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FORMULATION AND EVALUATION OF HERBAL NIOSOMAL GEL FOR PSORIASIS LIKE EFFECT

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ABSTRACT

Herbal dugs are used widely to cure a variety of diseases due to its increased efficiency and lesser side effects. Novel drug delivery system is an emerging drug delivery system used widely now a days. So combaining the herbal drug delivery with novel drug delivery system may enhances the therapeutic benefits. Niosomal formulations are already proven its potential in treating various diseases. In accordance with the improved therapeutic efficacy of niosomes, herbal drug can be incorporated in to it to produce a synergistic effect. The two plants *Wrightia tinctoria* and *Aloe barbadensis* having potent anti-psoriatic activity and their synergistic action when incorporated in to niosomes have been studied. Niosomes were prepared by thin-film hydration method and the drug extracts were incorporated in to it. Vesicle size of niosomes was analysed shows good results. Size and shape of niosomes formed, zeta potential was also determined. The Vesicle size and *invitro*- antioxidant studys were taken as criteria to optimize the formulation. Formula F3 was optimized having surfactant and cholesterol in the ratio of (2:1). The optimized formulation was converted in to a gel using carbopol 934 and various parameters like pH, viscosity, spredability, swelling index, *invitro*-antioxidant studies were evaluated. All the parameters showed better results. The gel formulated was then evaluated by *invivo* animal study. Skin irritation study was conducted and it shows the formulation was free from dermatological reactions. The anti-psoriatic effect was determined by PPD-induced psoriasiform rat model. The length of epidermal thickness, % orthokeratosis and drug activity were determined. From the result it shows that the herbal niosomal gel (F3) possesses significant inhibition in psoriasis which was comparable with that of standard dithranol gel.

KEYWORDS: Herbal drug; Niosomes; Psoriasis; *Wrightia tinctoria; Aloe barbadensis*.

INTRODUCTION

Herbal formulations have been used for decades due to its enhanced activity and lesser side effects. India has a very long history of safe and continuous usage of many herbal drugs in the alternative system of health like ayurvedha, yoga, unani, siddha, homeopathy and naturopathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods etc. Even allopathic system of medicine has adopted a number of plant derived drugs. These herbal drugs are used since ancient times and now a day there is a global recognition of plant potency which leads to the finding of herbal medicaments and are introduced into the market after successive preclinical and clinical trial. These are based on the fact that they are available from the nature itself in immense quantity to fulfill the needs of mankind. Due to the ability to cure a variety of disease with less side effects their value as medicament has increased day by day. Delivery of these herbal medicines can be only attained after suitable modification in order to produce sustained release and improved patient compliance. According to WHO about 80% of total world population depends upon herbal medicines.[1, 2]

While considering the better patient compliance and drug delivery there comes the importance of novel drug delivery system. Paul Ehrlich, in 1909, initiated the era of development for targeted delivery when he foreseen a drug delivery mechanism that would target directly to diseased cell. Conventional drug delivery involves the formulation of the drug into a suitable form, such as compressed tablet for oral administration or a solution for IV administration. These dosage forms have been found to have serious limitations while NDDS are developed to overcome the limitations of conventional drug delivery. A lot of novel drug carriers are available now a day such as niosomes, liposomes, nanoparticles, phytosomes and ethosomes. Novel drug carriers can be easily target the drug into the affected area inside the patient's body and can produce better cure. They can be used to deliver the herbal drug in a predetermined rate. Incorporation of herbal drug in to a novel carrier rather than simply giving the herbal drug to the patient can also improve their solubility, stability, pharmacological activity, tissue macrophage distribution, sustained delivery, decreased toxicity and protection from physical and chemical degradation.[2]

Psoriasis is an autoimmune skin disease present mainly on the flexural areas and also present in the folded skin like underarm. It can also be defined as a papulosquamous disease because it is characterized by the presence of thick scaly plaques all over the affected areas. It is a

long-term condition that affects about 1-3% of world population and is non-contagious. It might be connected with other inflammatory disorders like psoriatic arthritis, inflammatory bowel disease and coronary artery disease.

The main features of psoriasis include the abnormal epidermal skin proliferation with red colored raised patches are present with scales. Histological features of psoriasis include parakeratosis (absence of granular layer), Capillary loop dilation, acanthosis with regular elongation of rete ridges, spongiform pustules, munro microabcesses and marked infiltration of T-cells, neutrophils, macrophages and dendritic cells. Skin is made up of a large no of cells. The older skin cells are usually replaced with newer skin cells after they die. But in the case of psoriasis new skin cells are produced faster than the normal as a result of increased immune responses and are left as raised patches. Immunological factors are also known to play an important role in the pathogenesis of psoriasis.[3,4]

Dendritic cells and effector T-cells assumes a foremost part in the progress of psoriatic lesion. High proportion of T-lymphocyte is present in the inflammatory infiltrates, primarily CD4+ (helper/inducer) lymphocytes and CD8+ (suppressor or cytotoxic) subsets are known to happen. T-lymphocytes at its activated state with the expression of HLA-DR and IL-2 receptor is present in psoriatic lesion. Coordinated effort between innate immunity and acquired immunity results in production of cytokines, chemokines and growth factors that may contribute to inflammatory infiltrate in psoriatic plaques. The vital role of CD4+ T-cells in initiating and maintaining the pathogenic process of psoriasis are already known, but the cross-primed CD8+ T-cells are the primary effector cells. That will prompt epidermal hyperplasia and inflammation.

Antioxidant treatment is also suggested in the case of psoriasis. Imbalance between antioxidants and oxidants leading to oxidative stress that may account for the pathogenesis of psoriasis. Oxidative injuries due to reactive oxygen species such as (ROS) like superoxide anions, hydroxyl, hydroperoxyl radicals, and hydrogen peroxide ions are mainly targeting on skin and are present on the skin during various physiological and pathological processes. In case of normal cells there is a balance between oxidative damage and antioxidant protection. However excess ROS production or insufficient antioxidant protection will result in oxidative stress that may leads to the pathogenesis of psoriasis. The reported features of psoriasis includes an increased dimensions for chemo taxis and adhesion, and an increased ROS(reactive oxygen species) production in neutrophils. It has additionally been suggested

that generation of ROS from neutrophils, keratinocytes, and fibroblasts may prompts neutrophil activation, which assumes a vital part in the psoriatic process.

This increased production of reactive oxygen species during the inflammatory process in psoriasis due to the inadequate antioxidant mechanisms will lead to increased lipid peroxidation. The all total result obtained after further reactions are epidermal hyperproliferation.[5, 6,7]

Niosomes are non-ionic surfactant vesicles, in which it is formed by the self-assembly of non-ionic surfactants in aqueous medium resulting in a closed bilayer structures. The vescicle size of niosomes ranging from 10 to 1000nm. They are amphiphilic in nature, central core cavity act as aqueous phase and surfactant bi-layer act as non-polar phase. This permits entrapment of hydrophilic drug in the core cavity and hydrophobic drugs in the non-polar region present within the bilayer. Therefor both hydrophilic and hydrophobic drugs can be incorporated in the noisome. Among these carriers niosomes are the better drug delivery system due to its drug delivery potential, high chemical stability and economy.[8,9]

Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional cross linking between the particles or solvated molecules of dispersed phase. Gels are generally considered to be more viscous than jellies.[10]

MATERIALS AND METHODS

Collection of plants

The fresh leaves of *Wrightia tinctoria* (Roxb) and *Aloe barbadensis* (Linn), were collected from local vendors in Kannur district, Kerala (India) in the month of October 2017. The plant materials were identified and authenticated in the Department of Horticulture, Padannakad, Kasargod, Kerala.

Determination of physicochemical paramaters

After the collection of plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air-tight containers until further use. Physico-chemical parameters of both plants were determined such as total ash, acid insoluble ash, water soluble ash, moisture content by loss on drying method, water and alcohol soluble extractive values.

Preparation of plant extract[11]

The collected leaves were washed thoroughly twice under running tap water followed by double distilled water to remove all debris adhering in to it and to avoid other contaminated organic contents. They were kept away from direct sunlight and were shade dried for about 20 days to protect all the active compounds. These dried plant materials were then homogenized to a fine coarse powder using an electronic blender and then stored in air tight containers until further use. Various organic solvents viz. petroleum ether, chloroform, ethanol and water were used for extraction. 10gm of homogenized coarse leaf powders of *Wrighitia tinctoria* was soaked in different conical flasks containing 100mL of petroleum ether, chloroform, ethanol and water. Then it is allowed to stand for 30 min in a water bath with occasional shaking, finally, each sample extract (petroleum ether, chloroform, ethanol and water) was filtered through sterilized Whatman No:1 filter paper and concentrated to dryness. Thus the obtained dried extracts were stored at 4^{o}C in labelled sterile bottles until further use. To detect various biologically active constituents present in various solvent extracts the standard methods were followed.

Preliminary phytochemical analysis

Preliminary qualitative analysis of all the extracts (petroleum ether, chloroform, ethanol and water) was carried out by employing standard conventional procedures.

Soxhlet extraction of plant materials[11]

The collected plant leaves of *Wrightia tinctoria* was shade dried at room temperature and coarsely powdered. Required quantity of the leaf powder is loaded in a thimble made from filter paper. Then the thimble was placed inside the extractor and the required solvent(300 ml) was taken in a round bottom flask and placed in the heating element. Drug was then extracted by continuous hot extraction (soxhlation) using 95% ethanol in soxhlet apparatus for about 96 hours until the solvent present in siphon tube becomes colourless. Ethanol retained within the extract can be recovered by distillation process and it was then air dried and concentrated.

Drug excipient compatability study

The FT-IR spectrum of drug extracts with other ingredients was analyzed for compatibility study.

Formulation of drug loaded niosomes

Preparation of 7.4 phosphate buffer[12,13]

250.0 mL of 0.2 M potassium dihydrogen phosphate (27.2g in 1000 ml) was added to 196.7 mL of 0.2 M sodium hydroxide (8g in 1000 ml) and the volume was made up to 1000 ml.

Table No. 1: Composition of developed noisome.

Formulation code	Surfactant Used	Weight taken (in mg)				Surfactant: Cholesterol ratio
		Drug		Surfactant	Cholesterol	
		Wrighitiatincoria	*Aloe barbadensis*			
F1	Span 20	300	50	200	100	2 : 1
F2		300	50	300	100	3 : 1
F3	Span 60	300	50	200	100	2: 1
F4		300	50	300	100	3 : 1
F5	Tween 20	300	50	200	100	2: 1
F6		300	50	300	100	3 : 1
F7	Tween 80	300	50	200	100	2 : 1
F8		300	50	300	100	3 : 1

Preparation of niosomes[14,15,16]

Niosomes namely F1 to F8 were prepared by lipid film hydration technique with two different concentration of surfactant (2:1, 3:1) while cholesterol and drug level keeping constant. Surfactant(Span 20,60,Tween20,80), cholesterol and drug extracts were accurately weighed and dissolved in 15 mL mixture of chloroform : methanol (2:1 v/v ratio). Above mixture was sonicated for 1 min. Then it was vortexed in a round bottom flask at a temperature of 58-64°C to remove the solvent for about 30 min. The thin lipid layer formed inside the flask was then hydrated with 10 mL of 7.4 Phosphate buffer at 60°C for 1hr. The resultant dispersion was cooled in an ice bath and then left for 4hrs at room temperature for complete hydration and stored at 4°C overnight before use.

Preparation of niosomal gel[17, 18,19]

The optimized niosomal formulation was used for the preparation of 1% carbopol gel. The required amount of carbopol 934 (1% W/W) was weighed and sprinkled into a beaker containing water with continuous stirring using mechanical stirrer (at the minimum speed to avoid entrapment of air) to get a transparent dispersion. After successful completion of homogenization added niosomal suspension containing drug following by the addition of 10% propylene glycol. Required amount of preservatives were taken in a beaker and added to the above mixture after dissolved by heating it over a water bath. The above dispersion is then neutralized by triethanolamine to get a transparent thick gel. Then it is kept at 4°C

overnight to remove air bubbles. The composition of developed niosomal gel is shown in table.

Table No. 2: Composition of niosomal gel.

SL NO	Ingredients	Quantity
1	Niosomal dispersion	0.35%
2	Carbopol 934	1g
3	Propylene glycol	10%
4	Methyl paraben	.001%
5	Propyl paraben	.001%
6	Triethanolamine	3 Drop
7	Water	Q.S

Evaluation of niosomes[12,20]

Vesicle size of niosomes

Vesicle size of each formulation was determined by an optical microscope. Each formulation was spread uniformly on a glass slide and observed under the 45X magnification optical lens.

Vesicle shape of niosomes

The shape and morphological characters were obtained by SEM photographs of the optimized niosomes. The formulations were placed into circular aluminium stubs using double adhesive carbon tape and coated with gold in HITACHI ION SPUTTER E-1010 vacuum evaporator, it was observed in HITACHI SU6600 FE SEM (field emission scanning electron microscope) having acceleration voltage of 10.0kv and magnification of 60.0k-100.0k.

Zeta potential[21]

Zeta potential was determined using Malvern zetasizer nano essential. Zeta potential of the niosomal formulation is related to the stability of niosomal vesicle. The high value of zeta potential indicating the high degree of repulsion between the vesicle and excellent stability i.e. the dispersion will resist aggregation.

***In-vitro* antioxidant activity**[22, 23, 24]

Hydrogen peroxide assay

A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the samples or standards in methanol were prepared and added to PBS. After 10 min, absorbance was measured at 230nm against a blank solution that contain The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide. IC50 value is the concentration of the sample

required to scavenge 50% free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated using the following formula:

$$\% \text{ Scavenged } (H_2O_2) = (A_0 - A_1)/A_0 \times 100,$$

Where, A_0 - Absorbance of the standard (ascorbic acid), A_1- Absorbance of samples.

Evaluation of niosomal gel[25,14]

Physical examination: The herbal niosomal gel was prepared and evaluated for color, odor and transparency.

pH: The accurately weighed amount of gel (2.5g) was dispersed in 25 mL double distilled water. The pH was determined by using digital pH meter at room temperature.

Spread ability: The Spreadability of the gel formulations was determined by taking two glass slides of equal length. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of a certain load.

Viscosity: Viscosity of the gel was determined using Brookfield viscometer (DV-1 programmable rheometer) at 6 rpm. Gels were tested for their rheological characteristics at 25^{o}C. 200 g of the gel was taken in a beaker and spindle was dipped in it for about 5 minutes and then the reading was taken.

Homogenicity: Developed liposomal gel was tested for homogenicity by visual inspection after the gel has been set in the container. This was tested for their appearance and presence of any aggregates.

Extrudability studies: The gel formulation were filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of tubes was recorded and the tubes were placed between two glass slides and were clamped. 500gm were placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel was calculated as:

1) When it is greater than 90% then the extrudability is excellent.
2) When it is greater than 80% then the extrudability is good.
3) When it is 70% then the extrudability is fair.

Washability: The product was applied on hand and was observed under running water.

In-vitro antioxidant activity[22,23,24]

Hydrogen peroxide assay

A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the samples or standards in methanol were prepared and added to PBS. After 10 min, absorbance was measured at 230nm against a blank solution that contain The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide. IC50 value is the concentration of the sample required to scavenge 50% free radical. The above experiments were performed (in triplicate) and the percentage inhibition was calculated using the following formula:

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Where, A_0 - Absorbance of the standard (ascorbic acid), A_1- Absorbance of samples.

In-vivo study

In-vivo studies were performed on 24 rats after the approval of Institutional Animal Ethics Committee (IAEC).

Skin irritation study[26]

Albino wistar rats of either sex weighing about 150-200g were procured. Hairs on the dorsal portion of the rat were shaved and skin was washed properly one day prior to study. 0.5g of the herbal niosomal gel was used as the test substance was applied to the shaved dorsal region and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period, i.e., 1 hour, residual test substance was removed. Observations were recorded after removal of the patch. Control animals were prepared in the same manner and 0.5g of the niosomal gel base i.e.,niosomal gel formulated using all ingredients except the herbal mixture was applied to the control animals and observations were made as similar to the test animals. The gel was applied to the skin once a day for 7 days and observed for any sensitivity and reaction. If any was graded as A-None, B- Slight, C-Moderate, D-Marked and E-Very marked.

***In vivo* Anti-psoriatic Activity**[26, 27, 28]

Induction of psoriasis

PPD-induced psoriasiform rat model[26,27]

At the beginning of the experiment 24 rats were anesthetized and the spontaneous development of psoriasis in rat was conducted according to the modified method for psoriasiform lesions. The dorsal region of the rats was shaved. The shaved regions were carefully applied Di-n-Propyl Disulfide (PPD) with the recommended dose of irritant (5μL/cm^2/day) on 6 consecutive days. The rats were further randomized to four groups of six rats each (n=6).

Grouping and treatment of animals

Group I: Normal control

Group II: Positive control (left untreated)

Group III: Standard (Derobin ointment, 1.15% w/w)

Group IV: Test group (.35%w/w, optimized herbal niosomal gel)

The first group was taken as normal control. Second group was taken as positive control which was left untreated and the third group was standard group treated with marketed ointment (Derobin) - 1.15% w/w. The fourth group was treated with. 35% w/w optimized herbal niosomal gel daily, for 14 days to evaluate the therapeutic effect. During this period, animals were monitored daily to record the effect produced and photographs were taken before the treatment and after 14 days treatment. At the end of the entire treatment period, skin biopsies from experimental psoriatic rat before and after treatment were subjected to histological examination by haematoxylin and eosin stain. Histopathology slides were then analyzed for the evaluation of following parameters.[3,28,29]

Measurement of Percent Orthokeratosis (OK)

An anti-psoriatic drug that targets the epidermis can restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both. In case of psoriatic lesion the granular layer of epidermis is almost absent. This is known as parakeratosis condition and it is the most important feature of psoriasis. While granular layer formation around the epidermis is termed as orthokeratosis. Percent orthokeratosis is determined by,

$$\% \text{ Orthokeratosis} = \frac{\text{Length of continuous granular layer}}{\text{Length of scale}} \times 100$$

Measurement of Epidermal thickness (ET)

It was obtained by measuring the distance between the dermo-epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of the different animals was calculated. The change in epidermal thickness of standard and formulated ointment treated group was then calculated.

$$\%\ \text{Epidermal thickness} = \frac{\text{Epidermal thickness of treated group} - \text{Epidermal thickness of control group}}{100 - \text{Epidermal thickness of control group}} \times 100$$

Measurement of Drug activity

Drug activity is calculated by the percentage increase of orthokeratotic region.

$$\%\ \text{Drug activity} = \frac{\text{Mean OK of treated groups} - \text{Mean OK of control group}}{100 - \text{Mean OK of control group}} \times 100$$

Data analysis[29]

All the experimental results were expressed as mean ± SEM. Probabilities were obtained by the analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism software.

Stability study

The stability study was performed as per ICH guidelines. The formulated niosomal gel were filled in collapsible tubes and stored at different temperatures and humidity conditions like ambient temperature (R.T), refrigerator temperature (8±1ºC) for a period of three months and studied for appearance, pH and spreadability.[30,31]

RESULT AND DISCUSSION

Plant collection and authetification

The plants *Wrightia tinctoria*(Roxb) and *Aloe barbadensis* (Linn) were collected from Kasaragod and Kannur district, Kerala (India) in the month of October 2017 and were authenticated by Dr. A . Rajagopalan, Professor, Dept. of Hortriculture, Padannakad, Kasaragod, Kerala.

Physico-chemical parameters

After the collection of plant materials, they were shade dried and powdered coarsely in an electronic blender and stored in air-tight containers until further use. Physico-chemical

parameters of both plants were tabulated in Table no. 10. Parameters such as ash value, extractive values and moisture contents were estimated.

Table No. 3: Physico-chemical parameters of *W. tinctoria* and *Aloe vera.*

Sl.no	Test	*Wrightia tinctoria*	*Aloe barbadensis*
1	Total Ash(%w/w)	12.75±0.03	1.57±0.01
2	Acid insoluble Ash(%w/w)	0.63±0.01	9.24±0.01
3	Water soluble Ash(%w/w)	9.24±0.03	38.73±0.02
4	Water soluble extractive value(%w/w)	22.36±0.02	17.5±0.02
5	Alcohol soluble extractive value(%w/w)	16.52±0.01	9.32±0.01
6	Moisture content(%w/w)	21.23±0.02	12.25±0.02

Values were expressed in mean ± SD, (n=3).

Preliminary phytochemical screening

Table No. 4: Phytochemical test on various extracts of *Wrightia tinctoria* powder.

Compounds	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloids	+	+	+	-
Carbohydrates	+	+	+	+
Saponins	-	-	++	-
Terpenoids	+	+	+	-
Phenolic compounds	-	-	+	+
Tannins	+	+	+	+
Proteins	+	+	+	+
Steroids	+	++	++	-
Flavanoids	+	++	+++	-
Glycosides	+	+	+	+

(+++) Abundant, (++)Moderate,(+)Present,(-)Absent

Table No. 5: Phytochemical test on *Aloe barbadensis.*

Compounds	Petroleum ether	Chloroform	Ethanol	Aqueous
Alkaloids	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	-	-
Terpenoids	+	+	-	+
Phenolic compounds	-	+	+	+
Tannins	+	+	+++	-
Proteins	-	+	+	-
Steroids	+	+	-	+
Flavanoids	+	+	++	+
Glycosides	+	+	++	+

(+++) Abundant, (++)Moderate,(+)Present,(-)Absent

The phytochemical tests on petroleum ether, chloroform, ethanol and aqueous extracts of *Wrightia tinctoria* leaf powder was conducted revealed the presence of various

phytoconstituents such as alkaloids, carbohydrates, phenols, saponins, flavonoids, steroids etc. Among them ethanolic extract showed better results as compared to other three solvents. Therefore ethanol can be used as the solvent for further extraction process.

Soxhlet extraction of plant materials

The extraction of dried leaves of the plant *Wrightia tinctoria* was carried out by soxhlet extraction process using ethanol as the solvent. Extract obtained from the plant was then collected and concentrated. The concentrated extract was then weighed and kept in a desiccator which was previously filled with fused calcium chloride until it was used for the preparation of niosomal gel. Aloe gel was collected from fresh aloe plant leaves and used as such.

Table No. 6: Percentage yield of extract of *Wrightia tinctoria.*

Sl.No	Sample	Solvent (ml)	Weight sample taken(g)	Weight of extracts obtained(g)	%yield(w/w)
1.	*Wrightia. tinctoria*	Ethanol	50	9.26	18.52

Perccentage yield of *Wrightia tinctoria* in ethanolic extract was found to be 18.52%.

Table No. 7: The % yield of aloe gel obtained from fresh *Aloe barbadensis* plant.

Sl.No	Sample	Amount of sample taken(g)	Amount of extract obtained(g)	% yield
1	*Aloe barbadensis*	10	8.73	87.3

Perccentage yield of *Aloe barbadensis* in ethanolic extract was found to be 87.3 %.

Drug-Excipient Compatibility Studies by FT-IR

The drugs polymer interaction studies were carried out ascertain any kind of chemical interaction of drug with the excipients used in the preparation of gel formulation. The FT-IR spectra were obtained by using JASCO FT-IR 4700 L spectrophotometer. The FT-IR results obtained are shown below.

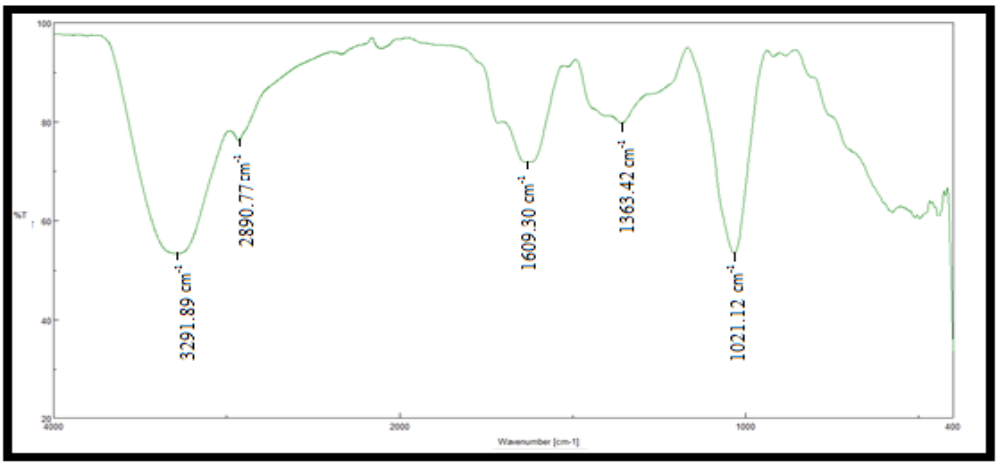


Figure 1: FT-IR spectrum of *Wrightia tinctoria.*

Sample A: 3291.89cm^{-1}(O-H stretching, H bond), 2890.77(C-H stretching), 1609.30(Aromatic, C=C bending), 1363.42(C-H_3 bending), 1021.12(strong, C-O stretching).

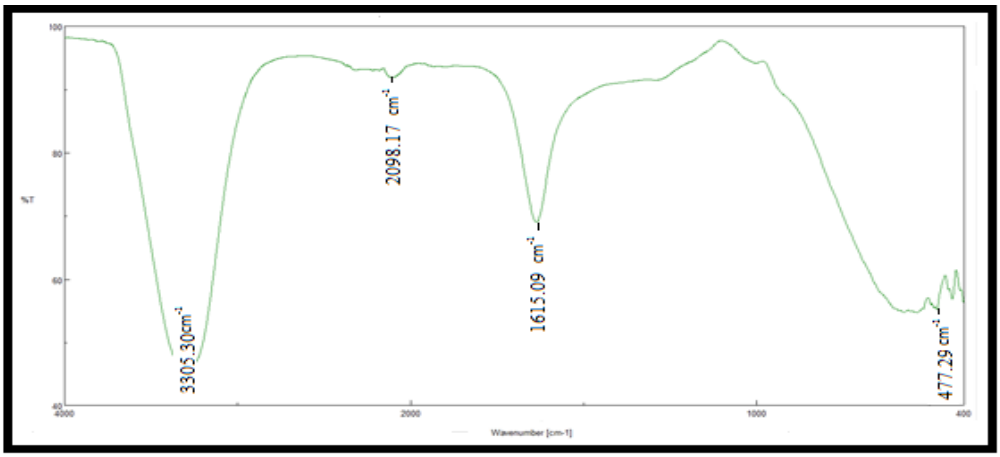


Figure 2: FT-IR spectrum of *Aloe barbadensis.*

Sample B: 3305.39cm^{-1}(Alcohol/phenol, O-H stretch), 1615.09cm^{-1} (N-H bending), 477.29 cm^{-1} (C-I stretching).

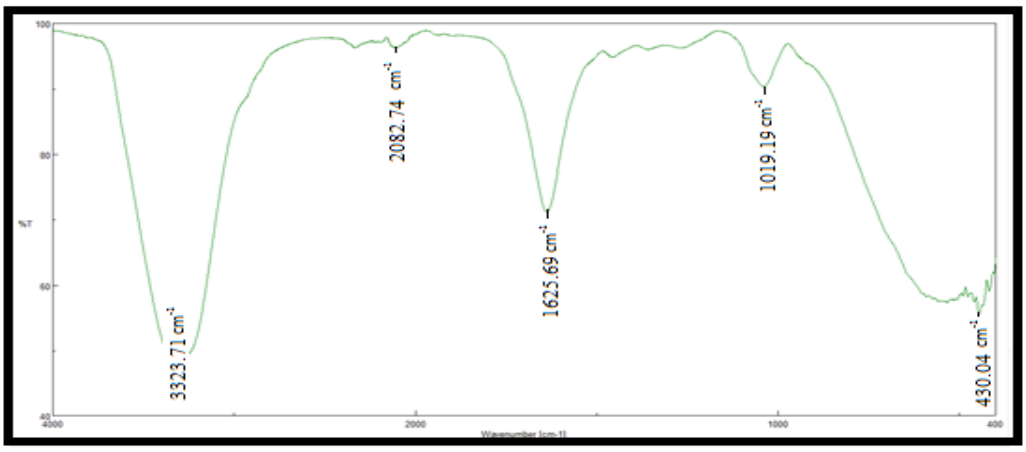


Figure 3: FT-IR spectrum of *Wrightia tinctoria* + *Aloe barbadensis.*

Sample C: 3323.71cm^{-1}(O-H stretching, H bonded), 1625.69(C=C stretch), 1019.19(C-O stretch).

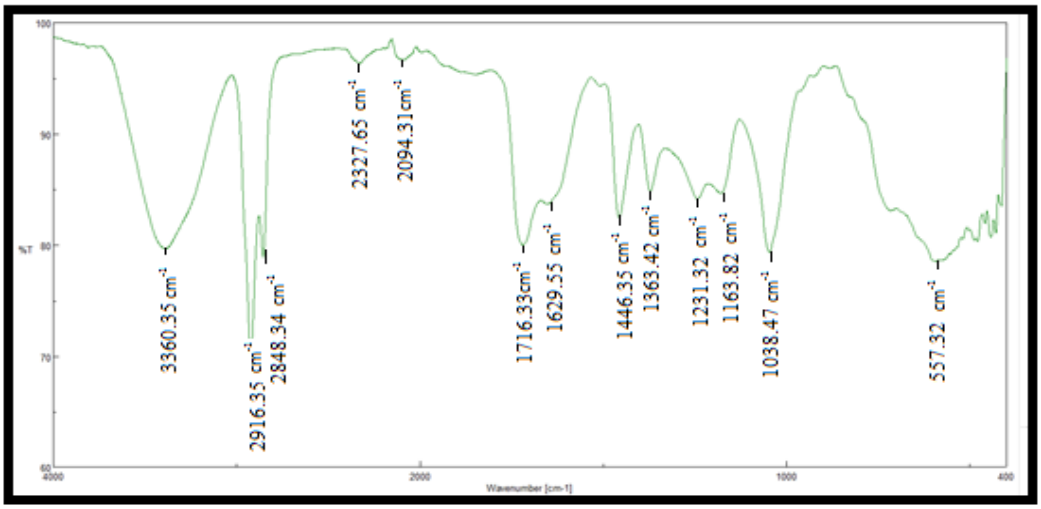


Figure 4: FT-IR spectrum of drug samples + Span 20 + Carbopol 934.

Sample D: 3360.35cm^{-1}(O-H stretching, H bond), 2916.80cm^{-1} (C-H stretching), 2848.3cm^{-1} (C-H stretching), 1716.33cm^{-1} (C=O stretching), 1446.35cm^{-1} (N-H bend), 1038.47cm^{-1}(C-O stretch).

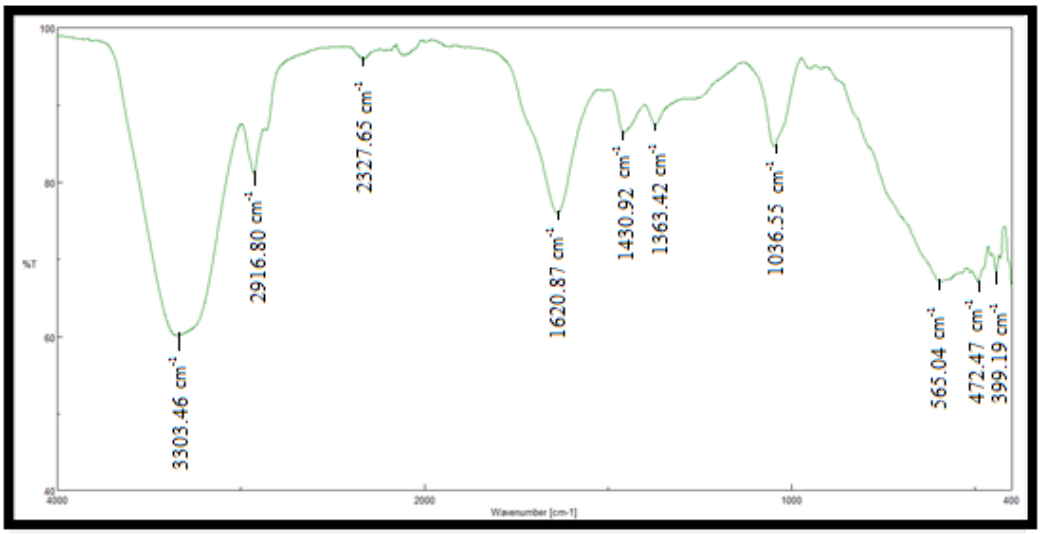


Figure 5: FT-IR spectrum of drug samples + Span 60 + Carbopol 934.

Sample E: 3303.46cm^{-1}(O-H stretching, H bond), 2916.80cm^{-1} (C-H stretching), 1620.80cm^{-1} (C=C stretching),1036.55 cm^{-1} (C-O stretching).

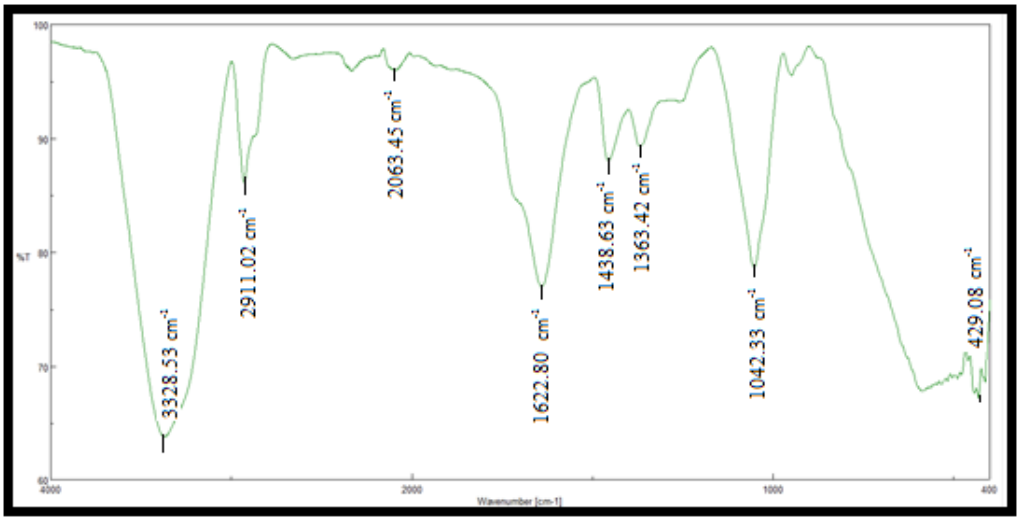


Figure 6: FT-IR spectrum of drug samples + Tween 20 + Carbopol 934.

Sample F: 3328.53cm^{-1}(O-H stretching, H bond), 2911.02cm^{-1} (C-H stretching), 1622.80cm^{-1} (C=C stretching),1438.63 cm^{-1} (C-Hbending), 1042.33 cm^{-1}(C-N amine).

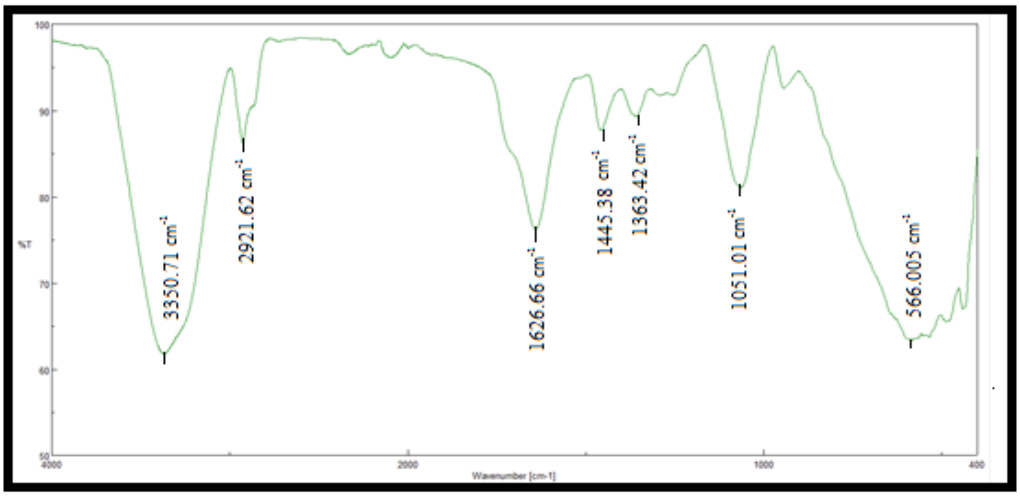


Figure 7: FT-IR spectrum of drug samples + Tween 80 + Carbopol 934.

Sample G: 3350.71cm^{-1}(O-H stretching, H bond), 2921.62cm^{-1} (C-H stretching), 1626.66cm^{-1} (C=C stretching), 1445.38cm^{-1} (C-H_3bending), 1051.01 cm^{-1}(N-H bend).

Formulation of drug loaded niosome

8 formulations named F1-F8 were prepared by different kinds of spans and tweens with varying ratios. The niosomal dispersion was formed as a dark brown color.

EVALUATION OF NIOSOME

Vesicle size of niosomes

Table No. 8: Vesicle size of noisome.

Sl.No	Formulation code	Mean particle size± SD(n=3)(µm)
1	F1	2.94±0.09
2	F2	3.95±0.04
3	F3	1.25±0.05
4	F4	2.08±0.08
5	F5	4.02 ± 0.07
6	F6	4.88 ±0.13
7	F7	5.11 ± 0.01
8	F8	6.13±0.02

Values are expressed in mean ± SD (n=3)

Vesicle shape of niosome

Shape and surface characteristic of formulated niosome (F3) was determined by Scanning Electron Microscopy. SEM photographs are shown in figure.

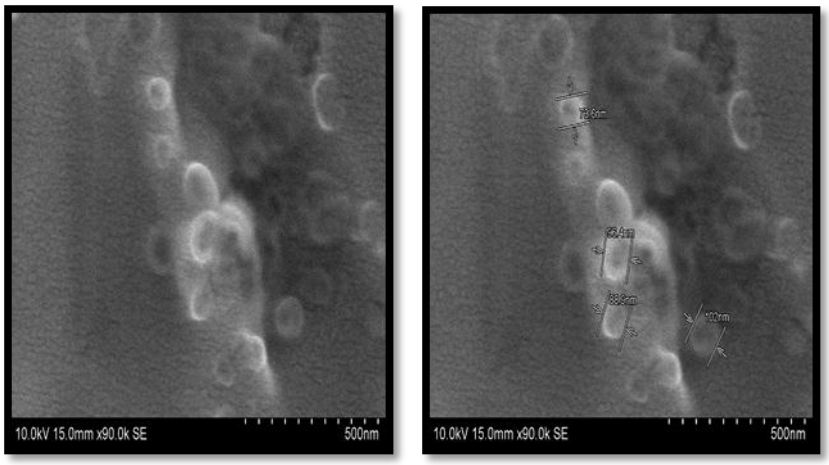


Fig 8: SEM photograph of formulated nisomes (F3).

Zeta potential of niosome

The zeta potential value of optimized niosomal formulation was found out byMalvern zeta sizer nano essential and the zeta potential was found to be -55.4. It indicates that it possess good stability. A higher zeta potential is an indicative of a stable colloidal system.

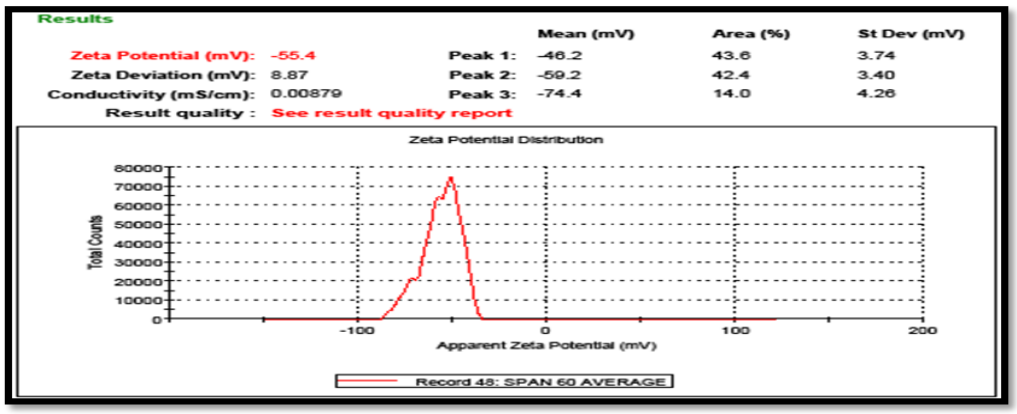


Fig 9: Zeta potential of noisome (F3).

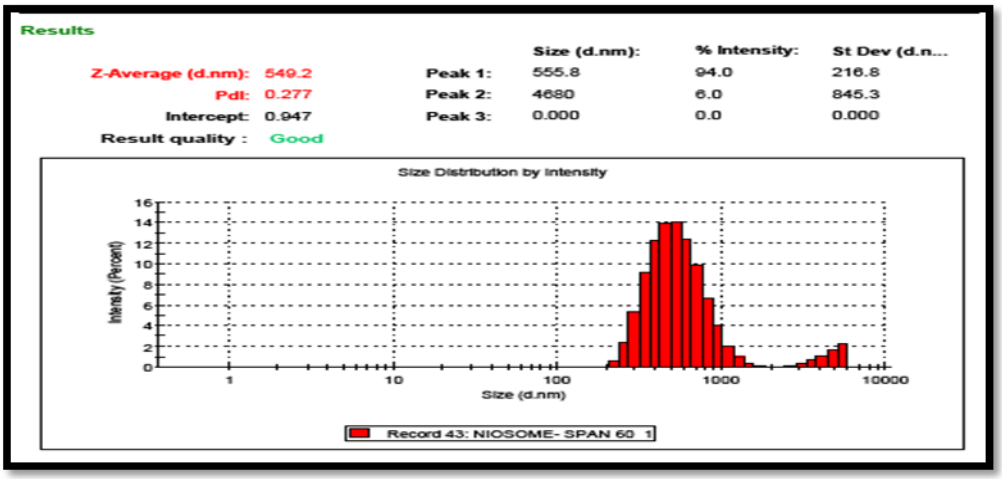


Fig 10: Statistical bar graph of particle size distribution in niosomal dispersion.

5.8. *In-vitro* antioxidant activity of niosomal dispersion

Hydrogen peroxide radical scavenging assay

The hydrogen peroxide radical scavenging activity of herbal niosomal dispersion was evaluated and compared with ascorbic acid and the results are given in table. The IC_{50}value of niosomal dispersion as well as ascorbic acid were calculated and plotted.

Table No. 9: Result of hydrogen peroxide radical scavenging assay.

Sl.No	Concentration	IC_{50} Values(µg/ml)				
		Ascorbic acid	F1	F2	F3	F4
1	20	18.22±0.01	23.27±0.02	23.94±0.03	21.04±0.02	21.85±0.02
2	40	26.31±0.01	36.36±0.03	38.27±0.01	31.65±0.04	33.14±0.02
3	60	32.12±0.01	41.16±0.01	43.52±0.02	38.13±0.03	38.67±0.01
4	80	43.23±0.02	50.16±0.01	52.67±0.01	46.54±0.03	48.25±0.03
5	100	52.14±0.01	59.86±0.02	61.14±0.01	54.14±0.03	56.14±0.02

Sl.No	Concentration	IC_{50} Values(µg/ml)				
		Ascorbic acid	F5	F6	F7	F8
1	20	18.22±0.01	27.46±0.03	32.45±0.1	33.57±0.02	35.18±0.01
2	40	26.31±0.01	40.74±0.01	42.35±0.01	42.87±0.01	43.65±0.01
3	60	32.12±0.01	46.97±0.01	50.16±0.02	53.45±0.01	53.88±0.01
4	80	43.23±0.02	57.84±0.03	61.55±0.02	61.97±0.02	64.65±0.03
5	100	52.14±0.01	64.98±0.01	66.76±0.03	68.26±0.02	72.56±0.03

Values were expressed as in mean ± SD, (n=3)

Optimization of niosome

The niosomal formulation(F3) was optimized based on the vescicle size of the niosomes formed, Vescicle shape obtained by SEM analysis and from the *invitro* anti-oxidant activity . F3 shows comparatively smaller vesicle size, shape and are suitable for niosomal gel preparation. It shows the lowest IC_{50} value indicates higher anti-oxidant activity.

5.10. Formulation of niosomal gel

The optimized niosomal dispersion F3 was used for the preparation of gel. The niosomal gel was prepared using carbopol 934.

Evaluation of niosomal gel

Physiochemical evaluation

The physical parameters such as color, odor and appearance were checked and results are showed in the given table.

Table No. 10:Physiochemical evaluation of the gel.

Parameters	Observation
Color	Yellowish brown
Odor	Characteristic
Appearance	Clear and translucent

Homogeneity

The optimized gel was tested for homogeneity by visual inspection and checked the presence of any aggregates. The gel possesses uniform distribution.

Extrudability studies

Extrudability of optimized niosomal gel was performed. about 80% of the gel was extruded from the collapsible aluminium tube. This indicated that the extrudability character of the niosomal gel (F3) was good.

Washability

The optimized gel was applied on the skin and was removed easily by washing with tap water.

pH

The pH of optimized niosomal gel formulations were determined by using digital pH meter.

Table No. 11: pH of optimized niosomal gel.

Formulation code	Samples			Average pH ± SD (n=3)
	1	2	3	
F3	5.81	5.83	5.79	5.81± 0.02

Values are expressed in mean ± SD (n=3)

Spreadability

The spredability of niosomal formulation was found to be 6.26±0.02 indicates better spredability. The value obtained was given in the table.

Table No. 12: Spredability of niosomal gel.

Formulation code	Trials			Average spredability(gcm/sec)
	1	2	3	
F3	6.23	6.27	6.28	6.26± 0.02

Values were expressed in mean ± SD (n=3)

Viscosity

The viscosity of the topical gel formulation refers to its consistency. The viscosity of optimized niosomal formulation was found out using Brookfields viscometer is as shown in the table.

Table No. 13: Evaluation of viscosity of niosomal gel.

Formulation code	Trials			Viscosity (cps)
	1	2	3	
F3	11432	11434	11432	11432.67±1.15

Values were expressed in mean ± SD (n=3)

Swelling index

Table No. 14: Evaluation of swelling index.

Time(hr)	Swelling index (%)
1	15.46±.17
2	20.68±0.03
3	29.65±0.1
4	37.27±0.04
5	43.5±0.06
6	50.66±0.02
7	57.49±0.09
8	61.77±0.2
9	64.47±0.06
10	69.38±0.06

Values were expressed in mean±SD(n=3)

***Invitro* antioxidant activity**

Table No. 15: Evaluation of *Invitro* antioxidant activity of niosomal dispersion and niosomal gel.

Sl.No	Ascorbic acid	IC_{50} value ofniosomal dispersion(F3) (µg/ml)	IC_{50} value ofniosomal gel(µg/ml)
1.	18.22±0.01	21.04±0.02	25.76±0.03
2.	26.31±0.01	31.65±0.04	36.34±0.04
3.	32.12±0.01	38.13±0.03	43.55±0.04
4.	43.23±0.02	46.54±0.03	53.65±0.03
5.	52.14±0.01	54.14±0.03	59.12±0.06

Values were expressed in mean±SD(n=3)

The hydrogen peroxide radical scavenging test was used ascorbic acid as a positive control and showed an inhibitory concentration 50% (IC50) of $52.14\pm0.01\mu g/ml$ for ascorbic acid. In the niosomal preparation and niosomal gel forms, the IC50 was obtained as$54.14\pm0.0301\mu g/ml$ and $59.12\pm0.0601\mu g/ml$ respectively. The anti-ocidant activity of niosomal gel was slightly decreased than niosomal dispersion.

***In-vivo* study**

Skin irritation study

Table No. 16: Skin irritation study of prepared topical niosomal gel formulation.

Sl.No	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Standard	A	A	A	A	A	A	A
2	F3	A	A	A	A	A	A	A

(A= No reaction)

The optimized niosomal gel F3 was free from dermatological reactions and did not produce any erythema and edema for about 7 days when applied over skin.

Anti-psoriatic activity

PPD-induced psoriatic rat model

Table No. 17: Effect of test and standard (Dithranol) on the degree of orthokeratosis, relative epidermal thickness and the drug activity.

Sl.No	Experimental groups	Degree of orthokeratosis (%)	Relative epidermal thickness (%)	Drug activity (%)
	Normal control	98.39±1.5	18.98±0.3	-
1	Positive control	19.85±0.32[a]	96.08±1.07[a]	-
2	Standard	64.31±1.09[ab]	49.7±0.24[ab]	55.47
3	Test (F3)	61.53±0.98[ab]	55.65±3.94[ab]	52

Values were expressed in mean±SD, (n=6 in each group). [a]p<0.05 significant difference in values when compared with normal control, [b] p<0.05 significant difference in values when compared with positive control(untreated).

According to the histological analysis conducted the Section shows skin with mild hyperkeratosis of outer layer of epidermis. Epidermal cells in some places shows mild hyperplacia with subepidermal region showing scattered inflammatory cells. Section revealed the presence of elongation of rete ridges, capillary loop dilation, and increased vascularity. The herbal niosomal gel (F3) shows considerable change in epidermal thickness compared to the control group (49.7±0.24 and 55.65±3.94). Parakeratotic condition was seen in psoriatic lesion. Formulation of granular layer is known as orthokeratic condition. Both standard and test produced considerable degree of orthokeratosis (64.31±1.09 and 61.53±0.98). The drug activity for standard and test were founded to be (55.47 and 52) respectively.

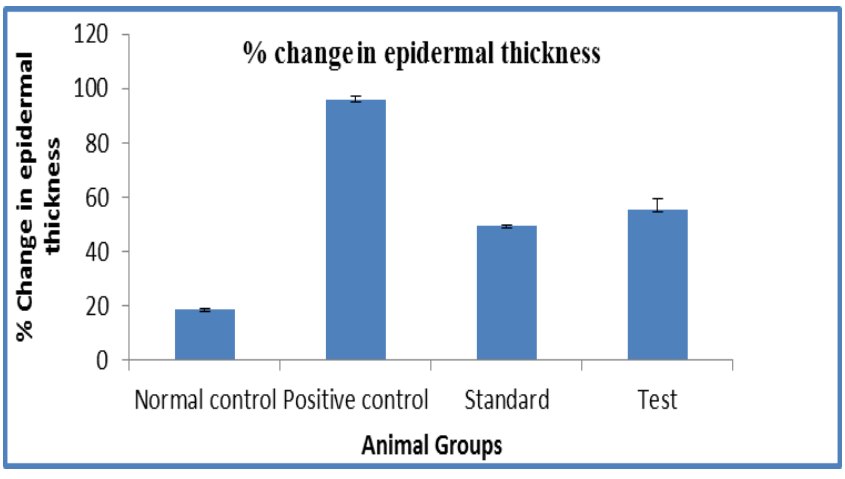


Fig 11: Effect of % change in epidermal thickness.

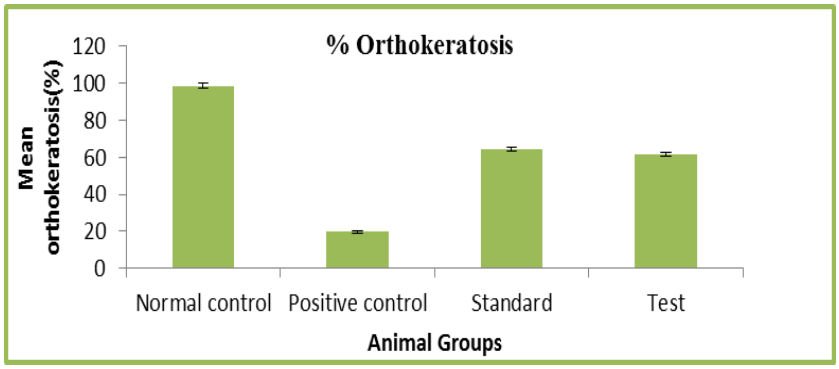


Fig 12: Each bar represents % Orthokeratosis.

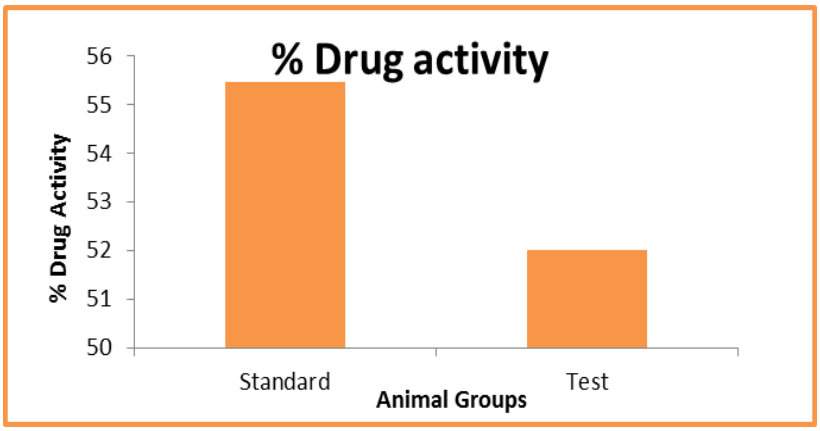
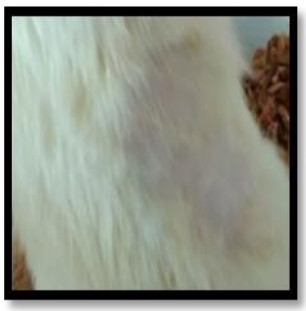
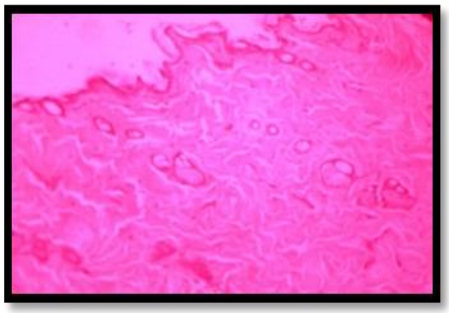
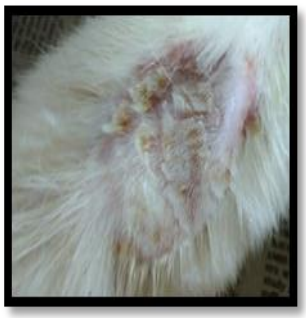
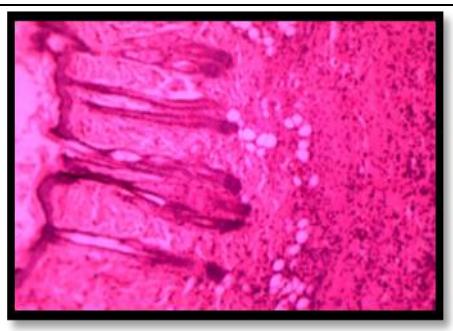


Fig 13: % Drug activity on different groups.

Gross morphology and histological features of normal rat skin		
		
		
Gross morphology and histological features of rat skin without treatment		

Gross morphology and histological features of rat skin

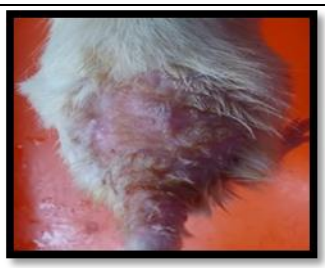
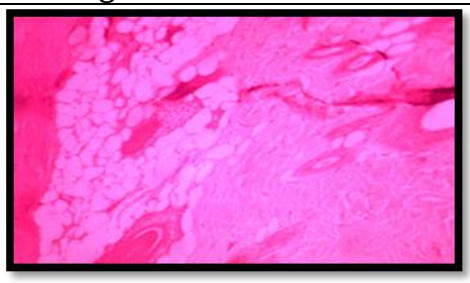
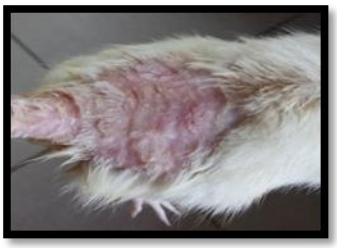
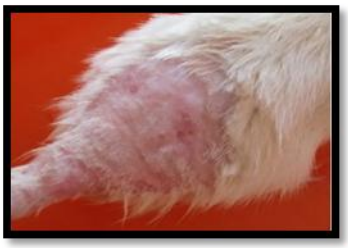
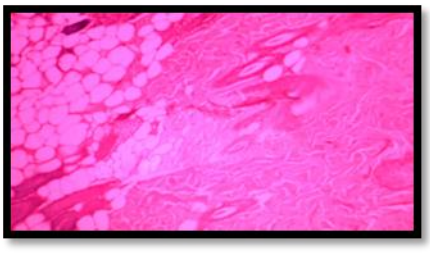
Before Treatment	After treatment	Histologic features
		
Gross morphology and histological features of rat skin treated with standard(Dithranol)		
		
Gross morphology and histological features of rat skin treated with test(F3)		

Fig 14: Stability studies of optimized formulation.

The stability studies of niosomal gel F3 was performed at room temperature 25°C±2°C and at refrigerator temperature of about 2-8°C±3°C and the results obtained are given in the following tables.

Table No. 18: Stability studies of niosomal gel at 25°C±2°C.

Sl.No	Evaluation Parameter	After one month observation	After two month observation
1	Color	Yellowish brown	Yellowish brown
2	Appearance	Clear and translucent	Clear and translucent
3	pH	5.76±0.01	5.74±0.03
4	Homogenity	Homogeneous	Homogeneous
5	Spreadability	6.2±0.01	6.18±0.02
6	Viscosity	11542.33±0.5	11543.75±0.03
7	Extrudability	Good	Good

Table No. 19: Stability studies of niosomal gel at 2-8°C±3°C.

Sl.No	Evaluation Parameter	After one month observation	After two month observation
1	Color	Yellowish brown	Yellowish brown
2	Appearance	Clear and translucent	Clear and translucent
3	pH	5.78±0.01	5.81±0.02
4	Homogenity	Homogeneous	Homogeneous
5	Spreadability	6.21±0.02	6.15±0.03
6	Viscosity	11474.9±0.5	11543.67±0.02
7	Extrudability	Good	Good

CONCLUSION

The Aim of present research work was to formulate and evaluate a topical herbal niosomal gel containing phytopharmaceuticals to be applied to treat skin diseases like psoriasis. Novel drug delivery system such as niosomes can be used to achieve localized drug action. *Wrightia tinctoria Roxb* and *Aloe barbadensis* Linn are the two plants that possesses potent anti-psoriatic effects.

The physicochemical and phytochemical investigations of the leaves of *Wrightia tinctoria Roxb* and *Aloe barbadensis* were conducted. From the preliminary phytochemical screening of both plants, the ethanolic extract shows intense presence of flavonoids,steroids and saponins may contribute greatly to the anti-psoriatic effect. The phytoconstituents were extracted by soxhlet extraction process. The preformulation studys were carried out inorder to develop niosomal gel containing drugs *Wrightia tinctoria Roxb* and *Aloe barbadensis* and polymer carbopol 934. The incompatibility between drugs and excipients were carried out by FT-IR spectroscopy. Niosomal gel was prepared by varying concentration of surfactants. All the evaluations were carried out including animal study.

To conclude, the findings of present investigation was an evidence that, the herbal niosomal gel containing *Wrightia tinctoria* and *Aloe barbadensis* was an excellent method to improve the drug delivery in the case of psoriasis. The drug loaded niosomes were stable in carbopol gel and the optimum HLB value of the surfactant span 60 made this formulation stable. There are less no of investigations were done related to herbal treatment in psoriasis. So that combaining novel drug delivery system with herbal drugs provide an excellent method to treat psoriatic skin lesion. The herbal drug loaded niosomal gel can be introduced in to the market for better therapeutic ailments. As far as the benefit of novel drug delivery in herbal drugs was concerned it can be used in the formulation technology. All total from the study it can be concluded that it is an alternative method to reduce the draw backs of conventional drugs already available for psoriasis.

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