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FORMULATION AND EVALUATION OF ALOEVERA HERBAL OINTMENT [ANTI-INFLAMMATORY & ANTI-OXIDANT ACTIVITY]

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ABSTRACT

In the present research Aloe Vera (Aloe-barbadensis) family of Liliaceae helps as anti-ageing, anti-inflammatory, anti-pyretic. In this study preparation of aloe Vera ointment helps to act as anti-inflammatory (to reduce inflammation on the skin) & (external wounds, insect bites, and sketch marks, itchiness) and also act as anti-oxidant. By adding all these ingredients like honey, clove oil to the aloe Vera extract the formulation enhance with the properties of analgesic and anti-oxidant and anti-inflammatory. We prepare the formulation with all suitable ingredients which enhance the healing process to the stress region of the skin and increase the surface skin smoothening with avoiding scar formation. This ointment can be evaluated by testing skin penetration, irritancy, pH, spreadability.

KEYWORDS: Aloe Vera, ointment, anti-oxidant, anti-inflammatory.

INTRODUCTION[1]

Ointments are semisolid(mushy) systems which are usually like rheological materials when shear stress is applied. They generally contain natural ingredients and are intended to be applied externally to the body. The vehicle of an ointment is known as the ointment base**.**

All ointments consist of a base which is primarily acts as a carrier for the natural ingredients. The nature of the base also controls its staging. Hence selection of ointment base is very important feature of their formulation. For researchers understanding of percutaneous absorption of ointment bases is essential to get familiar with skin structure in relation to drug absorption. Ointment bases are anhydrous and generally contain one or more medicaments in suspension or solution or dispersion.

Aloevera is a perennial succulent species that probably belonging to the family liliaceae. This plant has been also known as "the healing plant". Aloe vera has been used for traditional medical purposes in various skin disorders, it has been demonstrated that Aloe vera has growth promoting activities. Recently anti-fungal properties of aloe vera leaves were investigated by Casian. *In vitro*, extracts or components of Aloe vera stimulate the proliferation of several cell types. Many studies have shown that treatment with whole Aloe vera ointment, extracts resulted in faster healing of wounds and also several reports stated that Aloe vera ointment has a valuable impact on the wound healing and scars diminishing property. Inspite of the vast use of Aloe vera as a remedy to enhance wound healing, its mechanism in healing of wounds has not been studied in detail.

Topical application of antimicrobial agent is useful tool for the therapy of skin and soft tissue infections. It has several potential merits compared with systemic therapy. Firstly it avoid an unnecessary exposure of the gut flora (e.g. by the oral route), which may exert selection for resistance. Secondly it is expected that the high local drug concentration in topical application should overwhelm many nutritional resistance. Thirdly topical applications are less like than systemic therapy to cause side effects. At present there are several kinds of antimicrobial agent used in topical applications such as β-lactams, quinolones, aminoglycosides, macrolides, tetracycline and fusidic acid. Hence, in the present work an attempt will be made to prepare and evaluate topical ointment containing natural ingredients such as aloe vera, clove oil and honey has a major role for the effective management of different types of wounds.

Hence Clove oil has Aevidence that euginol in clove oil is effective in it is improves B.P reduce inflammation and fighting oral bacteria. Honey's wound care capabilities, a trait that can help an infected wound[2] properly heal in orderly fashion. Honey is an everyday household product that can provide numerous healing benefits.

Characteristics of an ideal ointment

1. It should be physically and chemically stable.
2. The base of ointment should possess no therapeutic action.
3. In ointment base, finely divided active ingredient should be uniformly distributed.
4. The ointment should be sooth and free from grittiness.

Advantages of ointment

1. They provide means of site specific application of drug on affected area, which avoids unnecessary non target exposure of drug thereby avoiding side effects.
2. They avoid first pass metabolism of drug.
3. Convenient for unconscious patients having difficulty in oral administration.
4. Comparatively they are chemically more stable and easy to handle than liquid dosage forms.
5. They are suitable dosage forms for bitter taste drugs.

Where for ointment bases (washable and non-greasy if oil-in-water (o/w))

- Wide range of compatibility
- Do not become rancid or support microbial growth;
- Nonirritating (to the same degree as lanolin, petrolatum, etc)
- Adhere well to skin
- Easily washed off.

Disadvantages of ointment

- Physico-chemically less stable than solid dosage forms.
- Unless acetyl alcohol is added, an aqueous solution can be added only to the extent of 5%.

MATERIALS AND METHODS

Ingredients	Manufacture	Category
Aloevera	Naturally occurring	Anti-inflammatory& antioxidant
Clove oil	Natural occuring	Anti- acne
Honey	Natural occurring	Anti- ageing
Petroleum jelly	Rajkamal industrial pvt Ltd	Lubricant
Hard paraffin	OEM manufacturers India	Absorbency
Cetyl alcohol	Godrej industries	Emulsifying and thickening agent
Glyceryl monostearate	OEM manufacturers India	Emulsifier
Propyl paraben	Prayosha health care pvt Ltd	Preservative

Preformulation studies

Preformulation studies are the first step in the rational development of dosage form of a drug substances.

The objective of preformulation studies are to develop a information about the drug substances, so that this information useful to develop formulation. Preformulation can be

defined as investigation of [3]physical and chemical properties of drug substances alone and when combined with excipients.

Preformulation investigation are designed to identify those physicochemical properties and excipients that may influence design, method of manufacture, and pharmacokinetic bio-pharmaceutical properties of resulting product. Following are the test performed for the preformulation study.

Natural ingredient Characterization

a) Solubility of drug
b) Physico-chemical characterization

Organoleptic characteristics

The colour, odour & taste of the drug were characterized and record using descriptive terminology.

Solubility of drug

Accurately weighed 1gm of sample was taken into beaker containing water, methanol, ethanol, di-chloro methane stir well and the observations are recorded.

METHODOLOGY

Preformulation Studies

It is one of the important prerequisite in development of any drug delivery system. Preformulation studies were performed on the drug, which included melting point determination, solubility and compatability studies.

Determination of melting point

The melting point of ingredients was determined by capillary method. Fine powder of itraconazole was filled in glass capillary[4] tube (previously sealed at one end). The capillary tube was tied to thermo meter & was placed in this tube on fire. The powder at what temperature is melted was noticed.

Solubility

Solubility of anti-inflammatory ointment was determined in PH 6.8 phosphate buffers, solubility studies were performed by taking excess amount of natural ingredients in different

beakers containing the solvents.[5] The mixtures were shaken for 24hrs at regular intervals. The solutions were filtered by using whattmann filter paper.

Uv-spectroscopy

Ultraviolet visible absorption (UV) The methanol extract of plant was analyzed in UV-Visible range between 200-780 nm using UV-Visible Spectrophotometer.

Infra-red spectroscopy (IR)

This technique is based on the fact that chemical substance shows specific absorption in infrared region. Under the influence of IR radiations the molecules shows various mode of vibration, which gives different absorption spectrum selective to the functional group present in molecule. The spectrum of compound is unique feature of molecular framework. The IR spectra of methanol extract of ointment were scanned on FT-IR spectrophotometer over the frequency range from 4000-400 cm-1.

Types of ointments

The various types of ointments are

1) Medicated ointments

2) Unmedicated ointments

Medicated ointments

- This ointment contains drug which shows systemic or local effects.

These are of several sub types

- Dermatologic ointments
- Ophthalmic ointments
- Rectal ointments
- Vaginal ointments
- Nasal ointments

Unmedicated ointments

These ointments do not contain any drugs. They are useful as emollient, protectants.

Formulation of ointment

The formulation components used were listed in Table 2. Oil in water emulsion of 20 and 60% of drugs were formulated. The emulsifier (glycerol monostearate) and other oil soluble components (petroleum jelly, Cetyl alcohol) were dissolved in the oil phase (Part A) and

heated up to 80° C. Extract and water soluble components (Methyl paraban, Propyl paraban) were dissolved in (Part B) and heated up to 80°C. After heating, the aqueous phase was added in portions to the oil phase[6] with constant stirring until cream is formed, And cream was formulated Having superb color i.e. Lemon yellow. Perfume was added when the temperature dropped to $45^0C \pm 50^0C$

Ingredients	F1	F2	F3	F4	F5
Aloevera: Clove: Honey	6:3.6:2.4	7.2:2.4:2.4	6.6:3.6:1.8	6.6:1.8:3.6	4.8:3.6:3.6
Petrolium Jelly	4.4gm	4.4gm	4.4gm	4.4gm	4.4gm
Hard Paraffin	2.2gm	2.2gm	2.2gm	2.2gm	2.2gm
Cetyl Alcohol	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml
Glyceryl Mono Stearate	0.4gm	0.4gm	0.4gm	0.4gm	o.4gm
Propyl Paraban	0.4gm	0.4gm	0.4gm	0.4gm	o.4gm
Methyl Paraban	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm
Activated Harcoal	0.01gm	0.01gm	0.01gm	0.01gm	0.01gm
Fragrance	Q. S	Q. S	Q. S	Q. S	Q. S

Method of preparation of ointments[7]

Trituration: In this finely sub-divided insoluble medicaments are evenly distributed by grinding with a small amount of the base followed by dilution with gradually increasing amounts of the base.

Fusion: In this method the ingredients are melted together in descending order of their melting points and stirred to ensure homogeneity. For herbal formulations, an ointment is made by emulsifying oil phase in water while the mixture is warm, then letting the formulation congeal at room temperature. During the preparation, the oil phase is heated to approximately 70ћ C and the water to 75ћ C. When added together and mixed in the presence of an emulsifying agent, the two phases quickly form a stable emulsion.[8] Upon mixing the two phases together, the mixture initially looks milky in appearance due to the presence of aloevera look like green color. Since the ointment is kept over a period of up to several weeks, preservatives such as propyl paraben or methyl paraben, must be used in ointment preparations. Dispensation may be in a plastic, but preferably, glass jar. Shelf life can be improved by storing the ointment in a refrigerator or in a cool place. (OR)

If heat is deleterious to the herbal components, then the ointment can be prepared without heat by incorporation method. A possible method for incorporating an aqueous herbal extract into an ointment base is to mix the extract with a small quantity of fat using a porcelain

mortar and then incorporating the resulting mixture into white petrolatum (petroleum jelly) using the same mortar for mixing.

Procedure

The extract were incorporated into the molten simple ointment base and allowed to congeal by stirring. After the ointment was formulated, they were packed in collapsible tube separately**.**

Evaluation of Ointment

The techniques involved are

Weight Variation Test

Select the sample of 6 filled containers and remove the any labeling that might alter weight during remove of the contents from the containers.

Thoroughly clean and dry out side of the container and weigh individually. Remove the contents from each container by opening and washing with a suitable solvent, take care to retain the closures and any other parts of each container.[9]

Dry and again weigh each empty container together with its corresponding parts. Difference between weights is the net weight of the contents of each container.

The average net weight of contents of 6 containers is not less than the labeled amount and the net weight of the contents of any single container is not less than 90% of the labeled amount, weight the labeled amount is more than 60gm but not more than 150gm.

P^H

P^H of prepared herbal ointment was measured by using digital P^H meter. The solution of ointment was prepared by using 100 ml of distilled water and set aside for 2hrs. P^H was determined in triplicate for the solution and average value was calculated.

Viscosity

The viscosity of the preparation should be such that the product can be easily removed from the container and easily applied to the skin. The rheological property was obtained by using brook field viscometer & cone plate viscometer.

Loss on drying

This is employed in IP and USP. Although the loss in weight, in the sample so tested, principally is due to water and small amount of other volatile material will be contribute the weight loss 1gm of ointment is placed digital moisture balance[10] instrument set the temperature 105°C and run the instrument up to constant weight. Finally read out the percentage loss on drying automatically.

Rheological Properties

The viscosity of the preparation should be such that the product can be easily removed from the container and easily applied to the skin. The rheological property was obtained by using brook field viscometer & cone plate viscometer.

Test of content uniformity

The net weight of contents of ten filled ointment containers is determined. The results should match each other and with the labeled quantity.[11] This test is also called minimum fill test

Test of rate of absorption

Diadermic ointments are those from which the drug moves into deeper skin tissues and finally into the systemic circulation. Such ointments should be evaluated for the rate of absorption of drugs. The ointment should be applied over a definite area of the skin by rubbing. At regular intervals of time, serum and urine samples[12] should be analysed for the quantity of drug absorbed. The rate of absorption i.e., the amount of drug absorbed per unit time should be more.

Test of rate of penetration

The rate of penetration of a semisolid dosage form is crucial in the onset and duration of action of the drug. Weighed quantity of the preparation should be applied over selected area of the skin for a definite period of time. Then the preparation left over is collected and weighed. The difference between the initial and the final weights of the preparation gives the amount of preparation penetrated through the skin and this when divided by the area and time period of application gives the rate of penetration of the preparation. The test should be repeated twice or thrice. This procedure is tedious and not followed anymore.

Using flow-through diffusion cell or micro dialysis method; the rate of penetration of the preparation can be estimated. Animal or human skin of definite area should be collected and

tied to the holder present in a diffusion cell.[13] The diffusion cell is placed in a fluid bath. Measured quantity of the preparation is applied over the skin and the amount of drug passed into the fluid is measured at regular intervals by analyzing the aliquots of fluid using a spectrophotometer.

Spreadability

It was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate two slides was measured as spread ability.[14] Lesser the time taken for the separation of two slides results in better spread ability. Spread ability was calculated by following the formula[15],

$$S=M \times L/T$$

Where,

S = Spread ability

M = Weight tide to the upper slide

L = Length of glass slide

T = Time taken to separate the slides.

Acute skin irritation study

The bases used in the formulation of ointments may cause irritation or allergic reactions. Non irritancy of the preparation is evaluated by patch test. In this test 24 volunteers are selected. Definite quantity of ointment[16] is applied under occlusion daily on the forearm for 21days. Daily the type of pharmacological action observed is noted. No visible reaction or erythema with edema and vesicular erosion should occur. A good ointment base shows no visible reaction.

Tube extrudability

It is usual empirical test to measure the force required to extrude the material from tube. More quantity extruded better for extrudability. The formulation under study was filled in clean, lacquered aluminum collapsible tube[17] with nozzle tube of 5mm opening and applies pressure on tube by the help of finger. Tube extrudability was then determined by measuring amount of ointment extruded through the tip when the pressure was applied on tube.

Invitro -DIFFUSION STUDIES

In In-vitro diffusion study of the ointment was carried out on Franz diffusion cell through a sigma membrane. In diffusion cell, sample (0.1g) was applied on dialysis membrane in donor compartment. And the entire surface of membrane kept in contact with the receptor compartment containing phosphate buffer (PB) pH 6.8 for 24 hours, prior to experiment.[18] The temperature was maintained at 37 ± 0.5°C. The time point for ointment was different. A sample of 1ml was withdrawn at predetermined time intervals, the solution was make up with the volume 5 ml of PB pH 6.8 and equivalent[19] amount of fresh dissolution fluid equilibrated at same temperature was replaced. The sample was diluted to 5 ml with PB at pH 6.8. The standard also was prepared as the same concentration of that of sample. The amount of drug permeated was determined using a UV- spectrophotometer at 340 nm.

Solubility

Soluble in boiling water[20], miscible with alcohol, ether, chloroform, both and dried for the temperature.

Washability

Formulation was applied on the skin and then easy extend of washing with water was checked.

Stability Study

Physical stability test of the ointment[21] was carried out for four weeks at various temperature conditions like $2^{o}C$, $25^{o}C$ & $37^{o}C$.The ointment was found to be physically stable at different temperature i.e $2^{o}C$, $25^{o}C$ & $37^{o}C$ with in four weeks.

RESULTS AND DISCUSSION

Table No. 1: Evaluation parameters.

S.no	Evaluation tests	Ranges
01	Appearance(color)	Green
02	P^{H} test	5-6
03	Rheological properties	180cp to 210cp. 185cp is better viscocity.
04	Spreadibility	5cms to 7cms, 6.5 is better spreadibility.
05	Non irritancy	No irritation
06	Rate of drug release	0.95 about 1cm
07	Solubility	Soluble in alcohol, ether, chloroform and boiling water.
08	Washability	Easily washable
09	Stability	Stable

Table No. 2: Evaluation parameters for topical formulations.

Parameters	F1	F2	F3	F4	F5
Color	Green	Green	Green	Green	Green
Viscosity	25413	1546	26451	12365	24561
Spreadability	20.10	24.52	36.12	21.56	50.24
Ph	5.4	5.6	5.2	6.1	6.5
Homogeneity	Good	Good	Good	Good	Good

Table No. 3: Drug release.

S.NO	Time	F1%CDR	F2%CDR	F3%CDR	F4%CDR	F5%CDR
1	0	1.12	8.17	7.25	9.12	5.26
2	2	21.5	15.4	14.3	26.4	23.1
3	4	30.1	19.6	21.5	38.5	24.3
4	6	45.2	26.5	27.3	45.2	29.1
5	8	61.8	29.5	35.1	24.5	30.5
6	10	21.6	38.2	29.5	40.5	38.6

CONCLUSION

In this experiment, we conclude that a non-irritant, stable and cosmetically elegant, skin care ointment containing Aloe vera and other added excipients has been developed and optimized on the basis of textural parameters. Texture parameters articulated that the overall elegance and aesthetic appearance of the formulation was more appealing compared to the marketed formulation. Thus, the developed aloe-vera ointment formulation has a potential for further application as safe topical preparation to treat various skin conditions and retain skin moisture without any irritation.

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