bottom flask with a long extension neck containing solvent mixture comprising of ethanol and chloroform (1:1). The round flask was rotated at a constant rpm of 150 dipped in a temperature-controlled water bath maintained at 40°C using a Rotary Evaporator. Solvent was removed under reduced pressure by using a vacuum pump. A thin film was formed after complete evaporation of solvent mixture. The lipids were redissolved in the organic phase and increase the solubility of lipids using chloroform. The aqueous phase was prepared by dissolving Mannitol in 25ml of deionized water with the aid of heat. The prepared aqueous phase was added to the above prepared organic phase. Sonicated the mixture for 15 mins with pulse rate of 3 seconds followed by 1 second interval (no pulse) and at amplitude of 80% in a bath-type (in cold condition). The mixture was sonicated until a homogeneous opalescent dispersion was obtained. The mixture was placed on the rotary evaporator to remove the organic solvent under reduced pressure {vacuum} at 20-25°C, rotating at approximately 180 rpm. A viscous gel was formed exhibiting an aqueous suspension. An excess deionized water was added to the formed suspension and evaporated for an additional 15 min at 20 °C to remove the traces of solvent. Liposomes formed were observed under an oil emulsion microscope (100X).

The composition of the formulations by reverse phase technique is same as in previous method given in Table 1.

Evaluation of formulated liposomes Percent Drug Loading

The mass ratio of drug to drug-loaded nanoparticles is known as drug loading. It reflects the amount of medication in nanoformulation that is truly present. A high drug loading with minimal nominal drug loss during formulation is ideal because it enhances entrapment efficiency and, as a result, dosage form performance.

HPLC was used to determine drug loading. Using deionized water, one ml of nanoliposomes formulation was dissolved in 1 ml ethanol, resulting in a volume of 10 ml. After that, the solution was sonicated for 5 minutes. After that, 0.45 m filters are used to filter the solution. The filtrate was then subjected to HPLC analysis.

Entrapment Efficiency

One of the most essential criteria for determining a medication delivery system's success is encapsulation efficiency. Encapsulation is a technique used in medication delivery systems to create a shell around a medicine to protect it from leaching out before it reaches its intended destination. The percentage of drug successfully entrapped in the nanoparticle is known as encapsulation efficiency, and it reflects the amount of medicine supplied per amount encapsulated. The lipid concentration, solubility of the lipid in the solvent, rate of solvent removal, solubility of organic solvent in water, and other parameters all influence nanoparticle encapsulation efficiency.

To separate the free drug, a remi cooling centrifuge was used to spin the liposomal formulation (10 mcg/ml) at 4000 rpm for 18 minutes at 4°C temperature. The suspended liposomes and free drug on the centrifuge tube's wall are found in a supernatant. At 4°C temperature, the supernatant was centrifuged for 30 minutes at 15000 rpm. As a result, a clear supernatant and liposome pellet solution was produced. The liposome pellet was re-dispersed in 1 mL ethanol before being diluted with distilled water to a final concentration of 10 mL. The medication was released once the solution was sonicated and the liposomes were disrupted. For the drug entrapment, the discharged drug was determined. Using an HPLC instrument, the quantity of AKBA was determined. Percentage entrapment efficiency was determined as

$$\text{Percentage Entrapment Efficiency} = \frac{Wc}{Wt} \times 100$$

Where amount of oil content (entrapped) in the liposomes is denoted as W_c and total amount of drug in the dispersion is denoted as W_t.

Based on the results of drug loading and entrapment efficiency for prepared nanoliposome formulations, particle size analysis and Zeta potential was evaluated.

Particle size analysis

Liposome particle sizes, were assessed using dynamic light scattering methods, which measure the intensity of scattered light created by existing particles moving in the sample. Particles with diameters ranging from 0.3 nm to around 10 m can be studied using the light scattering method.

A Zetasizer equipment was used to assess particle size at a temperature of 25 degrees Celsius. By using this approach, the mean particle diameter and particle size distribution may be calculated. Horiba scientific Instruments supplied the software that was used to conduct the investigation. Prior to analysis, samples were put in a refrigerator at a temperature of 4 degrees Celsius. All measurements were carried out in triplicate to ensure accuracy. In order to estimate the diameter of particles, the average particle size was employed.

Zeta potential

The zeta potential is a physical property that each particle in a colloidal system can show. The zeta potential is significant because its value can be linked to the stability of colloidal dispersions. Colloids with a high zeta potential (negative or positive) are electrically stable, whereas those with a low zeta potential coagulate or flocculate. The arbitrary value of 25mV (positive or negative) can be used to distinguish low-charged surfaces from high-charged surfaces. The zeta potential of nanoliposomes was determined using the Horiba scientific equipment. A zeta potentiometer was used to determine the zeta potential. The material was placed in the cell, and an electrode was inserted and connected to the zeta metre under the microscope. The experiment was conducted out at a temperature of 25°C.

Animal Study

Anti-Psoriatic: Oxazolone mice model

After the approval from the Institutional Animal Ethics Committee (Protocol No. PESRTBCOP/IAEC, 2022R-96).The experimental work was performed at animal house of Rajaram and Tarabai Bandekar college of Pharmacy, Ponda, where Albino Wistar rats of 200-250 g weight were used to determined psoriatic action. The animals were divided in five groups. Group I as normal, group II as control, group III as BS extract, and Group IV as BS liposomes.

Sensitization and Elicitation (Challenge Application) Procedure: The animals were sensitized for six days by applying 100 µl of 1.5% oxazolone in ethanol to their abdomen area. On days 7, 10, 13, and 16, 20 µl of % oxazolone in a combination of acetone and olive oil (4:1) was applied to both sides of the mouse ear after 7days of sensitization.

Measurements: Ear thickness was estimated using digital Vernier Calipers at several time periods during the investigation. To determine swelling ear responses, ear thickness was evaluated before the sensitization phase (Day 7) and after each elicitation on days 10, 13, 16, and 19. Mouse ears were removed, fixed in 10% buffered formalin solution, embedded in paraffin, cut into 5 µm slices, and stained with hematoxylin-eosin 72 hours after the last administration of oxazolone using conventional techniques. The epidermal thickness was evaluated as the distance

between the bottom of the stratum corneum and the foundation membrane in the interfollicular epidermis during the histological examination, after the microscopic fields were photographed. Inhibition of epidermal thickness was calculated.

***In-vitro study* of inflammatory Cytokines by ELISA method of extracts and their Nanoliposomes**

Psoriasis, a chronic inflammatory skin condition, affects 2% to 3% of the world and causes severe morbidity. The cause is unknown, however it is thought to be a hereditary, complicated autoimmune inflammatory disease. TGF-β1 levels are raised in plasma and lesion scales in psoriasis. This release and dendritic cell activation through pattern-recognition receptors may create Th17 cells in skin-draining lymph nodes that can induce IL-23R expression on developing Th17 cells. IL-23R expression enhances Th17 cell survival and proliferation by increasing IL-23 responsiveness.

Through toll like receptor (TLR) signaling, macrophages identify pathogen-associated chemicals like LPS (a bacterial endotoxin) and activate innate immunity. Dendritic cells produce IL-23 after lipopolysaccharidem-induced TLR 4 activation. Thus, IL-23 pathways are crucial to psoriasis immunopathogenesis, but many additional cytokines are implicated.

Type 2 IL-10 has immunosuppressive and anti-inflammatory properties. IL-10 inhibited dendritic, monocyte, and macrophage antigen-presenting cells. UV radiation, a proven psoriasis treatment, also reduced inflammation by increasing IL-10 in keratinocytes. In psoriatic skin, low IL-10 levels were thought to cause disease flares.

Promising treatments for several inflammatory disorders include inhibiting unfavorable macrophage activation or selectively neutralizing macrophage product overproduction.

A) Cell line, Reagents and kits

RAW 264.7 cell line was obtain by the National centre for cell science (NCCS) Pune, India. Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate buffer saline were purchase from Invitrogen (Carlsbad, USA). LPS purified from Escherichia (serotype 026:B6) was purchase from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade. Enzyme-linked immunosorbent assay (ELISA) kits were purchase from Ray Biotech Inc, USA.

- **Cell culture and culture conditions**

RAW 264.7 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 µg/ml streptomycin and 50 units/ml penicillin. The cells were incubated at 37°C in the presence of 5% CO_2 and sub-cultured every 2 days.

- **Cell viability assay**

The viability of the cell was evaluated by MTT assay. The samples were prepared by sonicating 1gm with 100 ml of methanol for 1 hr resulting in 10 mg/ml of solution. The 1 ml of solution further diluted with DMSO to produced 1000 µg/ml concentration. The RAW 264.7 cells were seeded into 96-well cultured plate at a density of 5×10^4 cells/well and incubated overnight at 37°C and 5% CO_2 for attachment. The cells were then treated with sample of concentrations range of 0.625 – 1000 µg/ml with (1 µg/ml)or without LPS and incubated for 24 hrs. After incubation, the culture medium was removed and 100 µl of fresh DMEM and 20 µl of MTT (5 mg/ml in PBS) solution was added to each well. Following 4 hrs incubation in the dark, the media was discarded again and 100 µl of DMSO was added to each well for the solubilization of formazan deposits. The optical density of the cells at 570 nm were measured using an ELISA plate reader (Bio-Rad Laboratories, CA, USA) and the experiment was carried out in triplicate.

- **Measurement of pro-inflammatory and anti-inflammatory cytokines (IL- 23 and IL-10) production :**

The RAW 264.7 cells were seeded at a density of 2×10^4 cells/well in 24 well cultured plates and incubated for 24 hrs at 37°C and 5% CO_2 for adherence. The adhered cells were incubated for 24 hrs, with the indicated concentrations of test samples (500 mcg/ml) in the absence or presence of LPS (1µg/ml). The cell culture supernatant was harvested after 24 hrs of incubation of cells with LPS and samples. These supernatants were tested for quantitation of pro- and anti-inflammatory cytokines (IL-23 and IL-10) using mouse-specific enzyme immune-assay kit (RayBiotech Inc, USA) according to manufacturer's instructions.IL-23 is a key cytokine for promoting inflammatory responses in a variety of target organs. The most important function ascribed to IL-23 is its role in the development and differentiation of effector Th17 cells via activation of STAT3. Interleukin 10 (IL-10) is also potent anti-inflammatory cytokine that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis.

B) ELISA

Briefly, the ELISA plates (96 well) were coated with specific mouse IL- 23, IL-10 antibodies (100 µl/well) and incubated at 4°C for overnight. The assay diluents (200 µl/well) were used to block the non-specific protein-binding sites present in the plate. Immediately, 100 µl of culture supernatant or respective standard was added into the suitably coated wells and incubated at room temperature for 2 hrs. After incubation, the plates were washed 5 times thoroughly with wash buffer [phosphate-buffered saline (PBS) containing 0.05% Tween-20]. About 100 µl of detecting solution (detection antibody and streptavidin horse-radish peroxidase) was added into each well.

The plates were properly covered with a plate sealer and incubated for 1 hr at room temperature and again wash 5 times thoroughly using wash buffer. Substrate solution of 100 µl, tetramethylbenzidine (TMB) was added to each well

and the plate was further incubated (without plate sealer) for 30 min in the dark at room temperature. Finally, 50 µl of stop solution (2N H_2SO_4) was added to each well. ELISA results were recorded at 450 and 570 nm with an ELISA reader (Bio-Rad Laboratories, CA, USA). The absorbance at instruction. The concentration determined for three wells for each cytokine and values were derive from the standard curve and express as pg/ml.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES:

Physical properties of the oil: The result of various physical properties of Frankincense oil are given in Table no. 2

Table no. 2: List of physical properties of the Frankincense oil

Sr.No.	Physical property	Observation	Inference
1	Colour	Pale yellow, light yellow	Complies to standard
2	Odour	Lemon like, sweet woody, balsamic undertone	Complies to standard
3	Density	0.873g/mlat25˚C(lit)	Complies to standard
4	Solubilityat37˚		
	Water	Insoluble	Complied to standard
	Ethanol	Soluble	Complied to standard
	Methanol	Soluble	Complied to standard
	Dichloromethane	Soluble	Complied to standard
	Ethylacetate	Soluble	Complied to standard
	Chloroform	Soluble	Complied to standard

Determination of purity of the oil by GC analysis:

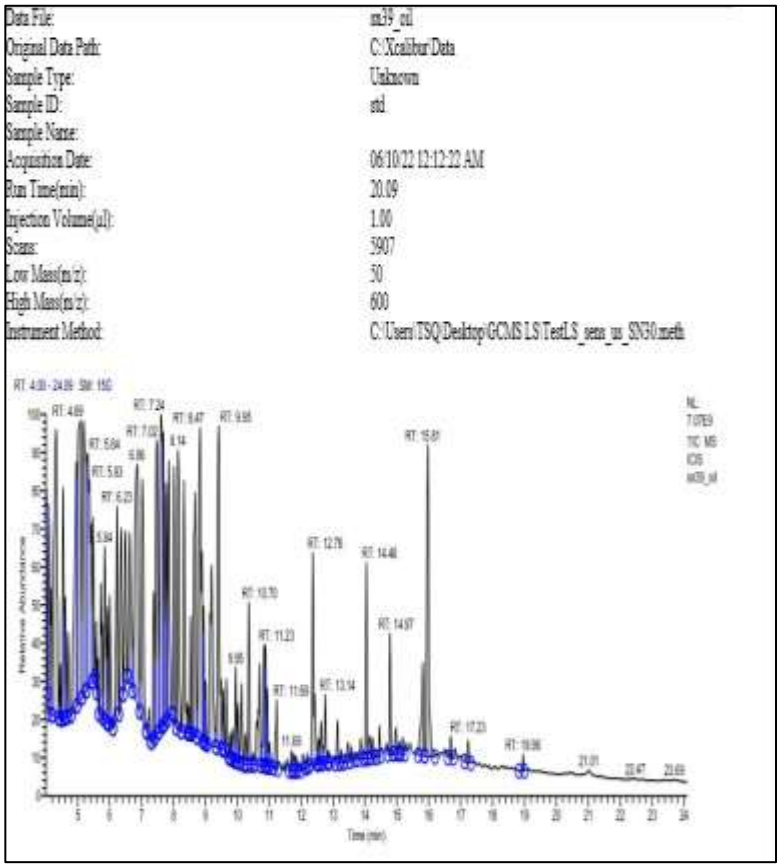


Fig No.6.1: GC analysis graph of Frankincense oil sample

HPLC METHOD DEVELOPMENT AND VALIDATION

The aim of ICH guidelines is to validate the developed analytical method and the method is suitable for its intended purpose. The procedure to be validated includes Identification tests; Quantitative tests for impurities' content. The purpose of validation is to provide some guidance on how to consider the different validation characteristics for each analytical procedure. The study also ensures the quality of the API or formulations. In the present study the quality of AKBA in FO liposomes was evaluated.

Optimization of RP- HPLC method

The essential parameters that determine the optimization of any chromatographic method for analysis are good resolution, peak shape, theoretical plates, retention time, and asymmetry. Several circumstances of chromatographic procedures, such as varied compositions of mobile phase, flow rate, and various stationary phases, were optimised and evaluated for the assessment of AKBA in order to achieve all of these characteristics. With mobile phase, the produced peak was observed to be excellent, crisp, symmetrical, and well defined. Acetonitrile: water (0.10 percent OPA) in a 92:8 v/v ratio, with a flow rate of 2 ml/minute at 250 nm. The retention time of AKBA was observed at 10.15 min (Figure 1). Optimized characteristics are given in table 3.

Table No 3: Optimized Characteristics

Sr. No.	Parameters	Specifications
1.	Composition of Mobile phase	Acetonitrile: water (0.1% of OPA) in a ratio 92:8 v/v
2.	Column Specifications	FortisC18 (100 x 4.6 mm id with 2.5 µm)
3.	Flow Rate	2.0 ml/min
4.	Retention Time	10.15 min
5.	Symmetry	0.89

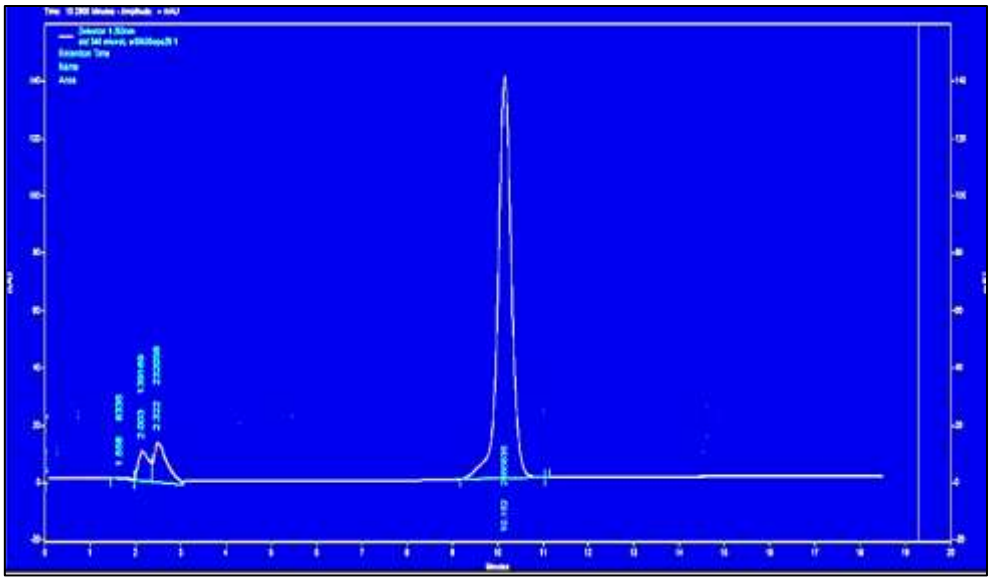


Figure 1: Chromatogram of AKBA

Method validation parameters

1. Linearity

The method's linearity was established by diluting the standard stock solution to obtain concentration ranges of 20 to 100 g/ml. The findings indicate that a strong connection existed between peak area and analyte concentration. The calibration curve was constructed and assessed using linear regression by graphing the AUC vs the analyte concentration (Figure 2).

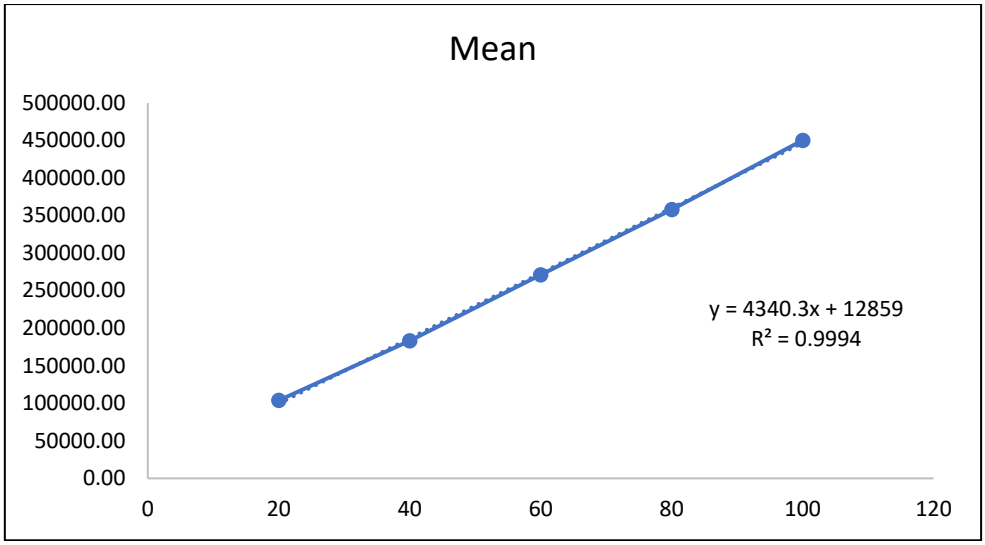


Figure 2: Linearity curve of AeKBA

2. Accuracy

At three distinct concentration levels, the medication recovered well, demonstrating that the procedure was accurate. In the pre-analyzed samples, a known quantity of standard medication (80, 100, and 120 percent) was added, and the samples were exposed to the suggested HPLC procedure. The % recovery values for the API at three different level (80%, 100% and 120%) were 99.60%, 93.43% and 101.58% found to be within the limits.

3. Precision

Method precision (repeatability)

Method precision was evaluated by repeatedly introducing 60 µg/ml concentration. The developed method was found to be precise as % RSD was found to be 0.45.

Intermediate precision

The analysis of three different concentration (40, 60, 80 µg/ml) of standard solution showed good reproducibility. The % RSD was found to be 0.11, 0.84 and 0.55 for interday precision and 1.17, 0.32, and 0.81 for intraday precision.

4. Robustness

Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate and wavelength. It was observed that there were no marked changes in the chromatograms. The developed method was found to be robust as the % RSD values were< 2.0 %.

5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

This data showed that the sensitivity of method to determine the AKBA. The LOD and LOQ were found to be 0.0405 µg/ml and 0.1229 µg/ml respectively.

DRUG AND EXCIPIENT COMPATIBILITY STUDY:

The physical appearance of the oil and excipient mixture was checked visually for any change for 15 days and the drug content was determined on 0^{th}, 7^{th} and 15^{th}day and it was found that there is no significant change in physical appearance and drug content of oil and excipient mixture. Hence the oil and the excipients were found to be compatible with each other.

Table No. 6: Evaluation of Drug and Excipient Compatibility

Ratio of Mixtures	Days	Physical appearance	Drug content
Mixture of the oil with Cholesterol (1:1)	Day0	White powder	95.18%
	Day7	White powder	93.50%
	Day15	White powder	92.12%
Mixture of oil with sunflower lecithin (1:1)	Day0	White powder	97.99%
	Day7	White powder	96.12%
	Day14	White powder	94.63%
Mixture of oil with DPPC (1:1)	Day0	White powder	98.50%
	Day7	White powder	97.87%
	Day14	White powder	95.54%
Mixture of oil with Soya lecithin (1:1)	Day0	White powder	97.59%
	Day7	White powder	96.09%
	Day14	White powder	94.32%

Formulation of Frankincense oil loaded nanoliposomes

Ethanol injection method and reverse phase techniques

The formulations composed of different ratio of Frankincense oil and lipids. The formulations were prepared by two different methods such as ethanol injection method (EI1- EI6) and reverse phase technique (RP1- RP 6). Among the selected lipids, liposomes formulated with sunflower lecithin (both concentrations) and DPPC with 5 mg by ethanol injection method delivered better EE and percent drug loaded data (EI4, EI3 and EI1). Similar combinations were found to be efficient in second technique (RP4, RP3, RP1). And thus, of these sixes' formulations particle size and zeta potential was evaluated.

Evaluation of liposomes

Entrapment Efficiency and Drug Loading

Entrapment efficiency (EE) is defined as the ratio of drug in nanoparticles to the total amount of drug added to the formulation. The EE percent is the amount of drug encapsulated in the liposomal structure. In the development of liposomes as drug carriers, excellent encapsulation and retention of the encapsulated API are critical. A high drug-to-lipid ratio is likely to save formulation costs while simultaneously lowering the lipid-induced toxicity. The percentage of encapsulation was obtained based on the linearity graph of drug obtained by the HPTLC method (mentioned above analytical method development) standard curve of the drug's formulation. The percentage encapsulation efficiency and drug loading given in table 10. The % entrapment efficiency and % drug loading ranged between 29 to 89% and 38 to 91%. The formulation EI4, EI3, EI1 and RP4, RP5, RP1 showed 89.31%, 83.79, 71.27 and 87.57, better entrapment efficiency and drug loading.

Thus, of these sixes formulations particle size and zeta potential analysis was carried out.

Table 10: Percentage Encapsulation Efficiency

Sr. No.	Formulation N=3	% Entrapment Efficiency	% Drug Loading
1	EI1	71.27	84.78
2	EI2	31.75	65.34
3	EI3	83.79	84.97
4	EI4	89.31	91.17
5	EI5	35.48	41.50
6	EI6	37.22	38.74
7	RP1	76.82	80.81
8	RP2	29.04	74.34
9	RP3	74.66	87.51
10	RP4	87.57	93.46
11	RP5	35.77	79.89
12	RP6	39.13	54.02

Table No.11: Particle size and zeta potential analysis of best six liposome formulations

Trial No.	Particle Size (nm)	Zeta Potential
EI1	89.3	-53.4
EI3	74.7	-64.6
EI4	69.4	-73.9
RP1	91.2	-75.3
RP3	191.5	-75.6
EI4	203.1	-85.9

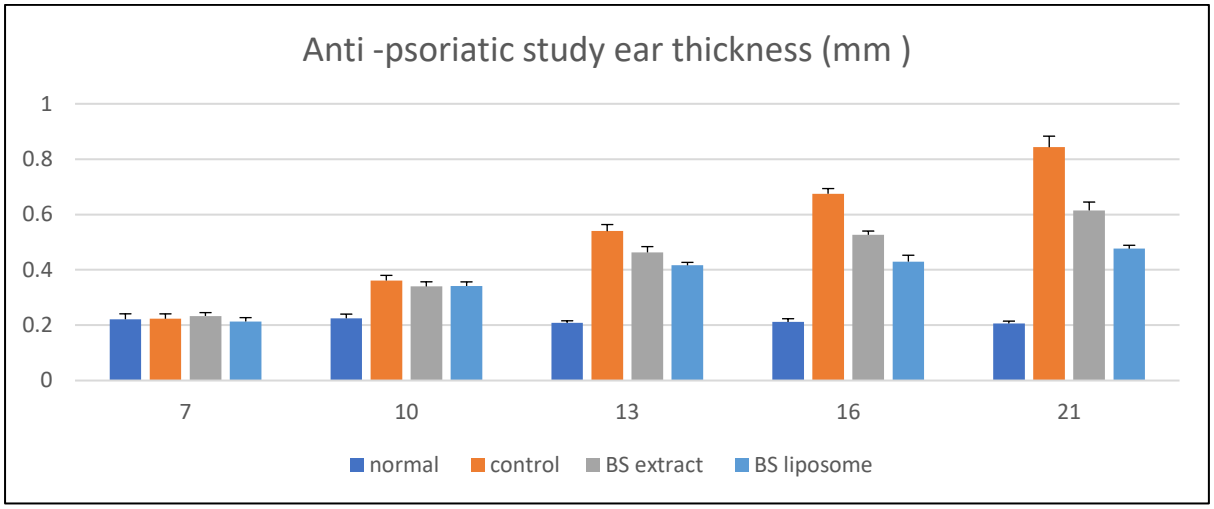
Particle size analysis and Zeta potential measurement

The particle size of nano-liposomes containing Frankincense oil were determined using Horiba Scientific. The particle size of the nano-liposomal formulation was ranges between 69.4 nm to 203.1 nm Table 11. The formulation EI4 showed lowest particle size that is 69.4 nm. The zeta potential was found to be in the ranges between -53.4 mv and -85.9 mv (Table 11). The formulation EI4 showed better zeta potential of -85.9, however the size is more (203.1), thus if compared EI4 is better among all formulations terms of all parameters that have been evaluated.

The surface charge of the nano particle determines the zeta potential which is a paramount factor for the stability of nano formulations. This is turn is majorly responsible of the primary absorption of drug (which is in nano delivery system) onto the cell membrane. Once absorption is done, the particle size play the vital role in the endocytotic uptake rate. Thus, particle size and zeta potential are the key factor for drug penetration into cells and tissues and nanoparticle toxicity.

Animal Study

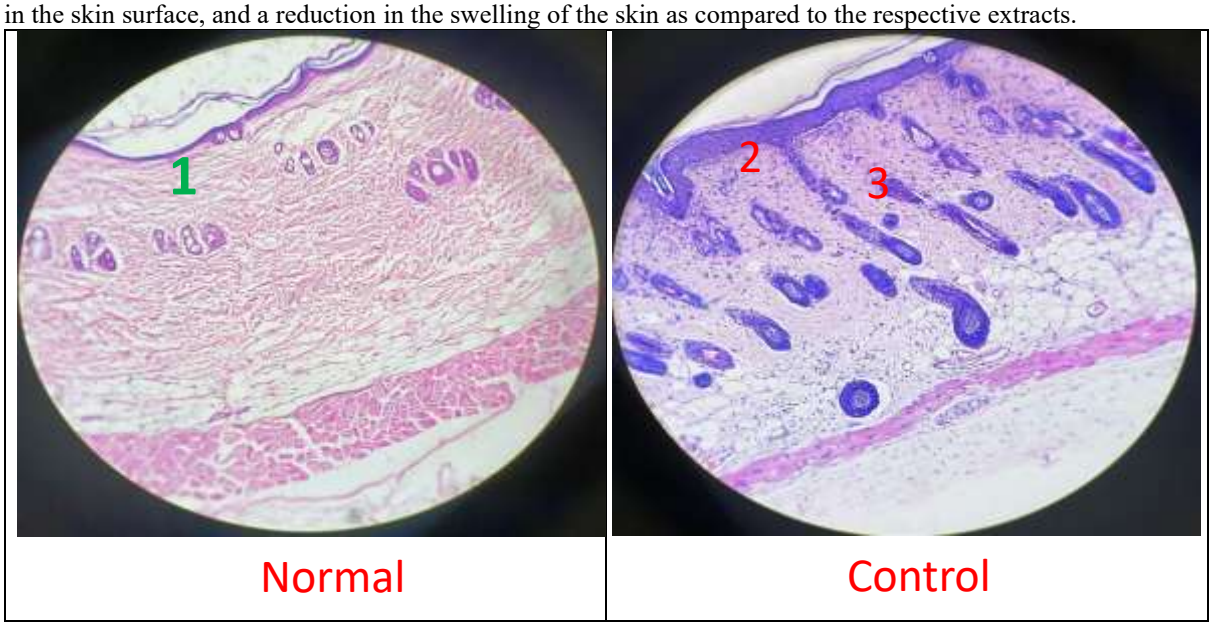
The effect of extract (BS) and formulations (BS liposomes) was evaluated by topical application of formulations to oxazolone induced psoriasis in mice for 16 days. The animals were sensitized by applying oxazolone to the abdominal region of the mice. The formulations were applied after the challenge. The evaluation was performed by measuring the thickness of ear. After the completion of treatment with both the formulations showed significant decreased in ear thickness, indicating the anti-inflammatory potential of the extracts and formulations.

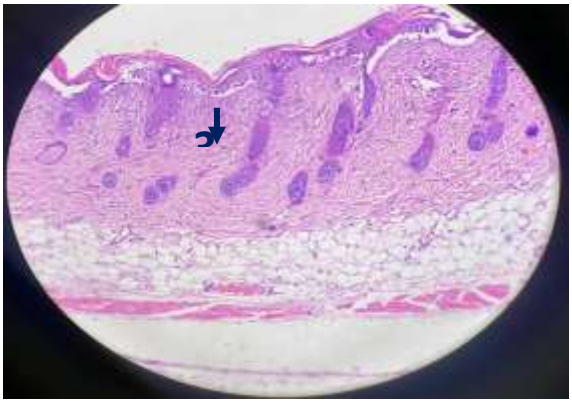


Determination of anti- psoriatic action of extracts and nanoliposomes by measurement of ear thickness

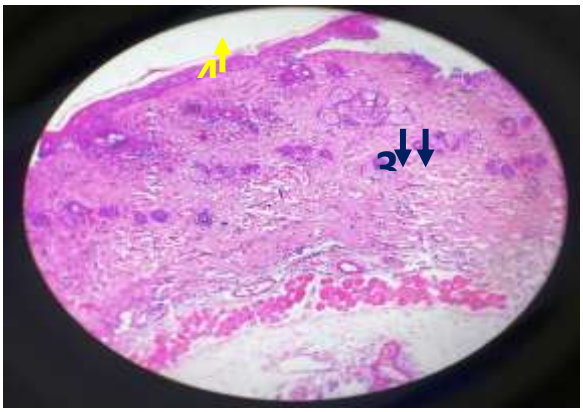
Histopathology Study

According to the findings of the histopathology study, the formulations of the active ingredient that were developed have increased efficacy, resulting in a reduction in the number of inflammatory cells, an improvement in the skin surface, and a reduction in the swelling of the skin as compared to the respective extracts.

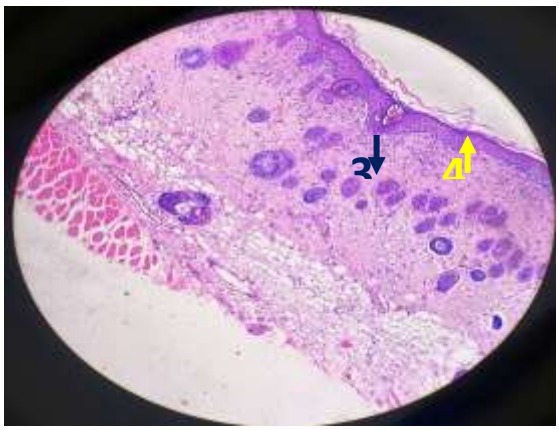




BS



BS liposomes



BS liposomes

1: normal epidermis,

2: thickened epidermis with psoriatic cells,

3: inflammatory cells,

4: keratinized layer

: slight reduction

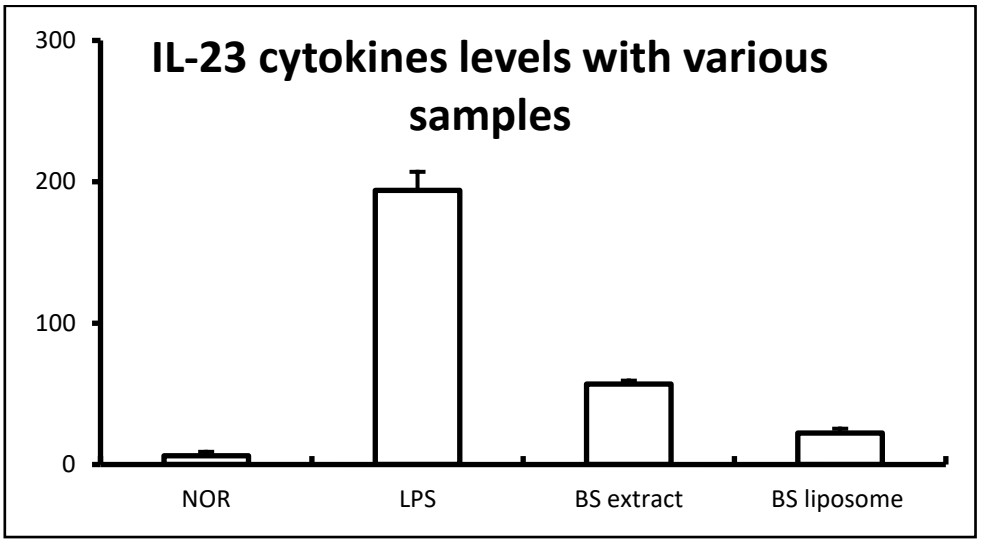
: more reduction

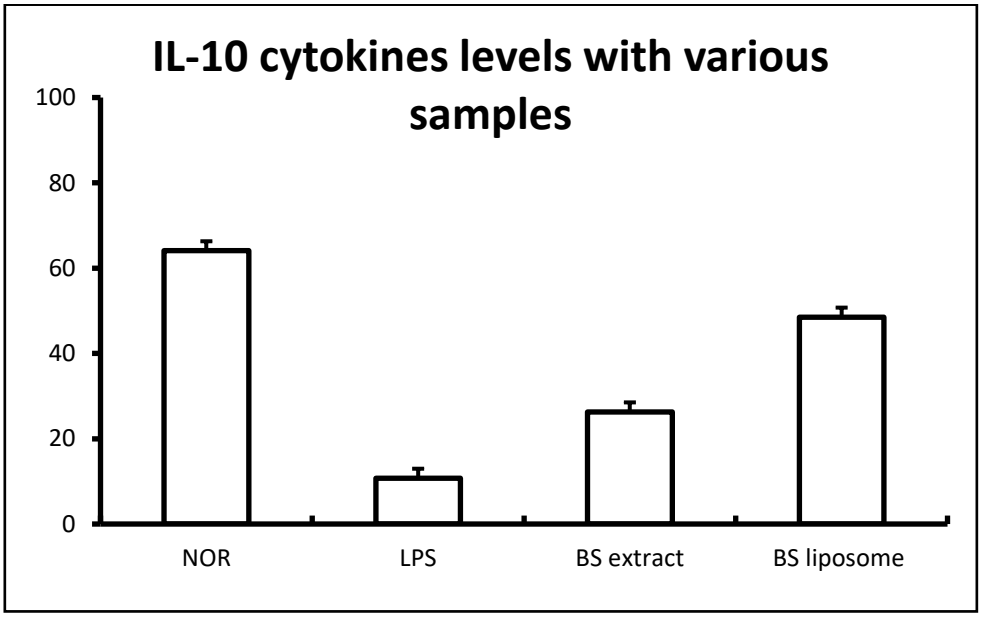
***In-vitro study* of inflammatory Cytokines by ELISA method of extracts and their Nanoliposomes**

The increase in the plasma concentrations of Tumour necrosis factor-α (TNF-α), Vascular endothelial growth factor (VEGF), ProstaglandinE2 (PGE2) and Leukotriene (LTB4) values, results in to a chronic inflammatory condition.

The comparative effect of extracts and their respective liposomes on production on these cytokines (IL-23 and IL-10) were analyzed by ELISA. To investigate the effects of samples on psoriasis, a Raw 264.7 cell macrophagic cell inflammatory model was established, wherein cells were treated with LPS for 1 h. The anti-inflammatory cytokine IL- 10 was elevated by liposomes at 500 mcg/ml (48.51% and 46%). The maximum suppressive effect

of liposomes on IL- 23 evaluated was approx 22% at 500 mcg/ml. As the formulation regulated the production of cytokines which are related to psoriasis etiology or treatment. It can be concluded that the formulation has potent anti-psoriatic action.





CONCLUSION

Psoriasis is an intricate, multifaceted condition that seems to be affected by genetic and immune-mediated factors. Psoriasis is a medical condition that is caused by an increase in the presentation of antigens, activation of T-cells, and the release of T-helper cell type 1 cytokines. This leads to the formation of thick, scaly, red plaques on the skin and, in certain individuals, arthritis may also occur. Psoriasis is linked to indicators of widespread inflammation, such as elevated levels of CRP.Psoriasis accelerates the proliferation of skin cells due to an underlying inflammatory response. Consequently, the body generates fresh skin cells at regular intervals. Subsequently, these cells accumulate on the outer layer of the skin and transform into psoriasis plaques and scaly protrusions. *Boswellia serrata* is used in traditional ayurvedic medicine to treat inflammatory diseases. Liposomes have the ability to improve the solubility of drugs, control their distribution, and provide the option to modify their surface in order to achieve continuous release that targets specific areas [12]. Commonly, they are employed for the purpose of enclosing drugs, with dimensions ranging from 50 to 150 nm, rendering them appropriate for drug administration through different pathways. Liposomal formulations have been studied in the context of psoriasis to improve the targeted distribution of medicinal drugs to the skin, which could enhance effectiveness and minimize adverse effects. The in vivo and cytokine study states that the prepared nano formulation of *Boswellia serrata* found to effective against psoriasis.

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