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Formulation and evaluation of an Aloe vera -Licorice combination topical gel: a potential choice for wound healing

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Abstract

Wound healing is a natural body response to injury and consists of three steps; Inflammation, proliferation and remodeling. Natural products have always been attractive in pharmacy and drug delivery and have shown higher patient compliance in some treatments. Aloe vera and licorice extract have each been used to improve and accelerate wound healing. It seems that combination of these two natural products may show better and effective results. Aloe vera gel and licorice extract powder were standardized with their major and important components, Glucomannan and Glycyrrhizin. Three different polymers in three different concentrations were used to prepare topical gels containing Aloe vera gel and licorice extract powder. Gels were examined for different properties such as appearance, pH, viscosity, spreadability, drug content and in vitro release. Optimized formulations contained carbopol 2% (F3) and CMC 3% (F5) with pH (5.92 and 5.69), spreadability (51 and 55 mm), a shear thinning manner and in vitro release within 8 hours proper for topical use. Preclinical studies should be processed to determine the suitability of these gels for wound healing.

Keywords: *Aloe vera*, Licorice, Topical gel.

1. Introduction

Wound healing is a natural body response to injury and consists of three steps; Inflammation, proliferation and remodeling (1, 2). Different chemical (dexpanthenol, phenytoin, zinc oxide) and natural (*Ginko biloba* (2), *Aloe vera* (3) and licorice (1)) agents have been used to improve and accelerate wound healing, separately. *Aloe vera* is a tropical plant of Liliacea family containing a viscose gel in its green leaves. In addition to its wound healing effect, Aloe vera gel possesses some other beneficial properties such as antibacterial and antifungal effects (4). It is reported that wound and burn healing effect is related to tannic acid and a type of polysaccharide (4-7) especially Glucomannan which is a fibroblast activator and proliferation stimulant and leads to increase in collagen production and secretion (4). Previous reports have mentioned 50.8% wound diameter reduction having been recorded in animals receiving topical *Aloe vera* gel 25% (3). Orally administered Aloe gel has also shown accelerating effects on wound healing in diabetic rats (7).

Licorice (*Glycyrrhiza glabra*) is a plant with traditional application in peptic ulcer and infections and newly reported usage in wound healing and as an antibacterial agent (2, 8, 9). The reported wound healing effect of licorice is related to its most active component, Glycyrrhizin, which may increase "interleukin 2" as the essential factor for wound healing (2) and shorten healing time (9).

Several reports have shown that combina-

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tion of licorice extract and other herbal extracts improved healing potential of each substance alone. Therefore, by considering the reported wound healing effects of both *Aloe vera* and Licorice, the aim of this study is to prepare and evaluate a topical dosage form for simultaneouse co-administration of these two natural agents for possible higher wound healing efficacy.

2. Materials and methods

2.1. Materials

Aloe vera mucilage was purchased from Barij Essence Company (Kashan, Iran). Licorice extract was gifted by Shirin Daru Company (Shiraz, Iran). Konjac Glucomannan was purchased from Richest Group (China). Na-Carboxy methyl cellulose (CMC) (viscosity=400-800cps), hydroxymethylpropyl cellulose (HPMC) and Carbopol 934 were purchased from Sigma-Aldrich (St Louise, USA). Propylene glycol, glycerol, ethanol, chloroform, methyl paraben, propyl paraben, were purchased from Merck (Germany). Congo red indicator was purchased from Samchun (Korea).

2.2. Aloe vera Gel standardization

A specific and sensitive colorimetric assay reported previously was used to determine glucomannan amount in commercial *Aloe vera* gel (10, 11).

2.2.1. Preparation of Glucomannan standard

One hundred milligrams of Konjac Glucomannan in 20 ml ethanol was stirred 4 hours and then centrifuged 15 min at 4000 rpm. The supernatant was removed and the precipitant was mixed with ethanol and centrifuged for second time. After removing supernatant, the precipitated powder was mixed with water and freeze dried for 24 hours (10).

2.2.2. Glucomannan standard curve validation

Different concentrations of standard polysaccharide (800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) were prepared from stock solution using serial dilution. 0.4 ml Congo Red indicator (0.01%) was added to 4 ml of each concentration. Absorbance levels were measured by UV/visible Spectrophotometer (PG Instrument, T-80, Germany),at 540 nm (10). The measurement was carried out in triplicates in a day and in 3 different days to find the intra- and inter-day variations, respectively. The mean data were used to prepare calibration curve. Linearity, inter-day and intra-day percision and accuracy were determined to validate the method.

2.2.3. Assay of Glucomannan in Aloe vera gel

The Aloe vera gel was dissolved in 100 ml distilled water and filtered. 4 ml of congo red reagent (0.01 %) was added to 0.4 ml of this solution while being mildly vortexed. The mixture was left at room temperature for 20 min. Then absorbance was measured at 540 nm and the Glucomannan content was determined using calibration curve equation (10).

2.3. Licorice extract powder standardization

2.3.1. Preparation of Licorice extract powder

One gram of powdered licorice was refluxed four times for 15 min with four successive 15-ml portions of 70% ethanol. Each portion was decanted and filtered through the same filter paper. The alcohol was evaporated, 20 ml of warm water was added, the mixture was transferred into a separator, the flask was washed with two 10-ml portions of boiling water, and the washings were added to the original mixture in the separator. After cooling, 5 ml ethanol and 1 ml 6 N H2S04 were added; then the mixture was shaken very gently with successive portions of 20, 20 and 10 ml chloroform. Each chloroform fraction was separated as completely as possible, taking care not to pass down any precipitated matter discarded. The aqueous phase with any suspended insoluble matter was quantitatively transferred into a boiling flask, using 5 ml of distilled water to wash off any clinging matter on the separator walls. Ten milliliters of 6 N H2S04 was added, and the mixture was heated on a boiling water bath for 75 min. The hydrolyzed mixture was transferred quantitatively into a separator, while hot, , and the flask was washed with 5 ml of distilled water. The aqueous acid solution was extracted with four 20-ml and one 10-ml portion of chloroform. Each chloroform fraction was filtered through a small piece of cotton surmounted by 2 g of anhydrous sodium sulfate. The chlo-

roform filtrate was collected into a 100-ml measuring flask, and the volume was completed with chloroform (12).

2.3.2. Coupling Experiments

A 10-ml aliquot was transferred into a separator containing 20 ml of pH 9.2 universal buffer solution, 2 ml of dye solution, and 10 ml of chloroform. The mixture was shaken continuously for 1 min. After separation of the two phases, the aqueous phase must be blue in color. If it has a greenish or yellow color, additional 1-ml aliquots of the dye solution must be added and the mixture must be shaken. The chloroform layer was separated into another separator containing 5 ml of pH 9.2 buffer. Then the mixture was shaken and, after complete separation, the chloroform layer was separated into a 100-ml measuring flask (without filtration or dehydration). The contents of the first and second separators were re-extracted with l0, 5, and 5-ml portions of chloroform in the same manner, and the combined chloroform extract in the measuring flask was completed to volume with ethanol.

2.3.3. Glycyrrhizin standard curve validation

Different concentrations of standard glycyrrhizin (25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 µg/ml) were prepared from stock solution using serial dilution in chloroform. After a coupling process, absorbance levels were measured by UV/visible Spectrophotometer at 640 nm (12). The procedure was carried out three times a day in 3 different days to find the inter-day & intra-day variations. The mean data were used to prepare calibration curve. Linearity, inter-day and intra-day precision and accuracy were determined for validation of the method.

2.3.4. Glycyrrhizin content of licorice extract powder

Absorbance level of determined amount of licorice extract powder after a coupling process was measured by UV/visible Spectrophotometer at 640 nm (12).

2.4. Preparation of Aloe vera -Licorice combined gel formulation

Considering preliminary studies, three different polymers (Carbopol, HPMC and CMC) in three different concentrations were applied to prepare topical gel containing fixed amounts of Aloe vera gel and licorice powder (Table 1).

2.5. Evaluation of Aloe vera -Licorice gel

Prepared Aloe vera-licorice gels were evaluated for appearance, pH, drug content uniformity, viscosity, spreadability and in-vitro release. All experiments were repeated three times.

2.5.1. Physical appearance and pH evaluation

Gel appearance and homogeneity was evaluated visually. A calibrated digital pH meter at constant temperature was used to determine the pH values of aqueous solutions of the prepared gels.

2.5.2. Viscosity and rheological behavior

Viscosity was measured by placing 1 g of sample in sample holder of Brookfield viscometer at 0 to 100 rpm. All experiments were

Table 1. Compositions of Aloe vera-Licorice topical gel formulations.

Content (%)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Carbopol	0.15	1	2	-	-	-	-	-	-
HPMC	-	-	-	-	-	-	1	3	5
CMC	-	-	-	1	3	5	-	-	-
Aloe vera gel	15	15	15	15	15	15	15	15	15
Licorice powder	9	9	9	9	9	9	9	9	9
Triethanolamin	1	1	1	-	-	-	-	-	-
Propylene Glycol	10	10	10	20	20	20	10	10	10
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06

performed in triplicates

2.5.3. Spreadability

Spreadability was measured by spreading 0.5 g of the gel on a circle premarked on a glass plate and then a second glass plate was employed. 0.5 kilograms of weight was permitted to rest on the upper glass plate for 5 min. The circle surface after spreading of the gel was determined (13). A commercial topical gel was examined to compare the spredability results.

2.5.4. Glucomannan and Glycyrrhizin content uniformity

The prepared gels were weighed accurately and dissolved in 100 ml of distilled water and filtered. The colorimetric determination of glucomannan and Glycyrrhizin which was mentioned in sections 2-2-3 and 2-3-4 was used for determining content uniformity (10, 12).

2.5.5. In vitro release study

The *in vitro* release of Glucomannan and Glycyrrhizin from gel formulations was evaluated by dissolution tester (Erweka, Germany). Five grams of gel was placed in a watch glass covered with aluminum mesh. The watch glass was then immersed in a vessel containing 100 ml of the release medium, phosphate buffer pH 5.5 at 37±0.5 ºC with a paddle speed of 50 rpm. Aliquots (5 ml) were withdrawn at specified time intervals of 30 min, 1, 2, 4, 6 and 8 hours and immediately replaced with fresh dissolution medium. The samples were divided into two parts and assayed for Glucomannan and Glycyrrhizin amounts using a validated analysis method. Experiments were carried out in triplicates (14).

2.6. Statistical analysis

All data were analyzed by one-way ANOVA test followed by Tukey's post-hoc test using SPSS version 16.0 software. $P<0.05$ was considered as statistically significant difference.

3. Results and Discussion

3.1. Aloe vera gel standardization

3.1.1. Glucomannan standard curve validation

Calibration curve data were created in the range of the expected concentrations 50 to 800 µg/mL. The equation was y=0.0004+0.0127x. The correlation coefficient (r^2) of the standard curve (0.9950) indicated linear relationship at selected range of Glucomannan concentrations. Inter-day, Intra-day precision and accuracy were 99.3±0.5, 98.1±0.6 and 96.7±3.3 %, respectively.

3.1.2. Glucomannan Assay of Aloe vera gel

Results indicated 0.36±0.014 mg Glucomannan per each ml of *Aloe vera* gel.

3.2. Licorice extract powder standardization

3.2.1. Glycyrrhizin standard curve validation

Calibration curve data were created in the range of the expected concentrations 3.125 to 50 µg/mL. The equation was y=0.0096+0.0167x. The correlation coefficient (r^2) of the standard curve (0.9962) indicated linear relationship at selected range of Glucomannan concentrations. Inter-day, Intra-day precision and accuracy were 99.7±0.3, 98.4±1.6 and 96.9±3.1%, respectively.

3.2.2. Glycyrrhizin content of licorice extract powder

Results indicated 7.36±0.1% Glycyrrhizin per each gram of licorice extract powder.

3.3. Aloe vera -Licorice gel Formulation preparation

Considering previous studies, *Aloe vera* gel has been used in concentration range of 10-70% in burn treatments and 30% in seborrheic dermatitis (15). According to glucomannan content and presence of licorice extract powder, a concentration of 15% was chosen for *Aloe vera* gel for the preparation of topical formulations. As it was reported, topical gel 1-2% licorice with 19.6% Gycyrrhizin content showed acceptable results in the treatment of atopic dermatitis (16). Therefore, based on the reported data and according to gel structure formation, the concentration of 9% was chosen for licorice extract powder.

Screening results of the studied formulations indicated that in F1, F7 and F8 formulations, gel structure was not stable and they were eliminated from further evaluations.

Table 2. Results of characterization of different gel formulations

	pH	Viscosity (cp)	Spreadability (mm)	Drug Content % (Glucomannan)	Drug Content % (Glycyrrhizin)
F2	5.83±0.23	2600	115±2.6	94.8±0.7	91.2±1.6
F3	5.92±0.19	21850	51±0.3	100.5±0.3	100.2±2.1
F4	5.95±0.16	3400	93±1.4	99.3±0.5	89.8±0.1
F5	5.69±0.1	185000	54±0.8	99.4±0.4	99.5±0.7
Commercial Gel	6.1±0.18	168000	61±0.3	-	-

3.4. Evaluation of Aloe vera -Licorice gel

Seven formulations were included in evaluation experiments. For better analysis all experiments were compared with a commercial topical diclofenac gel.

3.4.1. Physical appearance and pH evaluation

Gels' physical appearance was opaque and black due to the presence of licorice powder. Homogeneity of all gels was acceptable except for F6. pH of gel formulations was in the range of 5.69-6.01. According to previous studies, the suitable pH to avoid risk of irritation upon application to the skin is 5-6 (14). Therefore, pH of all gels was in the acceptable range.

3.4.2. Viscosity and rheological behavior

An important factor in gel characterization is viscosity due to its effect on gel spreadability. It is also an influential parameter on patient compliance. The shear thinning rheological behavior is preferred due to its low flow resistance when applied at high shear conditions. It means that gel viscosity decreases with increasing rate of shear (13). Viscosity of all gel formulations and the commercial topical gel used in this study were evaluated and reported in Table 2. As it is seen, viscosity of F5 is more similar to the commercial gel. The viscosity of F9 could not be determined. As it is seen in Figure 1, rheological behaviors of different formulations indicated a shear thinning pattern.

3.4.3. Spreadability determination

Considering previous reports, spreadability of F3 and F5 was in an acceptable range (17). The gel spreading helps towards the uniform application of the gel to the skin, so the prepared

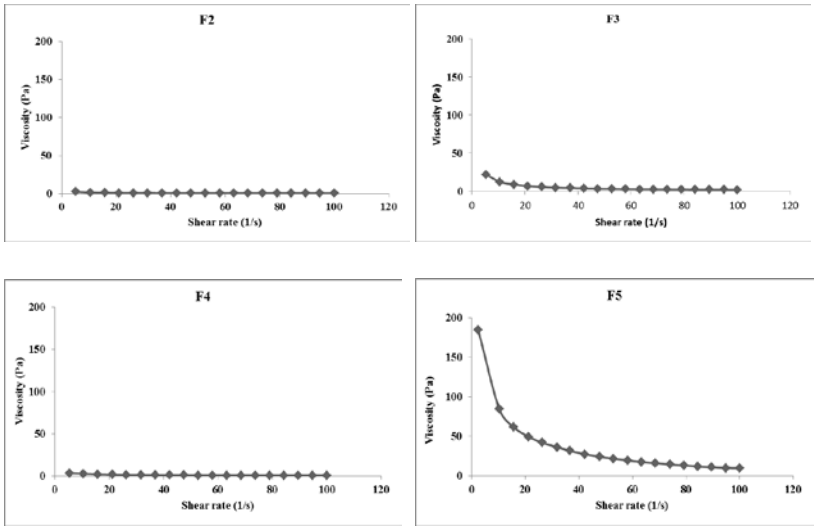

Figure1. Viscosity / shear rate rheological profile of selected formulations.

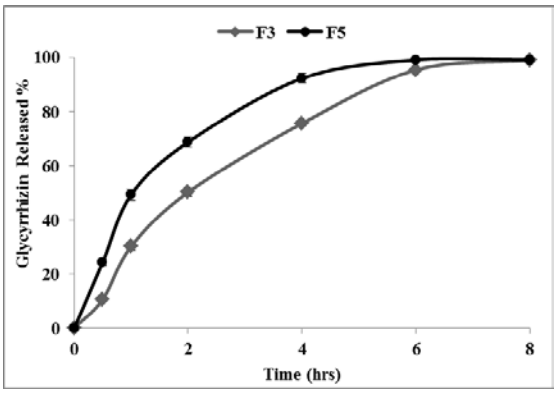

Figure 2. *In vitro* release of Glycyrrhizin from F3 and F5

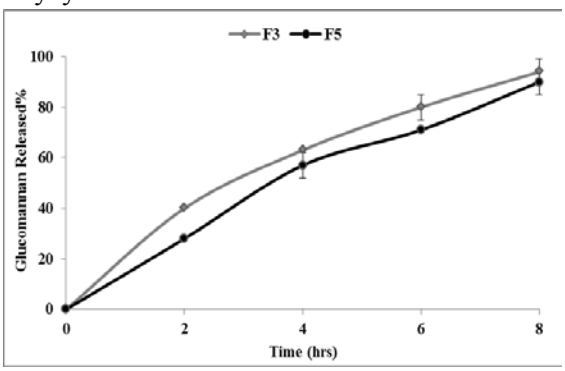

Figure 3. *In vitro* release of Glucomannan from F3 and F5.

gels must have a good spreadability and satisfy the ideal quality in topical application (13). According to statistical results, spreadability of F3 and F5 is significantly different (p=0.038) while in case of F5 it is significantly (p=0.114) similar to the commercial gel.

3.4.4. Glucomannan and Glycyrrhizin content uniformity

Glucomannan and Glycyrrhizin content in selected formulations were determined. Results were within permissible range indicating the uniformity of drug dispersion in the gels (Table 2).

3.5. In vitro release test

Considering the evaluating factors mentioned, the two final selected formulations were F3 and F5. As it is shown in Figures 2 and 3, Glycyrrhizin and Glucomannan were released from F3 and F5 within 8 hours. Similar to previous reports presence of propylene glycol could have increased the release rate (18). Glycyrrhizin is released in a higher rate compared to Glucomannan which may be related to its higher solubility.

4. Conclusion

Considering the importance of wound healing or tissues restoration integrity, topical formulations designed to accelerate wound healing is desirable. Aloe vera and licorice extract powder Topical gel was prepared with good physicochemical properties. Taking into account the reported wound healing, anti-bacterial and anti-inflammatory effects of Aloe vera and licorice, it seems that the presence of both active ingredients in lower concentrations would be preferable and more effective than each alone. Meanwhile, preclinical studies of these formulations need to be done.

Conflict of Interest

None declared.

5. References

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