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Research Article

Formulation Development of Cream Incorporating Extracts of *Glycyrrhiza glabra* (Licorice)

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Abstract

Purpose: Creams are semisolid preparations that can be either oil in water or water in oil type and are usually medicated and intended for external application. Present study describes the formulation development and characterization of cream using *Glycyrrhiza glabra* (licorice) with the intension of developing a cream with whitening effect.
Methods: Two stable bases (formulation 1[F1] and formulation 2[F2]) with different ratios of virgin coconut oil (F1-41.2% and F2-48.6%), Tween 80 (F1-22.3% and F2-24.3 %), and distilled water (F1-36.5% and F2-27.1%) were identified based on previous research done at our laboratory and their stability was studied. Methanolic licorice extract in five different concentrations (1-5% w/w) were incorporated into the selected cream bases. Before incorporating in to bases, whitening effect of the licorice extract in terms of tyrosinase inhibitory activity was investigated. Characterizations such as microscopic analysis, pH and viscosity were measured and stability studies such as visual observations in accelerated temperatures, freeze thaw and centrifugation tests were also conducted.
Results: Licorice extract showed 81.5% inhibition for anti-tyrosinase assay. According to the microscopic analysis, formulated creams were oil in water emulsions. The pH of the formulations varied with the temperature and creams showed higher stability at lower temperature (at 8 °C). Viscosities of the creams of F2 containing high virgin coconut oil ratio were greater than the creams of F1 having lower virgin coconut oil content. According to accelerated stability studies all the cream bases of F1 and F2 were stable at 8 °C compared to room temperature.
Conclusion: Methanolic extract of licorise can be incorporated with virgin coconut oil, water and Tween 80 to formulate a cream, which is in oil in water nature. Out of the creams that were kept in accelerated temperatures, the creams that were kept in 8 ^{o}C were more stable in both the formulations but further improvements are needed to improve the shelf-life of the emulsions.

Keywords: Virgin coconut oil, Tween 80, Licorice, Formulation, Stability, Characterization.

Introduction

Creams are semisolid dosage forms composed of oil phase, aqueous phase and emulsifying agent.(1) Cream is an emulsion that has high viscosity and is semisolid in nature.(2)

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According to literature, base for cream could be prepared using virgin coconut oil (VCO) as the oil phase, distilled water as aqueous phase and nonionic surfactant Tween 80 as the emulsifying agent.(3) Instability such as creaming or sedimentation, flocculation, coalescence, Ostwald ripening (disproportionation) and phase inversion are problems that are encountered during the formulation of cream.(4) In order to find out the shelf life of the emulsions, stability tests such as thermal stability, freeze-thaw, centrifugation testing, visual observation at accelerated temperatures and characterization such as microscopic analysis, pH and viscosity measurements are done.(5)(6)

Licorice is known as sweet wood and is a member of the Papilonaceae. It grows about 1.5 m tall, with an elongated taproot. Hydrophobic extract of licorice contains flavonoids and glabridin, which has an inhibitory effect on melanogenesis due to tyrosinase inhibitory activity.(7) The de-pigmenting efficacy of glabridin has been shown by various researchers to be greater than that of hydroquinone.(8) According to previous research, percent yield of licorice root in 50% methanol is 33.6%, in 60 % acetone is 17.1 % and in distilled water is 12.6%.(9)

The main objective of this study was to formulate cream, study the characterization and evaluate the stability and compatibility of the formulations.

Methods

VCO was purchased from Lunuwila Coconut Research Institute Bandiripuwa Estate, Lunuwila, Sri Lanka. Tween 80 and methanol were obtained from Analytical Instruments Pvt. Ltd. Distilled water was used as the external phase and dried root of licorice (*Glycyrrhiza glabra)* was obtained from the local market in Fort, Colombo and was authenticated at Botany division, Bandaranayake Memorial Research Institute, Mahagrama, Colombo.

The ratios of ingredients for preparing the oil in water (O/W) stable bases were adopted from a previous study conducted in our laboratory.(10) In this study the herbal cream was prepared by first dissolving the licorice extract in aqueous phase and then Tween 80 was added drop wise in to licorice mixture while mixing in the magnetic stirrer at 600 rpm for 15 minutes. After mixing well, the mixture was kept overnight for complete dissolution. On the next day, prepared mixture of plant extract in aqueous phase and Tween 80 was added drop wise into VCO while mixing in the magnetic stirrer at 600 rpm for 20 minutes. The resulting mixture was further mixed by high shear homogenizer at 1200 rpm for 20 minutes. 40 g of five different concentrations (1%, 2%, 3%, 4% and 5%) of licorice extract containing creams of two different formulations (F1 and F2) having different ratios of oil phase (41.16% and 48.6%) and aqueous phase (27.10% and 36.52 %) were formulated (Table 1 and Table 2).

Table 1: Composition of cream of formulation 1 (w/w) containing licorice extract

	1%	2%	3%	4%	5%
Licorice extract	1	2	3	4	5
Distilled water	36.155	35.790	35.424	35.059	34.694
Tween 80	22.097	21.874	21.650	21.427	21.204
VCO	40.748	40.337	39.925	39.514	39.102

Table 2: Composition of cream of formulation 2 (w/w) containing licorice extract

	1%	**2%**	**3%**	**4%**	**5%**
Licorice extract	1	2	3	4	5
Distilled water	26.829	26.558	26.287	26.016	25.745
Tween 80	24.047	23.804	23.561	23.318	26.016
VCO	48.114	47.628	47.142	46.656	46.170

Tyrosinase inhibitory activity was done to assess the whitening effect of the licorice extract before adding to the bases.

After the formulation of the cream of five concentrations, their stability was checked through freeze thaw, centrifugation and visual observation at the pharmaceutical laboratory of the Department of Pharmacy, Faculty of Allied Health Science, University of Peradeniya.

Visual observation- 5 g of each formulation was kept at different storage conditions [8 °C (in refrigerator), 25 °C (in oven), 40 °C (in oven)] and physical characteristics of creams (color, creaming, phase separation, sedimentation, coalescence and flocculation) were observed on 1^{st}, 3^{rd}, 7^{th}, 15^{th}, 25^{th}, and 40^{th} day.(1)

Centrifuge testing- Centrifugation test for formulated cream was done on 1^{st}, 3^{rd}, 7^{th}, 15^{th}, 25^{th} day. 5 g of each formulation was placed in a centrifugal tube and was centrifuged in a centrifuge for 3 minutes at a "g" centrifugal force of 1200 and 25 ^{o}C and appearance was observed under naked eye.(6)

Freeze thaw test- The samples were kept first in the freezer at -5 ^{o}C for 24 hours, and then the frozen sample was left in the room temperature for 24 hours to thaw. This is one cycle, and after every four cycles the cream was evaluated for phase separation.(11)

Characterization

Formulated creams were tested for characterization such as dye test, pH value, viscosity, and organoleptic valuation. Dye test, pH value, and organoleptic evaluations were carried out at the Pharmaceutical Laboratory of the Department of Pharmacy, Faculty of Allied Health Science, University of Peradeniya and viscosity measurements were carried out at the Laboratory of Mechanical Engineering, Faculty of Engineering, University of Peradeniya.

Microscopic analysis- Methylene blue was mixed with the cream. A drop of the cream was placed on a microscopic slide and it was covered with a cover slip, and was examined under the Premostar microscope. (12)

pH- The pH meter was calibrated using standard buffer solutions with a pH of 4.01,

7.01 and 10.01. 0.5 g of the cream was weighed and dissolved in 50.0 mL of distilled water and its pH was measured.(13)

Viscosity- Viscosity of the freshly prepared stable formulations was determined by Redwood Viscometer. Time taken to flow 50 mL of sample through the viscosity meter into a 50 mL measuring cylinder was measured using a stop watch.(10)

Organoleptic evaluation- The appearance of the cream was evaluated by its color, state, odor and texture.

Results

The yield of the methanolic extract of licorice is 12.7%. Licorice extract showed 81.5% inhibition for Anti-tyrosinase assay.

Formulated herbal whitening cream of licorice was whitish yellow in colour and had a pleasant smell which is a mixture of the characteristic smell of VCO and licorice. When considering the colour intensity of the different formulations, F2 was darker than F1 and with the increase in concentration an increase in colour intensity was observed.

According to preliminary stability studies for 40 days, formulated cream showed higher stability at 8 ^{o}C compared to room temperature or 40 ^{o}C (Table 3). At room temperature, the cream with F2 was only stable for 25 days and cream with F1 for 15 days indicating lower stability of F1 over F2. This observation was also supported by the centrifugation test (Table 4) done for the sample kept at room temperature.

Table 3: Days of stability of the creams that were kept in accelerated temperatures

	Stability in days		
Formulation	**8 ^{o}C**	**Room temperature**	**40 ^{o}C**
F1	40	15	7
F2	40	25	25

Table 4: Days of stability of creams that underwent centrifugation

	Formulation 1					**Formulation 2**				
Cream licorice%	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Days	7	7	3	3	3	15	7	7	25	15

Creams of F1 and F2 were unstable during the 1st cycle of freeze thaw test.

According to microscopic analysis formulated creams were oil in water (O/W) emulsions. According to viscosity measurements F2 had higher viscosity than F1. According to pH measurements pH decreased with number of days and with temperature. For statistical analysis, Mann Whiney U analysis was conducted to analyse the pH at different accelerated temperatures and different formulations of licorice, as the number of samples was smaller to carry-out ANOVA test. According to the Mann Whitney U analysis, there was no significant difference in pH observed when the same cream (F1 or F2) was kept at different temperatures. When F1 and F2 were compared, a significant pH differences was

observed in samples kept in room temperature and at 40 ^{o}C, but there was no significant pH difference observed between F1 and F2 when kept at 8 ^{o}C.

Discussion

This study was focused to develop emulsion formulation using natural substances such as VCO and distilled water which are not harmful to the human skin and are readily available in Sri Lanka. Tween 80 is an emulsifier that is used to stabilize oil phase and aqueous phase which become phase separated when mixed together. Base selection was done from past literature.(10) Higher VCO concentration was selected since formulations with higher water content and lower VCO content showed instability according to the study conducted previously. Therefore VCO concentration of 48.6 % and 41.16% were used for the formulations. According to the microscopic analysis formulated herbal creams were O/W emulsions. For this characterization study, water soluble methylene blue dye was used. Oil in water formulations are preferred for topical preparation as they are non-greasy and have high penetration of active ingredient due to low interfacial force. However O/W are best suited for active ingredients that are hydrophobic which dissolve in oil phase and are protected by the external hydrophilic phase.(2) Rate of drug release occurs in a controlled manner that is favorable pharmaceutically. O/W emulsions are prepared using VCO as oil phase, distilled water as aqueous phase and Tween 80 as the surfactant. Tween 80 has the HBL (Hydrophobic-Lipophilic Balance) value of 15 which means it is hydrophilic emulsifier that strongly dissolves in aqueous phase and forms O/W emulsion.

Viscosities of the emulsions were measured by Redwood viscometer and compared using Redwood seconds which gives an idea about the kinematic viscosity in Redwood viscometer type 1. There wasn't much variation in viscosity due to increase in concentration of licorice in both the formulations. Therefore viscosity is not affected by the concentration of licorice. The stability of the emulsions improved with the increase in viscosity and it may be due to the surfactant concentration. This has been proved in this study as F2 was more stable than F1.

According to the pH study, the concentration of the licorice extract influences the pH of the formulation. There was a decrease in pH with the increase in the concentration; this could be clearly observed in both the formulations in all the temperatures. This may be due to acidic nature of licorice extract due to the presence of glycirrhizinic acid. On the first day, pH of the formulations were higher than the pH of standard topical preparations and during the stability study period there was a decrease in the pH in all five concentrations of the two formulations. This may be due to the intrinsic factors of licorice and acidification of VCO with time. There was a difference in pH with temperature during the stability period of each concentration. Less variation in pH was observed in the formulations at 8 ^{o}C. Therefore the creams of both the formulations that were kept in 8 ^{o}C were all stable. A considerable variation in pH could be seen in the formulations kept in 40 ^{o}C. High pH difference could be observed with increase in temperature this may be due to formation of aldehydes, epoxides, acids, alcohols, and other hydrocarbons from unsaturated fatty acids in the presence of heat. Fluctuations in the pH may be due to temperature changes at the time of the measurement of pH which is

also a contributing factor. The pH is also an indicator for stability. Decrease in pH indicates instability of formulations and it is the cause for the changes in organoleptic properties of the formulations as well.

Accelerated stability studies are important so that long term stability of the formulations could be predicted and changes in consistency could also be observed. According to WHO specification accelerated studies are conducted for 6 months and long term stability studies are done if any instability observed within the time period of 6 months.

Conclusion

From the study conclusion could be made based on the parameters such as pH, viscosity, organoleptic evaluation and visual observation in accelerated temperatures of the F1 and F2 of licorice extract. The F1 is stable for 7 days and F2 of licorice was stable for 25 days at room temperature. Out of the creams that were kept in accelerated temperatures the creams that were kept in 8 ^{o}C were more stable in both the formulations but further improvements are needed to improve the shelf-life of the emulsions with extended stability studies.

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