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Formulation and Characterization of Polyherbal Cream for Skin Manifestations

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Abstract

Present study was aimed to formulate a stable w/o herbal cream wherein light liquid paraffin constituted the oily phase and *Aloe vera* gel and turmeric extract were incorporated in the aqueous phase and were mixed together with continuous stirring to form a homogeneous polyherbal cream. The cream was evaluated for a spectrum of pharmaceutical parameters namely viscosity, spreadability, rheology, electrical conductivity, pH and stability. The viscosity of the cream was 11000 cps at 100 rpm that upsided to 64000 cps at 10 rpm. The spreadability coefficient was 18.89 ± 1.11 g.cm/sec suggesting easy spreading and without grittiness. The formulation was physically stable for the test period of 8 weeks that was confirmed by the color, phase separation, liquefaction evaluation and electrical conductivity test. The cream possessed significant antibacterial activity ($P < 0.05$) against most of the skin disease causing microbes. It also displayed anti-inflammatory activity in carragenan induced paw oedema model in rats. Thus the present research concluded that the herbal cream can be effectively used to treat common dermatological problems that lead to local infection and inflammation.

Introduction

Skin is a highly flexible, self-repairable covering that provides a protective barrier to the internal organs/tissues/cells from the external environmental and stress factors. It requires moisture to stay smooth and supple in order to perform its physiological function effectively (Mikari and Mahadik, et al 2010). It is susceptible to trauma, or infections due to its exposure to chemicals, radiations, variable temperatures that lead to dryness, rashes, fungal and bacterial infections causing redness and inflammation[1].Certain principles of skin care have to be emphasized such as gentle cleansing, adequate hydration and moisturization of the skin, preventing friction and maceration in body folds, and protection from irritants and bright sunlight (Das et al, 2009). The most protective and preventive step taken against dry skin formation and related disorders is the use of emollients or moisturizing creams and lotions preferably with antiseptic properties.

Aloe vera exhibits diverse range of pharmaceutical activities, including anti-inflammatory, anti-oxidant, anti-ageing, anti-cancer and immunomodulatory, that are mediated by reactive oxygen species (Xiu et al., 2006). The action of aloe gel as a moisturizing and anti-inflammatory agent is a very popular concept. It has been reported for its wound healing properties and is supported by various clinical investigations (Akhtar et al, 2011). Turmeric is used for the treatment of wounds, cuts, burns, galactose-induced cataract formation and ulcers. Curcumin the active compound of turmeric is a polyphenol that exhibits anti-inflammatory action by

inhibiting leukotriene formulation, inhibiting platelet aggregation and stabilizing neutrophilic lysosomal membranes. Curcumin is reported to suppress the growth of several microbes like *Streptococcus, Staphylococcus, Pseudomonas, Candida albicans* and *Aspergillus niger* that cause various skin infections (Ahmad et al., 1998).

Topical delivery is an attractive route for drug delivery, both for local and systemic treatment. In the formulation of topical dosage forms, attempts are made to utilize drug carriers that ensure adequate localization or penetration of the drug within or through the skin in order to enhance the local and minimize the systemic effects, or to ensure adequate percutaneous absorption (Jani et al., 2010). There has been a renewed interest in the emulsion based drug delivery systems for targeting deeper layers of skin. From an emulsion based topical system, the therapeutic properties of the constituents are claimed to be accentuated. Water-in-oil (w/o) emulsions are more often employed for the treatment of dry skin and emollient applications (Magdy, 2004).

Thus the aim of the study was to develop a topical polyherbal formulation consisting of *Aloe vera* (Family- Liliaceae) juice and turmeric (*Curcuma longa*, Family- Zingiberaceae) extract for treating the bacterial and fungal infections and related inflammation and itching.The objective was to evaluate the formulation for its pharmaceutical as well as pharmacological properties.

Material And Methods

Plant materials used for the study were collected from botanical garden and authenticated by, Professor and Director Dr. K.N Modi Institute of Pharmaceutical Sciences and Research. Light liquid paraffin was procured from Ranbaxy Fine Chemical Ltd., New Delhi. Stearic acid, triethanol amine, glycerin, hard paraffin wax, methyl paraben, propyl paraben were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore. Glycerylmonostearate and cetostearyl alcohol were from CDH, Pvt. Ltd., Mumbai and microcrystalline wax was from KK India Petroleum Specialities Pvt. Ltd., Mumbai. The test microorganisms *Staphylococcus aureus* (MTCC 2943), *Pseudomonas aeruginosa* (MTCC 1688), *Candida albicans and Aspergillus niger* were procured from INTECH, Chandigarh, India. Carrageenan was procured from SD Fine chemicals, Mumbai, acetylsalicylic acid from British Drug House and Piroxicam gel was from Cipla, Ahmedabad.

Plant Extract/Juice

The *Aloe vera* leaf was cut at the base of the plant, lower leaf was sliced opened and 100 gm. juice was collected in a beaker. The turmeric rhizome was dried in shade and powdered with help of electric grinder. The powder (100 gm.) was macerated with 250 mL of 80% v/v ethanol with ocassional stirring and was filtered to get the liquid extract.

Preparation of cream

The ingredients were weighed as per the details given in Table 1.The oily phase (Part A) that consisted of the emulsifier (stearic acid) and other oil soluble components was heated to 75±1° C on a water bath shaker (Hicon, New Delhi, India) with constant stirring. The water soluble components were added to water (Part B) and heated to the same temperature followed by addition of turmeric extract, *Aloe vera* extract, methyl and propyl paraben with continous stirring. To the heated aqueous mixture, oily phase was incorporated with continuous stirring on magnetic stirrer (Jindal Scientific Industries Pvt. Ltd) until the emulsion cooled down. The concentration of the aloe vera and turmeric extract was maintained at

Table 1: Formulation ingredients of the herbal cream

PART A (Oily Phase)		PART B (Aqueous Phase)	
Ingredient	**%w/w**	**Ingredient**	**%w/w**
Light liquid paraffin	27.85	Triethanolamine	1.71
Stearic acid	8.57	Glycerin	10.71
Glyceryl monostearate	7.50	Methyl paraben	0.21
Cetostearyl alcohol	4.28	Propyl paraben	0.21
Microcrystalline wax	0.21	*Aloe vera* extract	2.10
Hard paraffin wax	1.00	Turmeric extract	2.10
		Distilled water	q. s.100%

1% by weight of the formulated w/o cream base.(Amgad et al 2015)

Evaluation of Cream

Formulation properties

The formulation properties of the cream were studied visually and characteristics like physical state, colour,odour and overall appearance was recorded.

Viscosity

The viscosity of the formulation was determined at room temperature using Brookfield Viscometer equipped with spindle No.7 (Brookfield Engineering Laboratories, Inc., Stoughton) at different rotational speed. The formulations were placed in 10 ml beaker and the spindle was lowered perpendicularly taking care that the spindle did not touch the bottom of the beaker.The readings were recorded after they became constant.

Rheology

The rheological properties were determined using the small sample adaptor of the rheometer (Brookfield DV-III,Brookfield Engineering Laboratories, Inc., Stoughton USA).Shear stress was measured against increasing shear rate and viscosity was measured against time at a speed of 100 rpm and constant temperature.

Spreadability

The formulation was sandwiched between two slides, of dimension 20 ×5 cm, by placing a weight of 100 g uniformly on the slide.The weight was removed and the excess of cream was scrapped off. The slides were fixed to a stand at a 45° angle without the slightest disturbance so that only the lower slides was held firmly by the clamp, allowing the upper slide to slip off freely under a weight of 20g. The time taken for the upper slide to separate from the lower glass plate under the direction of the weight was noted. Experiment was done in triplicate and spreadability was calculated as follows:

$S = (W.L)/T$ ..... Eq. (1)

Where, S = spreadability, L = length of glass plate, W= weight tied to the upper plate and T= time (sec).

Stability

Stability studies were performed under different conditions to observe the effect of these on the storage of emulsions. The samples were kept at 8± 1°C (in refrigerator), 25±1°C + 60 % RH, 40±1°C + 60%RH and 40±0.1°C and 75% RH in stability chamber (Thermolabs scientific equipments Pvt. Ltd, Vasai). Physical characteristics like color, creaming and liquefaction were determined at various intervals for 28 days. To serve the purpose better, various tests related to physical analysis, type of emulsions, pH determination, electrical conductivity and centrifugation were also conducted.

In vitro antimicrobial activity

Preparation of test and standard solution(s)

The test sample was prepared by dissolving the cream in ethanol (95% v/v). Kanamycin was used as standard and was dissolved in DMSO to get a final concentration of 10 µg/ml. Ethanol (0.1 ml) was used as solvent control as it was used for the extraction process, so that we can confirm that the antibacterial activity was not due to solvent.

Preparation of inoculums(s)

The suspensions of all the microorganisms were prepared as per Mac-Farland Nephelometer Standard. A 24 h old culture was used for the preparation of suspension. The suspensions were made in sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted. The microbes selected for carrying out antimicrobial studies were *Staphylococcus aureus, Pseudomonas aureginosa, Aspergillus niger* and *Candida albicans.*

Procedure

The *in-vitro* antimicrobial activity was conducted by agar well diffusion method. This method is based on diffusion of antimicrobial component from the reservoir hole to the surrounding inoculated agar medium, so that the growth of the microbe is inhibited as zone around the hole. 0.1 ml of inoculum (prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms accurately measured (0.1 ml) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of 2 h for effective diffusion of test and standard. Later, they were incubated at 37 ºC for 24 h in an incubator for bacterial strains and 28 ºC for fungal strains. The presence of definite zones of inhibition around the cup indicated antimicrobial activity. The solvent control was

run simultaneously to assess the activity of ethanol, which was used as a solvent for extracts. The diameter of the zone of inhibition was measured (n=3) and recorded.

Anti-inflammatory activity

Animal procurement

Wistar rats weighing (200-250 g) of either sex were procured from Dr. K. N. Modi Institute of Pharmaceutical Education and Research, Modinagar. They were kept in departmental animal house in well cross ventilated room at 22 ± 2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1018C/06/CPCSEA).

Carrageenan-induced rat paw edema

0.3g of cream containing 1%, 2% and 4% of the extract was applied to the plantar surface of the hind paw by gently rubbing 50 times with the index finger. Rats of the control group received only the cream base. 0.5% Piroxicam gel was used as standard. Formulations were applied 1h before the carrageenan injection. 50 µl of a 1% w/v suspension of carrageenan in saline (prepared 1h before each experiment) and was injected into the plantar surface of right hind paw of the rat. The paw volume was measured immediately after carrageenan injection and at 1, 2, 3 and 4 h intervals after administration of the noxious agent by using a plethysmometer (model 7159, UgoBasilearese, Italy). The percent inhibition of oedema volume between treated and control groups was calculated as follows:

Percent inhibition = (1-Vt/Vc) x 100 Eq…….(2)

Where Vc and Vt represent the mean increase in paw volume in control and treated groups, respectively (Baquerizol et al. 1999).

Results And Discussion

Formulation properties

The formulation properties of the cream, on visual observations were a homogeneous white colored semisolid with characteristic odor of the raw materials.

Viscosity

The prepared cream at least rpm of 10 exhibited a viscosity of 64000 cps (Table 2) that indicates that the formulation has the desired viscosity required for semisolid formulation for proper packaging. It was found that the viscosity decreases as the rotational speed of viscometer increased suggesting that greater the shearing the lower viscosity favours easy spreadability further confirmed by spreadability and rheological testing.

Table 2: Viscosity of formulated cream at different rpm

rpm	Viscosity (cps)
100	11000
50	19600
20	41000
10	64000

Rheological data

Shear stress was tested at increasing shear rates (Figure 1), and viscosity was measured at 100 rpm (Figure 2). The figures illustrate pseudoplastic behavior of the formulation, as an increase in shear rate resulted in large increment in shear stress. Viscoelastic systems having high viscosity under low shear rate and vice versa are preferred for topical delivery. The viscosity plays a important role in the application of creams as very low viscosity results into easy flow off the surface and high viscosity presents problem in spreading (Baquerizol et al. 1999). Figure 2 shows no change in viscosity in formulation at a constant rate of 100 rpm demonstrating the ease of storage.

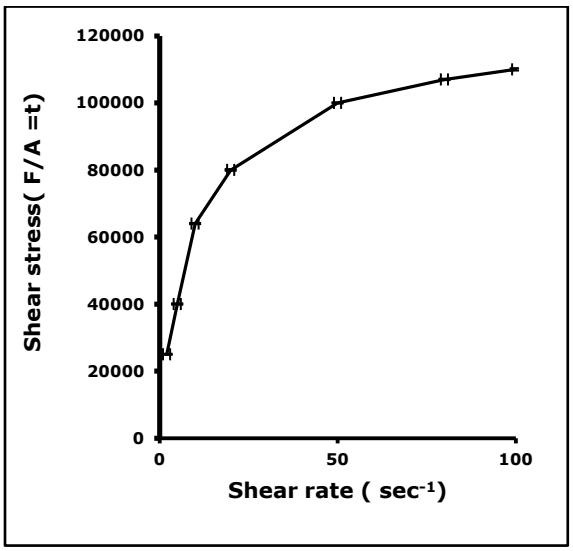

Figure 1: Rheological characterization of prepared cream

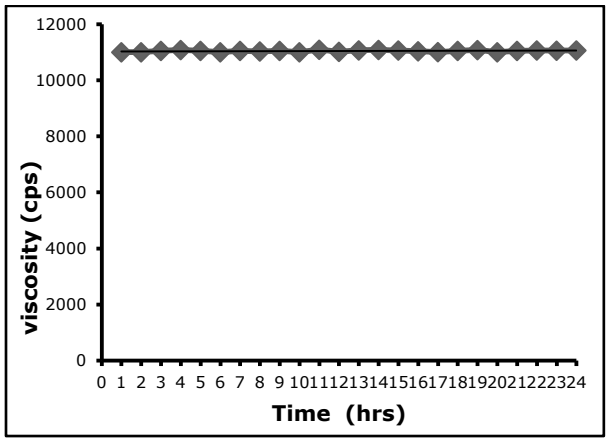

Figure 2: Shear rate vs. shear stress at constant rotational speed (100 rpm)

Spreadability

Spreadability plays a considerable role in patient compliance and ensures uniform application of cream to a larger area of the skin. The spreadability of the formulation was calculated as 18.89 ± 1.11g.cm/sec. The low value of spreadability coefficient of the cream was sufficient suggesting easy spreading and no signs of grittiness. The lower value of spreadability indicates the lesser work required to spread the cream over the skin, which means formulation was easily spreadable by applying small amount of shear.

Stability

In this study, the formulation was stored at different storage conditions and various stability indicators pertaining to emulsion were assessed. There was no change in the color of formulation at the end of observation periods suggesting physical stability and apparently no chemical reaction between the ingredients. The external phase of the emulsion constituted of paraffin oil which is a colorless, transparent, tasteless, non-fluorescent liquid; and is mixture of hydrocarbons (Henriette, 1995) is one of major stability providing element.

Viscosity is a useful process indicator of emulsion quality, as it is highly sensitive to changes in the emulsion due to variations in process and formulation parameters. As soon as an emulsion is prepared, time and temperature-dependent processes occur to effect its separation leading to the decreased viscosity which results in increased liquefaction (Herbett et al., 1988). No liquefaction was observed in formulation kept at 8°C and 25°C + 60%RH and 40°C+ 60% RH during the observation period of 28 days. However, liquefaction was observed in the formulation stored at 40°C +75% RH from 21st day of observation but there was no further increase in liquefaction till the end of the study period but it can be suggested that the formulation should be stored in well closed container.

No phase separation was observed in any of the formulations stored different conditions for 28 days. This indicated stable formulation, considering phase separation as a parameter of stability. Depending on the conditions, emulsions may be more stable at lower temperature due to increased phase viscosity (Derick, 2000). No phase separation on centrifugation was seen in any of the formulation kept under different storage conditions. It can be said that proper homogenization speed during emulsion formulation prevented the formulation breakage during stress testing (Abdurahman and Rosli, 2006).

According to many authors, determination of conductivity is often used to determine the nature of an emulsion and to control its stability. Modifications of conductivity value allows the detection of creaming, sedimentation or phase inversion (Emsap and Seipmann, 2002). No electrical conductivity was seen in the formulation kept under different storage conditions.

The formulation intended for application to skin should have near neutral pH value (Masmoudi et al. 2005). In this study the pH of freshly prepared cream was found to be 7.13, which can considered optimum for topical application. The pH values of the formulation kept under the mentioned storage conditions are detailed in Table 3 and negligible variation was observed on aging. At the end of the study, the pH values of the formulation at 8°C, 25°C, 40°C and 40°C + 75% RH were 6.87, 6.53, 6.49 and 6.57 respectively as can be seen in table 3. By using two-way analysis of variance (ANOVA) at 5% level of significance, no significant difference in change of pH of the formulation was found at different time and temperature.

Table 3: The pH values of the formulation after storage at varying conditions

Time	pH			
	8˚C	25˚C/ 60%RH	40˚C/60%RH	40˚C/75% RH
0 h	7.13	7.13	7.13	7.13
12 h	6.95	7.32	6.90	6.91
24 h	7.43	7.46	7.32	7.38
36 h	7.61	7.39	7.58	7.51
48 h	7.67	7.61	7.33	7.46
72 h	7.31	7.43	6.89	6.97
7 d	7.69	7.65	7.51	7.46
14 d	7.70	7.61	7.49	7.29
21 d	7.23	6.92	7.03	6.79
28 d	7.34	7.87	7.49	7.57

(RH = relative humidity, h = hours and d = days)

Antimicrobial activity

The degree of response of the test sample was different for different selected microbes. The zones of inhibition ranged from 7.8 ± 0.5 to 16.3 ± 0.8 as detailed in Table 4. The test sample exhibited very good activity against *S. auresus, A.niger* and *C.albicans* whereas it showed only moderate activity against *Pseudomonas aeruginosa.* It can be suggested that the prepared herbal cream possessed definite antibacterial activity against the microbes that are reported to be major cause for various skin manifestations and should be effective in treating the exact in-vivo disease pathology.

Table 4: Zone of inhibition of the prepared formulation against various microbes

Microbial species	Mean zone of inhibition ± SD	
	Standard	Test
Staphylococcus aureus	21.67 ± 0.51	16.33 ± 0.82
Aspergillus niger	18.56 ± 0.25	11.33 ± 0.65
Candida albicans	15.73 ± 0.75	8.73 ± 0.32
Pseudomonas aeuroginosa	18.58 ± 0.32	7.65 ± 0.53

Anti-inflammatory activity

The mean increase in paw oedema volume was about 0.68±0.01 ml in the vehicle treated control rats. The formulations containing the extract of (Aloe vera and Curcuma longa, AVCL) significantly (P < 0.001) reduced the mean paw oedema volume at 1, 2, 3 and 4 h after carrageenin injection by 48.57%. The prepared formulations exhibited anti-inflammatory activity as compared with the control group. However, the standard drug, Piroxicam gel (0.5%) showed highly significant (P<0.001) anti-inflammatory activity with the percent inhibition of 84.28%. The results are shown in Table No 5**.** The Carrageenin induced paw oedema model in rats is known to be sensitive to cycloxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. The oedema and inflammation induced by carrageenin is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 2.30 h and from 2.30 to 6 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site (Rao et al., 2005). It is well known that carrageenan induced paw oedema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3-4 h after carrageenan injection) kinins and prostaglandins are involved (Di Rosa et al. ,1971). As seen in this experiment, the ability of this cream to suppress inflammation when it is applied after the onset of inflammation is likely to be due to the genuine anti-inflammatory activity.

Table 5: Anti-inflammatory data of the formulated herbal cream on the carrageenan induced paw edema model in rats.

Treatment	Paw Volume In Ml±SEM (% Inhibition Of Paw Edema)			
	1 H	2 H	3 H	4 H
Control	0.68±0.01	0.69±0.02	0.70±0.02	0.70±0.02
Standard	0.21±0.01[c] (69.11)	0.21±0.01[c] (69.56)	0.18±0.01[c] (73.91)	0.11±0.01[c] (84.28)
AVCL-1%	0.61±0.02[a] (10.29)	0.61±0.02[b] (13.04)	0.47±0.02[c] (31.88)	0.36±0.02[c] (48.57)

The values are mean ± SEM of 6 readings; [a]: P<0.05, [b]: P<0.01, [c]: P<0.001 compared to control group.

Conclusion

The herbal cream was successfully developed that met the relevant pharmaceutical characteristics form. The cream possessed definite antibacterial activity against the microbes reported to be major cause for various skin manifestations and should be effective in-vivo. The experiments concluded the ability of the cream to suppress inflammation even after the onset of inflammation.The developed herbal cream is a potential candidate for conducting further clinical studies.

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