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EVALUATION OF THE WOUND HEALING ACTIVITY OF A NOVEL ALOE HYDROGEL IN ALBINO RATS

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ABSTRACT

This paper describes a methodology to produce films composed of carbopol and Aloe vera gel were prepared and characterized for application in the treatment of several types of wounds. The *in -vivo* study of aloe hydrogel was performed on albino rats using excision wound model and it was found that the formulation has heal the wound in 16 days which was almost equal to standard ointment. The present research work accomplished the combination of anti-bacterial therapy and a healing promoting moisturization. It involved formulation of a new topical herbal hydrogel allowing for both antiseptic and moist wound healing for treatment and it also provide cooling sensation. Results shows that positive influence of *Aloe vera* on the transparency of the films, in both dry and wet state. Films were immersed in acetate buffer at pH 5.5 simulating the value of the skin, to evaluate its water absorption capacity. It was found that water absorption increases as the *Aloe vera* content increases, suggesting that *Aloe vera* enhances the hydrophilic properties of the films. Aloe Hydrogel was found to have better and faster wound healing effect than standard drug Povidone Iodine ointment on excision wound model.

KEYWORDS Wound healing, Excision wound, Hydrogel & Wound closure.

INTRODUCTION

Wound is the disruption of cellular and anatomic and functional continuity of living tissue, produced by physical, chemical, electrical or microbial insults to the tissue.[1] Significant fluid loss and extensive tissue damage, resulting from deep wounds, impair multiple vital functions performed by skin.[2] Wound infection, which further increases the local tissue damage, is a common complication[3], while systemic inflammatory and immunological responses might lead to a higher predisposition to life threatening sepsis and multi-organ

failure.[4] In such cases, early and appropriate clinical treatments are fundamental to reduce the mortality rates associated to the injury.[5] Based on the nature and repair process of wounds, they can be classified as chronic wounds and acute wounds. Acute wounds are tissue injuries that heal within 8-12 weeks. The primary causes of acute wounds are mechanical injuries (friction contact between skin and hard surfaces), burns and chemical injuries. In the case of burns, the temperature of the source and time of exposure is important to decide the degree of wound. Burn wounds need normally specialist care because of associated trauma. Healing is a complex and intricate process initiated in response to an injury that restores the function and integrity of damaged tissues.[6]

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases.[7] Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhorea, scabies, venereal disease, ulcers, snake bite, etc.[8] More than 80% of the world's population still depends upon traditional medicines for various skin.[9] Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process.[10]

In the present work we have formulated a herbal hydrogel intended for wound treatment. Hydrogels are hydrophilic polymeric network of three dimensional cross linked structures that absorb substantial amount of water. Cross linking facilitates insolubility in water because of ionic interaction and hydrogen bonding. It also provides required mechanical strength and physical integrity to the hydrogels. Thus, hydrogels can imbibe water nearly 10-20 times its molecular weight and hence become swollen.[11] General benefits include biocompatibility, can be injected, easy to modify, timed release of growth factors and other nutrients to ensure proper tissue growth, entrapment of microbial cells within polyurethane hydrogel beads with the advantage of low toxicity, environmentally sensitive hydrogels have the ability to sense changes of pH, temperature or the concentration of metabolite and release their load as result of such a change, and natural hydrogel materials are being investigated for tissue engineering, which include agarose, methylcellulose, hylaronan, and other naturally derived polymers.[12]

MATERIAL AND METHOD

Procurement and authentication of Plant: The Plant was procured from Botanical Garden NRI College Bhopal (M.P.) and all other chemicals are of LR Grade.

Preliminary Phytochemical analysis

A preliminary phytochemical screening was carried out for the extract employing the standard procedure to reveal the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, glycosides, carbohydrates, phytosterols and proteins.

Preparation of Aloe Vera Hydrogel

Carbopol has very good dispersion ability and forms gels rapidly. In brief, the gels (0.2, 0.5 and 1% w/w, respectively) were prepared. Carbopol resin (weight in grams) was dispersed in distilled water (volume according to the desired concentration of gel), then add sodium carboxymethyl cellulose then Aloe vera gel was added into it & add preservatives. The mixture was stirred until thickening occurred and then neutralized by drop-wise addition of 50% (w/w) triethanolamine, until a transparent Hydrogel appeared. Quantity of triethanolamine was adjusted to achieve gel with desired pH. Gels were stored for 24 hours at the room temperature to stabilize.Composition of Aloe hydrogel is mentioned in the Table No.2.

EVALUATION OF HYDROGEL

Appearance

The formulated hydrogels were observed for their visual appearance, colour, texture,feel upon application such as grittiness, greasiness, stickiness, smoothness, stiffness and tackiness.

pH

The pH of the hydrogel was determined by immersing electrode to a depth of 0.5 cm in a beaker containing hydrogel. The determinations were carried out in triplicate and the average of three reading was recorded.

Spreadability

It was determined by wooden block and glass slide apparatus. About 20g of formulated hydrogel were placed to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated by using the formula:

S = M.L / T

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other

Unit = g.cm/sec

Viscosity

The viscosity of formulated hydrogel was determined using Brookfield viscometer (spindle number LV-61) in triplicate and the average of three reading was recorded.

Viscosity = Dial reading x Spindle factor

Smoothness

The 5ml gel formulation taken in the 10ml test tube and visually checked for its transparency. The smoothness of the gel formulation was tested by rubbing between the fingers and observes whether the gel is smooth, clumped, homogenous or rough.

Percentage moisture loss

Percentage moisture loss from the formulations were determined. Two gram formulations was weighed accurately and kept in a desiccators containing 50gm anhydrous calcium chloride. After the formula as follow.

$$\% \text{ Moisture loss} = \frac{\text{Initial concentration- Final concentration}}{\text{Final weight}} \times 100$$

STABILITY TESTING OF HYDROGEL

After storage of Hydrogel at 40 °C, it was observed that Hydrogel are not stable at higher temperature. The gels precipitated down and on the top of the gel we could observe the gel "sweating" phenomena.The testing was performed at 40 °C for 1 month period. In regard to stability of aloe vera Hydrogel, the research literature shows that the stability depends on the type of gelling polymer and the composition of gel used. Some hydrophilic polymers like for example carboxymethylcellulose are not affected by the incorporation of aloe gel in Hydrogel.

IN – VIVO EVALUATION FOR WOUND HEALING ACTIVITY

Animals

Adult albino rats (wistar strain) of either sex weighing 180±200g were taken. They were placed in polypropylene cages in a controlled room environment (25°C±2°C) at a natural day

night cycle and they were provided with standard laboratory food and water . Approval for the study was obtained from Institutional Animal Ethical Committee (IAEC), Reg No. 1238/PO/Re/S/08/CPCSEA

To perform the experiment, the rats were divided into three groups consisting of six animals each.

Group I - CONTROL

Group II - TEST

Group III - STANDARD [Standard Povidone-Iodine (5% w/w) Ointment]

EXCISION WOUND MODEL[13]

For the excision wound study, animals were selected circular wound of about 2.5 cm diameter were made depilated dorsal thoracic region of rats were deplicated under light ether anaesthesia in semi-aseptic condition and observed throughout the study. Individually rats were kept in housed. The different groups were divided and treated with hydrogels systems on the wound once daily for 14 days starting from the day of wounding. The observations of percentage wound closures shall be made on 4th, 8th, 12th and 16th post wounding days and also epithelization, size and shape of scar noted. All the samples were applied once daily for 16 days, starting from the day of wounding and evaluated for the following parameter and results are mentioned in Table No.4

Wound contraction And Epithelization time: - Wound contraction was measured using a transparent paper at four days interval.

Calculated by the formula mentioned below:

$$\% \text{ CLOSURE} = \frac{\text{Wound area on corresponding day} - \text{Wound area on day zero}}{\text{Wound area on day zero}} \times 100$$

INCISION WOUND MODEL[14]

Under light ether anaesthesia, two para-vertebral incisions of 6 cm were made on either side of the vertebral column through the entire thickness of the skin with the help of sharp blade and the mean Percentage closure of excision wound area and the Period of epithelization was mentioned in Table No.4

RESULTS AND DISCUSSION

The preformulation studies were performed to develop a hydrogel as a dosage form containing Aloe vera as a potential dermatological formulation. The prepared formulation presented physicochemical properties compatible on its coetaneous administration, including the pH. The gel was isolated from the Aloe vera Leaves, and then it was subjected to various phytochemical analysis of its components so it was found that Aloe vera is rich in Glycosides, tannin, flavanoids, carbohydrates and steroids this was mentioned in Table No. 1.

TABLE NO. 1. Identification test of aloe gel

Test	Aq. Extract
For carbohydrates:- Molish test Benedict test	 (+) (+)
For Tannins **(**$FeCl_3$)	(+)
For Steroids (Salkowski Libermann–Bucher)	(+)
For Flavonoids Shinoda Test Hydrochloride Reduction Test	 (+) (+)
For Glycosides Baljet Test Legal Test Killer-Killani Test	 (+) (+) (+)
Lipid Test	(-)

Hydrogels were prepared by keeping the Carbopol: Sodium carboxy methyl cellulose polymer ratio (2:1) and hydrogel was formulated by using different composition of various ingredients was mentioned in Table 2.

Table No .2 Composition of Aloe Vera Hydrogel

Ingredients	Quantity
Carbopol	300mg
Sodium carboxy methyl cellulose	200mg
Propylene glycol	2ml
Aloe vera	5ml
Methyl paraben	100mg
Propyl paraben	100mg
Distilled water	50ml

The hydrogel characterized by its appearance, pH, viscosity, spreadability & %entrapment (Table 3) On the basis of results of spreadability and % entrapment studies.

Table No. 3: Various Evaluation Parameters

Evaluation parameters	Formualtion
Appearance	Transparent & translucent
pH	5.7
Viscosity	4200
%moisture entrappment	97.2%
Spreadability	5.1 g.cm/sec

Table 4: Mean Percentage closure of Excision wound area by Control, formulation and standard

	Mean Percentage closure of Excision wound area					Period of epithelization
Group						
	Initial	4thday	8thday	12thday	16thday	
Control	500	395.45	315.14	176.10	124.30	23 Days
Test	480.65	420.25	365.52	190.21	92.62	16 days
Standard	480.65	420.76	360.10	188.18	90.33	11Days

CONCLUSION

Wound healing activity of marketed formulation of Povidone- Iodine ointment and new herbal Aloe vera hydrogel was evaluated. It was found that the herbal Aloe vera hydrogel also showed the wound healing activity when applied on rats within 16 days the wounds heals and marketed formulation of Povidone –Iodine ointment applied on rats it heals within 11days. Hence,the herbal aloe vera hydrogel formulated is of much advantage when compared to the marketed prepration. Further studies will enhance its Healing properties in future research by encorporating Herbs in combination with others to better the healing action without itching burning sensation,. This Herbal Aloe vera also has cooling properties so it can also applies to normal itching, burning conditions.

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