Formulation development, optimization and evaluation of aloe vera gel for wound healing

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ABSTRACT

Purpose: To formulate and optimize a herbal gel of Aloe vera extract containing Carbopol 934 as gelling agent and to investigate the effects of topical application of Carbopol 934 gel containing Aloe vera extract on the healing of skin wounds surgically induced in Wistar rats. **Materials and Methods:** Different concentrations of viscosity enhancer Carbopol 934 were tried and finally gel that showed good spreadability and consistency was selected for wound healing property of herbal gel of Aloe vera. Excision wound model was used for the study. **Results:** The optimized gel was evaluated for different physicochemical properties and wound healing property. Differences in wound healing were observed between the various treatments when compared to the control group. Tissue hyperplasia was lower in the control group compared to the other treated groups. In animals group treated with gel, 80.14% healing was observed up to 14th day. While in untreated group I (control) animals showed 52.68% healing of wounds on 14th day. On the other hand, control group animals also showed inflammation and pus formation up to 5th day of study, while treated animals did not showed any observable inflammation and pus formation. Conclusion: Results shows prepared gel has promising effect on the wound healing process.

Key words: Aloe vera, spreadability, viscosity, wound healing

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INTRODUCTION

Skin serves as a barrier to water and various pathogens. Wounds and injuries destroy this barrier that normally prevents invasion of bacteria, fungi and viruses.[1] Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers. The human adult wound healing process can be divided into 3 distinct phases: the inflammatory phase, the proliferative phase, and the remodeling phase. Within these 3 broad phases there exist a complex and coordinated series of events that includes chemotaxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodeling.[2-4] In addition, angiogenesis, epithelization, and the production of new glycosaminoglycans (GAGs) and proteoglycans are vital to the wound healing milieu. The culmination of these biological processes results in the replacement of normal skin structures with fibroblastic mediated scar tissue.

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The objective of wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort and scarring to the patient. Understanding the healing process and nutritional influences on wound outcome is critical to successful management of wound patients.

Aloe vera belonging to the Lily (Liliaceae) family is a perennial succulent plant. This plant has been known as "the healing plant". Aloe vera has been used for traditional medical purposes in several cultures for millennia,[5-8] it has been demonstrated that Aloe vera has growth promoting activities. Recently anti-fungal properties of aloe vera leaves were investigated by Casian.[9] *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 gel, extracts resulted in faster healing of wounds.[10-13] Several reports state that Aloe vera gel has a beneficial influence on the wound healing in both normal and diabetic rats. Reports have stated that it exerts an immune stimulative effect by activating macrophages. Inspite of the wide use of Aloe vera as a remedy to enhance wound healing, its mechanism in healing of wounds has not been studied in detail.

The aim of the study is to formulate and optimize a herbal gel of Aloe vera extract containing Carbopol 934 as gelling agent and to investigate the effects of topical application of Carbopol 934 gel containing Aloe vera extract on the healing of skin wounds surgically induced in Wistar rats. Various physicochemical parameters of the gel that influences the properties of gel are also studied.

MATERIALS AND METHODS

Carbopol 934 was purchased from Loba Chemie (Loba Chemie Pvt Ltd., Mumbai, India), Carboxy Methyl Cellulose Sodium from Thomas Baker, Methyl Paraben Sodium and Propyl Paraben Sodium was Purchased from Titan Biotech Ltd. (Rajasthan, India), Sodium metabisulphite from SD Fine chemicals ltd., Mumbai, India.

Aloe vera (Aloe barbadensis) plant was obtained from Herbal Garden, Jamia Hamdard, Hamdard University, Delhi.

Extraction of Aloe vera extract

Thick succulent leaves of Aloe vera (*Aloe barbadensis*) plant obtained from Herbal Garden, Jamia Hamdard, Hamdard University, Delhi, were used. To obtain Aloe vera extract, the mucilaginous jelly obtained from the centre (the parenchyma) of the plant leaf of Aloe vera, the leaves of Aloe vera were collected, washed with water and a mild chlorine solution and were finally cut transversely into pieces. With a vegetable peeler, the thick epidermis was selectively removed and the inner gel-like pulp in the center of the leaf was separated with a spoon, minced, and homogenized in a mixer.

Animals

Studies were performed on 2-3 months old healthy male Sprague dawley rats (weighing 300 ± 20 g). Animals were allowed to acclimatize for one week before start of the experiments in controlled environment (centrally air-conditioned with 100% fresh air replacement) at ambient temperature of 22 ± 3°C with relative humidity of 50 ± 10%, and 12h light/dark cycle.

Animal grouping

Animals were randomly selected and divided into 2 groups, with 6 rats in each group. Animals in group 1 were without treatment and served as control. Animals in group 2 received herbal gel containing Aloe vera. Gel was applied on wound once daily upto 14 days starting from the first day of wounding.

Selection and Optimization of gelling agent

In order to optimize the concentration of gelling agent to achieve proper consistency of the gel formulations were prepared with different gel formers, Carboxy methylcellulose sodium, Carbomer 934, HPMC and different concentration of viscosity enhancer vis. 1.0, 2.0, 3.0 and 4.0 % were tried and finally gel that showed good spreadability and consistency was selected.

Preparation of Aloe vera gel

To prepare Aloe vera gel Sodium metabisulphite, Methyl paraben sodium and Propyl paraben sodium were dissolved in water. Gelling agent was added to it and stirred continuously till it got swollen completely. Triethanolamine was slowly added to the dispersion with continuous stirring which resulted in a stiff gel. Aloe extract was added to it and stirred for 15 min. Volume was made with water and stirred continuously till a uniform gel was formed [Table 1].

Evaluation of Gel

*p*H

1.0 g gel was accurately weighed and dispersed in 100 ml purified water. The *p*H of the dispersion was measured

Table 1: Composition of Aloe vera gel

Batch No.	Aloe Extract	Sodium meta bisulphate	Sodium CMC	HPMC	Carbomer 934	Triethanolmine	Methyl paraben sodium	Propyl paraben sodium	Purified water
	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
F1	75	0.200	1	-	-	-	0.020	0.002	Qs to 100
F2	75	0.200	2	-	-	-	0.020	0.002	Qs to 100
F3	75	0.200	3	-	-	-	0.020	0.002	Qs to 100
F4	75	0.200	4	-	-	-	0.020	0.002	Qs to 100
F5	75	0.200	-	1	-	-	0.020	0.002	Qs to 100
F6	75	0.200	-	2	-	-	0.020	0.002	Qs to 100
F7	75	0.200	-	3	-	-	0.020	0.002	Qs to 100
F8	75	0.200	-	4	-	-	0.020	0.002	Qs to 100
F9	75	0.200	-	-	1	1.2	0.020	0.002	Qs to 100
F10	75	0.200	-	-	2	1.5	0.020	0.002	Qs to 100
F11	75	0.200	-	-	3	1.8	0.020	0.002	Qs to 100
F12	75	0.200	-	-	4	2.1	0.020	0.002	Qs to 100

using digital pH meter, which was calibrated before use with standard buffer solution at 4.0, 7.0 and 9.0. The measurements of *p*H were done in triplicate and average values were calculated.

Spreadability

One of the criteria for a topical formulation to meet the ideal qualities is that it should possess good spreadability. It is the term expressed to denote the extent of area to which formulation readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. To determine the spreadability of formulation, 0.5 g of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.

Extrudability

To determine extrudability a closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 sec was determined. The average extrusion pressure in g was reported.

Viscosity

The viscosity of the formulations was determined as such without dilution by R/S CPS Plus Rheometer (Brookfield Engineerring Laboratorie, Inc., Middleboro, MA,USA) using spindle #C 50-1 having diameter of 50 mm using software RHEO3000.

Homogeneity

The developed formulations were tested for homogeneity by visual inspection after the gel had been filled in the container. They were tested for their appearance and presence of any aggregates.

Experimental wounding / wound creation:

Excision wound model

All animals were anaesthetized by open mask method with anesthetic ether before wound creation. Hairs were removed from dorsal thoracic central region of anaesthetized rats and the area sterilized with 70% alcohol. Full thickness from the demarcated area was excised to produce wound measuring around 340 mm2. The wound was left undressed to the open environment. In this model wound contraction and wound closure time were monitored. Wound contraction was measured as percent contraction in every two days after wound formation.

Percent wound contraction and epithelialization time

After wound creation the wound margin were traced at 2 days intervals on transparent graph paper having a millimeter scale and measurements were continued up to 14 days. After every two days healed area was calculated by subtracting initial wound area to the unhealed area. Contraction was represented as percent wound contraction and epithelialization time was observed after complete healing.

Statistical analysis

All values were expressed as mean ± SEM and the statistical significance of differences among groups in term of rate of wound healing were evaluated. A value of $p < 0.05$ was considered significant. Statistical computations were calculated using PASW statistics 18 software (USA).

RESULTS AND DISCUSSION

Optimization and selection of gel

One of the main ingredients of the formulation is the gelling agent. The concentration of viscosity enhancer or gel former is of immense value as a less concentration will lead to simple solution or lotion with very low consistency, while high concentration may lead to formation of gels with high viscosity leading to non-uniform distribution of drug and problem with handling of gel. Different gel formers were tried in order to select the best gelling agent. Gels containing aloe vera juice extract and Sodium CMC showed phase separation and were rejected. Aloe vera gels containing 1.0 % of carbomer 934 form a very thin gel that liquefies within 06h of preparation. With 2.0% gelling agent somewhat better gel was obtained but the problem of liquification after 24h was observed. Gel containing 3.0% of carbomer 934 formed uniform and smooth gel that does not liquefy upon keeping. At 4.0 % of carbomer gel was very thick and more sticky that could not be properly spread out. With HPMC the gels formed are poor in consistency and very thick as indicated by spreadability and extrudability values. Thus, 3.0% of carbomer 934 was selected as the optimized concentration of gelling agent.

The pH of the formulation was determined in order to be sure that the formulation can be used without the risk of irritancy to the skin. The *p*H was found to be 6.6 ± 0.5 for gel which was very near to the neutral *p*H, thus the formulation can be used without the risk of irritancy to the skin. This also indicated that the selected ingredients of the formulation did not alter the *p*H of the formulation.

The Spreadability of formulations was found to decrease with increasing the concentration of gelling agent. The value of Spreadability for optimized gel was found out to be

Table 2: Evaluation of Gel

S No	Parameter	F5	F6	F7	F8	F9	F10	F11	F12
1	*p*H	6.3	6.4	6.6	6.5	6.4	6.4	6.5	6.6
2	Spreadability* (cm)	8.8	6.5	6.3	4.5	15.4	11.7	8.6	5.3
3	Extrudability(gm/cm^2)	15.3	18.5	20.3	28.2	#	#	18.1	22.3
4	Viscosity (mPas)	315	360	384	415	266	295	330	396
5	Homogenity	Poor	Poor	Poor	Poor	Poor	Good	Good	Good

* Spreadability is determined within 1 h of preparation of gel
\# Sample liquefies before analysis

8.6 cm indicating that the lotion is easily spreadable by small amount of shear. The results indicated that the formulation can be applied easily without being runoff. This assures that the formulation maintain a good wet contact time when applied to the site of application [Table 2].

Wound healing activity of Aloe vera gel

The percent wound contraction was determined using the following formula:

$$\%\,Wound\ contraction = \frac{Healed\ Area}{Total\ Wound\ Area} \times 100$$

Measurement were taken in every two days interval and continued till 14th day.

In wound healing study [Table 3], wound contraction progresses in wound treated with formulation and control group. In animals group treated with gel, 80.14% healing was observed up to 14th day. While untreated group I (control) animals showed 52.68% healing of wounds on 14th day. On the other hand, control group animals also showed inflammation and pus formation up to 5th day of study, while treated animals did not showed any observable inflammation.

Table 3: Effect of Gel on wound healing

Post wounding days	Wound Area (mm^2)	
	Control (Group I)	Treatment (Group II)
0	343.54 ± 16.20 (0)	341.25 ± 17.66 (0)
2	276.32 ± 5.30 (19.56)	253.85 ± 5.46 (25.61)
4	255.45 ± 4.92 (25.64)	236.14 ± 3.01 (30.80)
6	235.76 ± 2.72 (31.37)	197.47 ± 2.85 (42.13)
8	199.46 ± 2.59 (41.93)	158.68 ± 2.41 (53.50)
10	187.68 ± 2.53 (45.36)	135.52 ± 2.73 (60.28)
12	174.35 ± 3.15 (49.24)	96.39 ± 3.39 (71.75)
14	162.53 ± 3.12 (52.68)	67.75 ± 1.75 (80.14)

Value are mean ± SEM of six animals ($n = 6$) in each group. Number in parenthesis indicates percentage of wound contraction

DISCUSSION

The greater part of the world's population relies on traditional medicine for their health care. This is also the case in the treatment of wounds. In developing countries, formulations prepared from plants have been widely used for the treatment of soft tissue wounds and burns by medical personnel trained in western medicine as well as by traditional practitioners.

Skin wound healing is a dynamic response to injury that results in wound contraction, wound closure and restoration of the functional barrier.[14] It has three overlapping phases: inflammation, granulation tissue formation and remodeling. During the wound healing process, especially the transition from granulation tissue to scar tissue formation, collagen remodeling occurs, which involves the degradation of collagen with the formation of larger collagen bundles and an increase in the number of intermolecular cross-linkages. This process is controlled by matrix metalloproteinases. They are proteolytic enzymes discharged by fibroblasts, macroghages, epidermal cells and endothelial cells. The tensile strength of a wound can be related to its collagen formation and maturation. On the other hand, the strength of the repaired wound tissue is the result of the remodeling of collagen and the formation of stable intra-and inter-molecular cross-linkages.[15]

This study examined the effects of Aloe vera gel on the healing of skin excisional wounds in rats. Aloe vera may have a direct effect on the wound healing process as a whole, which is manifested as an increase in rate of contraction of wound area. Subramanian *et al.* also confirmed the effect of Aloe vera on increasing wound contraction and collagen synthesis and attributed this to the mannose-6-phosphate known to be present in Aloe vera leaf gel.[16] Mannose-containing products have been shown to increase macrophage activity and therefore stimulate fibroblast activity and collagen synthesis.[17,18]

The prepared gel has promising effect on the wound healing process. Further studies need to be performed to understand the exact mechanism of wound healing by Aloe vera gel.

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