Formulation and evaluation of post laser herbal cream

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Abstract

Aim and Objectives: Herbal creams offer several advantages over other cream. The purpose of the present research work was to formulate and evaluate post laser moisturizing herbal cream. A moisturizing cream (oil in water)-based formulation containing extract, namely beta-vulgaris extract (1%) and *Glycyrrhiza glabra* root extract (1%), intended for post laser therapy was developed with an aim to provide moisture and UVA/UVB protection. **Methodology:** Post laser treatment was assessed by previously reported ice bags, menthol packs, and stubborn anti-scarring ointment method. By discovering different types of formulations, such as oil in water, we were able to create several moisturizing creams, respectively, classified from F1 to F12, by incorporating different concentrations of natural emollients by stirring method. Further, the formulated cream was evaluated for various stability parameters. **Result:** The preparation was stable under normal storage conditions and also passed through different storage conditions at room temperatures: 25°C, 40°C, and 2–8°C. The initial physicochemical parameters of formulations, i.e., pH was near about 5.8, which lies in the normal pH range of the skin, viscosity, spreadability, extrude ability and stability, moisture content, centrifugation, and specific gravity were also examined. Herbal moisturizing cream did not produce any skin irritation, i.e., erythema and edema when applied over the skin. The cream is for all skin types, especially moderate to dry. It relieved and cooled the treated area. This cream facilitated re-pigmentation by stimulating melanocytic proliferation and removed stubborn scars and wrinkles.

Key words: Beta-vulgaris extract, Herbal formulation, *in vitro* anti-inflammatory activity, licorice extract, TiO_2

INTRODUCTION

Dermatology is a unique area of medicine because diseases that affect the integumentary system manifest externally and are constantly on public display. In addition to providing medical therapy, dermatologists are consulted for the improvement in the appearance of many of these disfiguring conditions.[1] New research is being conducted on the use of laser alone and in conjunction with standard medical therapy in the treatment of patients with skin disease. In general, women are more likely to seek laser therapy for the treatment of their disease. Lasers including infrared wavelengths and pulsed dye lasers; light devices including blue light, red light, and broadband light; and photodynamic therapy with aminolevulinic acid and methyl aminolevulinic acid have been shown to be effective in the treatment of acne vulgaris. Acne scarring has been best treated with lasers, including non-ablative infrared lasers, fractional non-ablative and ablative laser resurfacing, and most recently needle-based radiofrequency

devices. Albert Einstein is often credited for the development of Laser Theory. In 1916, the term "stimulated emission" in his theory "Zur Quantum Theories der Strahlung" was published. The healing effects of the laser were not discovered until over 50 years later. "Leon Goldman" a dermatologist came up with a experiment of the first use of laser in medical history to remove unwanted tattoos by applied a laser on the tattooed area and then the tattoo disappeared.[2] Traditional ablative laser resurfacing is associated with adverse side effects, including prolonged erythema, edema, burning, milia, acne, crusting, and hypo- and hyper-pigmentation. Today's skin care consumer is presented with a wide array of available products to treat complications, and thus, the choices for the individual consumer seem endless.[3,4] The laser treatment

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results in loss of water content. Moisturizers are supposed to block[5] the transepidermal water loss by occlusion phenomenon. The post laser creams have moisturizing action, anti-inflammatory action, anti-mitotic action, anti-pruritic action, photoprotective action, and antimicrobial action and specially improve skin quality.

The present work was undertaken with an aim to develop a moisturizing cream with herbal ingredients to overcome post laser side effects and to evaluate the formulation.

EXPERIMENTAL

Collection of plant material

The roots of *Beta vulgaris* were obtained and collected from Nurturing Green, Noida, Uttar Pradesh, India, and identified as *B. vulgaris,* and the roots and rhizomes of *Glyccrhizia glabra* were collected and identified by the National Herbarium of Cultivated Plant, National Bureau of Plant Genetic resources, New Delhi, India, wide reference NHCP/NBPGR/2017-13 and a specimen copy of plant was submitted to the Department of Pharmacognosy, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India.

Preparation of extracts

The aqueous extracts were prepared by cold maceration method by placing accurately weighed 500 g of coarsely powdered air-dried material in a glass-stoppered conical flask with 1000 ml of the distilled water for 6 h, shaking frequently, and then allowed to stand for 18 h at room temperature, filtered rapidly, taking care not to lose any solvent, transferred 25 ml of the filtrate to a tarred flat-bottomed dish and evaporated to dryness in rotary vacuum evaporator, and stored in a well-closed glass vials in refrigerator at 4°C.

pH value of the extracts

The pH value was determined potentiometrically by means of the glass electrode as reference electrode in digital pH meter (Eutech pH 7000 instrument). The glass electrode was standardized using 0.2N sodium hydroxide.[6-9]

Determination of heavy metals

An accurately weighed 1 g of powder was dried, grounded, and soaked in 10 ml of nitric acid (HNO_3). Samples were then heated with 3 ml of 60% perchloric acid until brown color fumes have stopped to evolve. Solutions were cooled and diluted with 20% HCl to 50 ml. Samples, hence, obtained were analyzed in atomic absorption spectrometer. The standard calibration curves were prepared. Results were obtained in parts per million (ppm) levels.[8]

Microbiological analysis of extracts[8]

Total viable aerobic bacterial count was carried out with Soyabean Casein Digest Agar Medium (SCDAM). 1 ml of sample was pipetted out from nutrient agar broth medium into pre-sterilized Petri plates and 15–20 ml of SCDAM. The contents were mixed properly for uniform distribution, and the plates were incubated in a bacteriological incubator at 37°C for 24 h. After incubation, a total number of bacterial colonies were counted using colony counter, and colony-forming unit (CFU)/ml was calculated using the following formula:

CFU/ml = (total counted on agar plates/weight of Initial sample) × Dilution

The total fungal count was carried out with Sabouraud glucose agar media and sterilized at 121°C for 15 min. 1 ml of sample was pipetted out and 15–20 ml of Sabouraud glucose agar added in pre-sterilized Petri plates. The contents were mixed properly for uniform distribution, and plates were incubated in bio-oxygen demand incubator at 28°C for 72 h. After incubation, a total number of fungal colonies were counted with the help of colony counter, and CFU/ml was calculated using formula:

CFU/ml = (Total counted on agar plates/Weight of Initial sample)×Dilution

FORMULATIONS

Oil in water emulsion base cream was formulated. Twelve batches were prepared including different phases A, B, C, D, and E, respectively. The emulsifier Emulkare ET and other oil soluble components were added to make phase A and were heated to 80°C. All the ingredients of phase B which were water-soluble components were heated to 85°C. Thereafter, Phase B was continuously stirred with Phase A for emulsification process, and Phase C was added till the emulsion reaches 45°C temperature. After proper mixing, Phase D and Phase E were added with continuous stirring. Perfume was added when the temperature reached below 45°C. The resulting white creamy and opaque emulsions were transferred in a well-labeled container. The compositions of formulations are given in Table 1.

EVALUATION OF THE CREAM

pH

The pH meter was calibrated using a standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 ml of distilled water, and its pH was measured with Eutech pH 7000 Instrument.

Phases and temp.	S. No	Ingredients	F1%w/w	F2%w/w	F3%w/w
			Table 1: Composition of formulation based on plant extracts		
	1	Almond oil	3	3	3
	2	Cetyl alcohol	0.5	1	1
	3	Jojoba oil	3	3	3
	4	CCTG	3	3	3
Phase A heated to 80°C	5	Simulsol 165	1.5	1.5	1.5
	6	GMS-SE	0.7	1	1.5
	7	Kokum butter	1	1	1
	8	Glycerin	5	5	5
	9	Disodium EDTA	0.02	0.02	0.02
Phase B heated to 85°C	10	DM water	74.49	74.54	74.95
	11	Zinc oxide	0.1	0.1	_
Phase C heated to 45°C	12	Emulkare ET	1	1.2	1
Phase D	13	Xiam PMX 200	2	2	2
	14	Aquaxyl	1	1	1
	15	Zemea	1	1	1
	16	Hydroxyethyl urea	1	1	1
	17	Beta vulgaris extract	0.5	0.5	1
	18	*G. glabra* extract	1	1	1
Phase E	19	2-Phenoxy ethanol	0.8	0.4	0.4
	20	euxyl k 220	0.2	0.1	0.1
	21	Perfume	0.1	0.1	0.05

Viscosity

A spindle was selected according to the consistency of emulsion and dipped in sample. The sample should be sufficient so that the mark in the spindle could be dipped into the sample, the sample should be sufficient so that the mark in the spindle should be dipped into the sample. Then, rpm (1.0, 0.5, and 0.3) was set and motor was switched on, and viscosity (Brookfield DV 1 Viscometer) was noted down. Viscosity was taken at room temperature of 25°C at spindle number 96 selected.

Spreadability

Spreadability may be expressed by the extent of the area to which the topical application spreads when applied to the affected parts on the skin. The therapeutic efficiency of the formulation also depends on its spreading value. Hence, it was found necessary to determine the spreadability of the formulation. For this purpose, ample (about 3 g) was applied in between two glass slides, and they were pressed together to obtain a film of uniform thickness by placing 1000 g weight for 5 min. Thereafter, a weight (10 g) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves over the lower plate to cover a distance of 10 cm is noted. The spreadability (S) can be calculated using the formula: $S = m \times L/T$ where S - spreadability, m- weight tied to upper glass slide, L - length moved on a glass slide, and T - time taken. The determinations were carried out in triplicate, and the average of three readings was recorded.[10]

Extrudability

50 g cream was filled in standard capped collapsible plastic jars. The weights of the jars were recorded. The jars were placed between two glass slides and were clamped. The cap of jar was removed and nobe was tightened to apply pressure. The amount of the extruded cream was collected and weighed. The percentage of the extruded cream was calculated.

Stability studies

The stability study was performed as per the ICH guidelines. The formulated cream was filled in plastic jars and stored at different temperatures (25°C, 40°C, and 2–8°C for 10 months).

Moisture content

5 g of cream was weighed accurately in a tare bottomed shallow dish. The cream was dried at room temperature to constant weight. Finally, the material was weighed, and %

w/w loss on drying with respect to the original cream was calculated.[11]

Centrifugation

Centrifugation of sample was done by taking equal weights of sample taken in each centrifuge tube, and it was kept in the centrifuge (Remi R-24 Centrifugation Instrument, Mumbai, India) and centrifuge was switched on sample was centrifuged for ½ h at 5000–10,000 rpm in a standard laboratory centrifuge, and then, it was observed for separation.

Irritancy test

1 cm² of area on the dorsal left-hand surface was marked. The cream was applied to the specified area, and the time was noted. Irritancy, erythema, and edema were checked for regular intervals up to 24 h and reported.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation: *In vitro* anti-inflammatory activity was carried out by the method of Chandra *et al*.[12,13] The reaction mixture (5 ml) consisted of 0.2 ml of bovine albumin, 2.8 ml of phosphate-buffered saline (PBS), (PBS. pH 6.4),and 2 ml of varying concentrations of betavulgaris and *G. glabra* extracts (1 mg/ml, 500 mg/ml, 250 mg/ml, and 100 mg/ml, respectively, for each). Similar volume of double distilled water served as control. Then, the mixtures were incubated at 37 ± 2°C in a incubator for 30 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm using vehicle as blank. Diclofenac sodium at the final concentrations (100–1 mg/ml) were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated using the following formula:

% inhibition = 100×{(abs C-abs S)/abs C}

Where abs S = absorbance of test sample and abs C=absorbance of control

The extracts concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration. Calculated values are given in the results.

RESULTS AND DISCUSSION

The aqueous extracts of *B. vulgaris* and *G. glabra* were dark brown and yellowish-brown in color, respectively. The pH of extracts of *B. vulgaris* and *G. glabra* was 6.9 and 7.1 which are very much suitable for human skin. The heavy metals (Ca, Mn, Mg, K, Cu, Zn, Fe, and Na) were found to be within their permissible limits in both the extracts. Total bacterial count and fungal count were found to be 10^3 g/ml and 10^2 g/ml and were also lower than permissible limit of 10^5–10^7 g/ml and 10^3 g/ml, respectively. The formulations (F1, F2, and F3) were tested for physicochemical parameters such as appearance, homogeneity, spreadability, extrudability, moisture content, and irritancy test. Cream was stable even after centrifugation for 30 min at 10,000 rpm. The results of evaluation of cream are tabulated in Table 2. The cream did not cause any irritation after application on dorsal surface of the hand. The pH of formulation was within the range of skin's pH. The viscosity of cream was measured at rpm (1.0, 0.5, and 0.3) at spindle no. 96 found to be 5,72,700–8,10,000 cps at 40°C for 10 months, 4,50,900–8,80,500cps at 2–8°C, and 5,11,500–7,28,900cps at 25°C with torque limits (62–82%, 47–82%, and 56–77%) lying in the limited range of viscosity and torque (1,00,000–20,00,000 cps and 40–90%), respectively. The oil in water cream was found easily soluble in water. The cream showed *in vitr*o anti-inflammatory activity with the reference to standard drug (diclofenac sodium) at varying concentrations of *B. vulgari*s and *G. glabr*a formulation (1000 µg/ml, 500 µg/ml, 250 µg/ml, and 100 µg/ml).The results of the $I_{C5}0$ value of *B. vulgari*s, *G. glabr*a, cream formulation, and diclofenac sodium were recorded as 124.15 ± 0.134 µg/ml, 383.37 ± 1.36 µg/ml, 203.56 ± 1.04 µg/ml, and 416.30 ± 1.29 µg/ml, respectively. The study gives an idea that the plant extract can be used as a lead compound for designing a potent

Table 2: Results of evaluation studies	
Physical parameters	**Observations**
Appearance	White, opaque
Ph	5.8
Homogeneity [A], By visual [B], By touch	Homogeneous smooth and consistent
Rubout [A] spreadability [B] extrudability	Easily spreadable moisturizes skin surface
Type of smear	Non-greasy
Emolliency	No residue left
Stability studies Viscosity (cps) [OVEN, FRIDGE, RT]	572700, 450900, 511500
Centrifugation	Stable
Moisture content	78.02±0.08
Irritancy test	No irritation

topical formulation against the inflammation caused by laser treatment. The formulation F3 was found to be the best as post laser treatment cream since, and it passes all preliminary studies and complies with the Ayurvedic Pharmacopoeia of India and European Pharmacopoeia.[14,15]

CONCLUSION

In this study, post laser treatment cream was constructed with highest moisturizing effect. Based on the study, the aqueous extracts of *B. vulgaris* and *G. glabra* were selected as suitable moisturizing agents for post laser treatment cream formulation and provide cure for all side effects of laser treatment. Herbal active-based cream formulation with high thermodyanmic stability was achieved. The formulated cream F3 containing both aqueous extracts of *B. vulgaris* and *G. glabra* was much better as compared to the available marketed products for post laser treatments.

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