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FORMULATION DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF HERBAL HYDROGELS FOR DERMAL WOUND HEALING

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ABSTRACT

In the present study two medicinal plants *Centella asiatica* (Umbelliferae) and *Aloe vera* (Liliaceae) reported to have significant wound healing potential were selected to be formulated as herbal hydrogels and were investigated for their wound healing activity. The main objective of the study was design and development of novel herbal hydrogels for wound repair. The dried finely ground herb *centella asiatica* was extracted in ethanol by successive solvent extraction process and the ethanolic extract was screened for its phytochemical constituents and gave positive report for the presence of Carbohydrates, Flavonoids, Alkaloids and Gums. *Aloe vera* leaves are collected and further processed to obtain Aloe vera gel. A Preformulation study was performed prior to the development of herbal hydrogels formulation. The Preformulation studies revealed that the herbal drug and selected polymers were compatible. Optimized formula was designed and further three formulations were prepared namely HHF-1, HHF-2 and HHF-3 respectively with polyvinyl pyrolidone K-30 and glutaraldehyde as cross linking agent. In-vitro characterization like P^H, Viscosity, Water absorption capacity, Skin irritation test was done for all the three formulations. From the three formulations HHF-2 formulation is having acceptable PH, excellent Viscosity -41991 Cps at 70% torque. Fair Water absorption capacity, possess antimicrobial activity against both *Staphylococcus aureus* and *E.coli* microorganisms as compared to standard and control, non-skin irritant and good Wound Healer.

KEYWORDS: HHF-Herbal hydrogel formulation, CPS-centipoises, FT-IR-Fourier transform infra Red spectrophotometer, PVP-Polyvinyl pyrolidone, CMC-Carboxy methyl Cellulose, RPM-Rotations per minute.

INTRODUCTION

A wound is defined as a defect or break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition. Based on the nature and repair process of wounds, they can be classified as chronic wounds and acute wounds (Boateng et al., 2008). 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 (Boateng et al., 2008). The wound healing process is the dynamic process take place by regeneration or repair of broken tissue. In these stages will both cellular and matrix compounds work to reestablish the integrity of damaged tissue and replacement of lost tissue. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing. Inflammatory cells also arrive along with the platelets at the site of injury providing key signals known as cytokines or growth factors. The fibroblast is the connective tissue responsible for collagen deposition that is needed to repair the tissue injury. In the present work herbal hydrogel formulations were prepared to accelerate wound healing process. Literature survey revealed that *Centella asiatica* has the antimicrobial, antioxidant activity, wound healing property and increase collagen synthesis and tensile strength. *Aloe vera* has the anti-inflammatory activity increase degree of cross-linking. *Aloe* also provides moisturizing activity. In the present study for easy application on wound hydrogels were prepared and evaluated for its *Invitro* characteristics along with their feasibility was checked by wound healing activity in wistar albino rats as compared to nitrofurazone cream.

MATERIALS AND METHODS

Preparation of *centella asiatica* extract

The leaves of *Centella asiatica* (L.) were collected from Nalla mala Hills of Srisailam, Kurnool dist, A.P and Aloe vera was collected from our college medicinal garden and authenticated from Leaf cleantech private limited, Ganesh, # 16, Krishna murthy layout, sanjaynagar, Bangalore, India. About 50gms of powdered leaves of *Centella asiatica* was

extracted with 400 ml of ethanol using Soxhlet apparatus for 8 hrs. The extract was concentrated to ¼ of its original volume by distillation as it was adapted to recover the solvent, which could be used again for extraction. The extract was evaporated to dryness at low temperature.

Preliminary phytochemical screening

The plant extract was subjected for phytochemical screening of Alkaloids, Glycosides, Phenolic compounds and Tannins, Saponins, Phytosterols, Proteins and amino acids, Carbohydrates, Gums and mucilages, and Flavanoids.

Preparation of *aloe vera* gel

The fully expanded leaves of *Aloe vera* were selected from the plants, washed with distilled water and were subjected to surface sterilization with 70% ethyl alcohol followed by 0.1% HgCl2. The parenchymatous covering of the leaves were peeled and the gel drained out. Slurry was formed with the help of pestle and mortar. It was sterilized by autoclave and further stored in a airtight container and placed in a refrigerator for further work.

Preformulation studies

Preformulation studies for the *Centella asiatica* extract, *Aloe vera* gel, PVP-K30, Sodium carboxy methyl cellulose and HHFormulation-1. Includes test for identification which consist of Physical appearance, Drug Excipient compatibility studies includes storing the extracts-excipients at 50^{o}C temperature for a period of 30 days, then it was subjected for compatibility study by Perkin Elmer FT-IR Spectrophotometer, Shelton, USA by potassium bromide (KBr) press pellet technique.

Formulation of herbal hydrogels

Three different formulations varying in their polymeric concentrations (PVP: Na CMC) namely HHF-1, HHF-2, HHF-3 was prepared by chemical crosslinking technique.

In-Vitro evaluation tests: The prepared herbal hydrogels formulations was subjected for its essential and significant in-vitro evaluation parameters which includes physical appearance, P^{H}, Water absorption capacity, viscosity, skin irritation test, antimicrobial studies and wound healing potential.

The physical appearance of the prepared hydrogels was performed for their colour, thickness, texture. P^H of the hydrogels was studied using calibrated P^H-meter. The degree of swelling can be described as water absorptivity of the hydrogels was measured gravimetrically by using the following equation (1)

$$\text{Absorption (\%)} = (Ws - Wd) \div Wd \times 100$$

Where, Ws and W_d are weights of swollen gel and dried gel respectively.

The viscosity of the three formulations HHF-1, HHF-2 and HHF-3 respectively was determined by Brookfield Viscometer LVDV-II+PRO. With conditions like 0.01 RPM, at temperature 29.5°C and at different % Torque

Primary skin irritation test was performed on a healthy rabbit under Institutional animal ethical committee reference no: 842/07/ac/CPCSEA weighing between 1.5 to 2.0 kg. The test was conducted on unabraided skin of rabbit. The control simple hydrogel was placed on the left dorsal surface of the rabbit; whereas the test hydrogel formulation was placed on the right dorsal surface of the rabbit. Before placing the gels, the unabraided skin was cleaned with rectified spirit. The gels were removed after 24 hours and the skin was examined for erythema or oedema.

The antimicrobial activity of HHF-2 Formulation was assessed by agar disc diffusion method. The zone of inhibition was measured in nutrient agar media, employing *S.aureus & E.coli* as test organisms. All the values are measured in triplicate.

The herbal hydrogel formulation HHF-2 was investigated for its Wound healing activity. This was studied using excision wound model as described by Mukherjee P K.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The alcoholic extract showed the presence of carbohydrates, glycosides, alkaloids and flavanoids. The details of qualitative chemical tests and phyto constituents present in the extract are shown in Table-1.

Preformulation studies

Physical appearance of *Centella asiatica* gives – Greenish in colour with gummy mass.

Aloe vera gel -- Colorless with sticky in nature.

The results of compatibility studies were shown in Fig-1 to 5.

Formulation of herbal hydrogels

Three different formulations HHF-1, HHF-2, HHF-3 were prepared by chemical crosslinking technique. The optimized formula was tabulated in Table-2.

In-vitro evaluation tests

The physical appearance of the prepared hydrogels was performed for their colour, thickness, texture. The PVP hydrogels are pale yellow, transparent and soft gel like, moreover, no noticeable differences was observed at before dry state. The thickness was increased from formulation 1 to formulation 2 (1.862 to 3.244) this indicates that the concentration of polymer increases with the increase in thickness. The physical appearance was shown in Fig: 6.

The p^H of the hydrogels formulation namely HHF-1, HHF-2 & HHF-3 was found to be 6.8, 6.7 and 6.8 respectively. This is near to the skin P^H. Hence HHF-2 is considered as ideal formulation for the treatment of skin disorders.

Formulated herbal hydrogels HHF-1 to HHF-3 has a good water absorption capacity it retain the moisture on effected area. The values are listed in Table-3.

Among all the formulations, HHF-2 formulations possess 41991cps viscosity at 70.0% Torque. The values are listed in Table-4. Hence it was considered as suitable formulation for wound dressing.

No skin irritation and oedema was noticed after placing the hydrogel formulation on to the surface of the skin of rabbits for a period of 24 hrs.

Antimicrobial investigation of selected HHF-2 formulations reveals that it has antimicrobial property against the S. aureus and E. coli. The results are shown in Table-5.

The results of wound healing activity of HHF-2 Formulation by excision wound model were presented in Table-6&7 and Fig-7 and 8. It was found that the test formulation shows period of epithelization within 16 days that is closer to the standard period of epithelization days.

CONCLUSION

The main objective of the study was design and development of novel herbal hydrogels for wound repair. We have selected two potent natural plants having wound healing property, soon after the collection of plant *Centella asiatica* it was subjected for the successive solvent extraction process and collected concentrated ethanolic extract of *centella asiatica*. Whereas, on other side Aloe vera leaves are collected and further processed to obtain Aloe vera gel. Phytochemical screening was carried out on *centella asiatica* extract and we obtained positive results for Carbohydrates, Flavanoids, Alkaloids and Gums. Then we attempted to carry out the Preformulation studies prior to the development of herbal hydrogel formulation. The Preformulation studies revealed that the herbal drug and selected polymers were compatible. Optimized formula was designed and further three formulations were prepared namely HHF-1, HHF-2 and HHF-3 respectively. *In-vitro* characterization like p^H, Viscosity, Water absorption capacity, Skin irritation test was done for all the three formulations. p^H for all formulations was obtained and the values are closer to the skin P^H. The water absorption capacity of three formulations states that, as the concentration of one of the polymer increase the water absorption capacity increase with respect to time. Due to increase in the percentage of water absorption capacity it was concluded that all the formulations having good moisture retaining ability. Whereas viscosity of all the formulation was carried out using Brookfield viscometer the formulation HHF-2 obtain satisfactory viscosity value i.e. 41991 Cps at 70% torque. The Skin irritation test was done by one of the herbal hydrogel formulation and the results are found that there was no noticeable erythema and oedema even after prolonged exposure of formulation on to the rabbit skin. After conducting some of the in-vitro parameters we have selected optimized formulation i,e HHF-2 and it was subjected to carry out antimicrobial activity and wound healing studies. Antimicrobial study reveals that the formulation HHF-2 shown good antimicrobial activity against both *Staphylococcus aureus* and *E.coli* microorganisms as compared to standard and control. The result was analyzed based on the zone of inhibition. The wound healing activity of HHF-2 showed prominent results with respect to standard and control. Finally our formulation HHF-2 is having acceptable PH, Viscosity, Water absorption capacity, Antimicrobial activity, non-skin irritant and good Wound Healer.

List of tables

Table 1: Qualitative chemical examination of ethanolic extract of *centella asiatica* leaves.

Sl. no.	Tests	Alcoholic extract
1	Alkaloids	
	• Mayer's test	+
	• Wagner's test	+
2	Glycosides	
	• Modified. Brontrager's test	+
	• Baljet test	+
3	Saponins	
	• Froth test	-
4	Phytosterols	
	• Libermann-burchard's test	-
5	Phenolics and tannins	
	• Ferric chloride test	-
	• Gelatin test	-
6	Proteins and amino acids	
	• Ninhydrin test	-
7	Fixed oils and fats	
	• Stain test	-
8	Carbohydrates	
	• Molish's test	+
	• Benedict's test	+
	• Fehling's test	+
	• Barfoed's test	+
9	Gums and mucilage	
	• Molish's test	+
10	Flavanoids	
	• Alkaline reagent test	+

(+) – Signifies present **(-)** – Signifies absent

Table 2: Composition of herbal hydrogels.

S. no.	Ingredients	Hhf-1	Hhf-2	Hhf-3
01	Polyvinyl pyrrolidone (pvp-k30)	1 g	1 g	1 g
02	Na cmc	2 g	4 g	6 g
03	*Centella asiatica* extract	500 mg	500 mg	500 mg
04	*Aloe vera* gel	500 mg	500 mg	500 mg
05	Glutarldehyde	2 ml	4 ml	6 ml
06	Triethanolamine	Q.s to neutralize the gel base	Q.s to neutralize the gel base	Q.s to neutralize the gel base
07	Glycerin	1 ml	2 ml	3ml
08	Methyl parabene	0.02g	0.02g	0.02g
09	Na benzoate	0.03g	0.03g	0.03g
10	Purified water	60 ml	80ml	140ml

Table 3: Water absorption capacity (%) of different formulations.

S. no.	Time (min)	Water absorption (%) of HHF-1	Water absorption (%) of HHF-2	Water absorption (%) of HHF-3
01	5	31.32%	33.36%	37.25%
02	10	40.12%	42.84%	44.23%
03	15	46.24%	49.26%	51.26%
04	20	52.42%	54.23%	55.62%
05	30	62.46%	64.21%	66.41%

Table 4: Viscosity of three hydrogel formulations.

Sample no.	Code of the sample	RPM	Temperature (˚C)	% Torque	Viscosity (cps)
01	HHF-1	0.01	29.8	25.3%	15177
02	HHF-2	0.01	29.7	70.0%	41991
03	HHF-3	0.01	30.1	70.0%	42111

Table 5: Antimicrobial activity of HHF-2.

Test Organisms	Zone of inhibition (mm)		
	Simple hydrogel (Control)	Nitrofurazone cream (Standard)	Test formulation (HHF-2)
Staphylococcus aureus	4±0.01	20±0.8	17±0.6
E.coli	3±0.9	20±0.6	16±0.5

Table 6: The effect of formulation on excision wound healing in rats.

Time (days)	Wound contraction (%)		
	Simple hydrogel (control)	Nitrofurazone cream (standard)	Test formulation (hhf-2)
4	2.12±0.499	5.546±0.488**	0.13±0.033
8	5.991±0.933	24.966±0.973**	7.460±0.5141*
12	12.291±0.808	33.353±0.888**	18.466±0.5141**
16	24.121±0.025	94.96±1.502**	49.883±0.994**

*Significant difference between treatment groups and the control group ($P<0.05$), ** ($P<0.01$).

Table 7: Comparison of period of epithelization.

Groups (n)	Period of Epithelization (Days)
Simple Hydrogel (Control)	22.27±0.67
Nitrofurazone Cream (Standard)	18.66±0.70*
Test Formulation (HHF-2)	16.88±0.68*

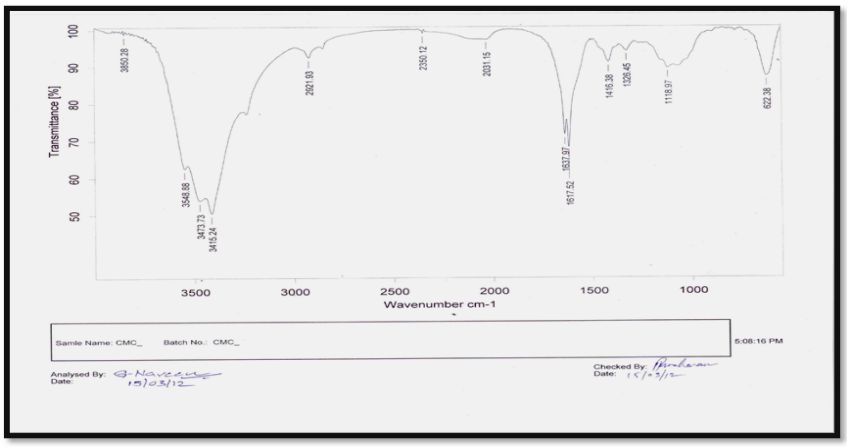

Fig: 1 FT-IR Spectrum of Na CMC.

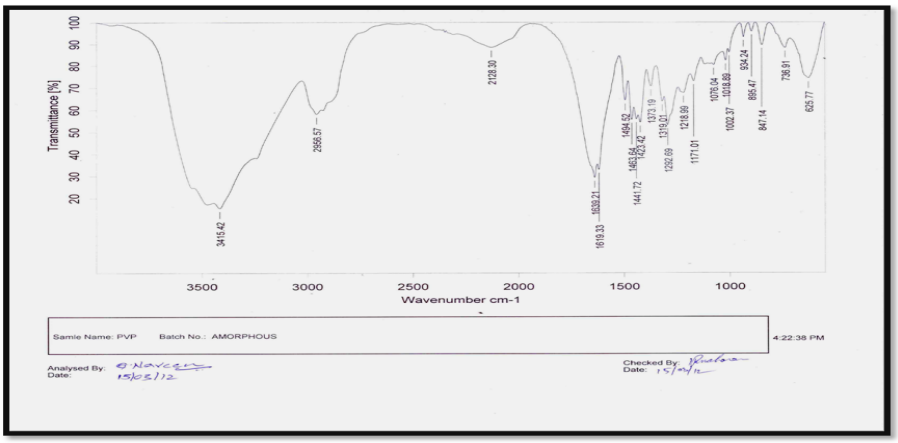

Fig. 2: FT-IR Spectrum of PVP K-30.

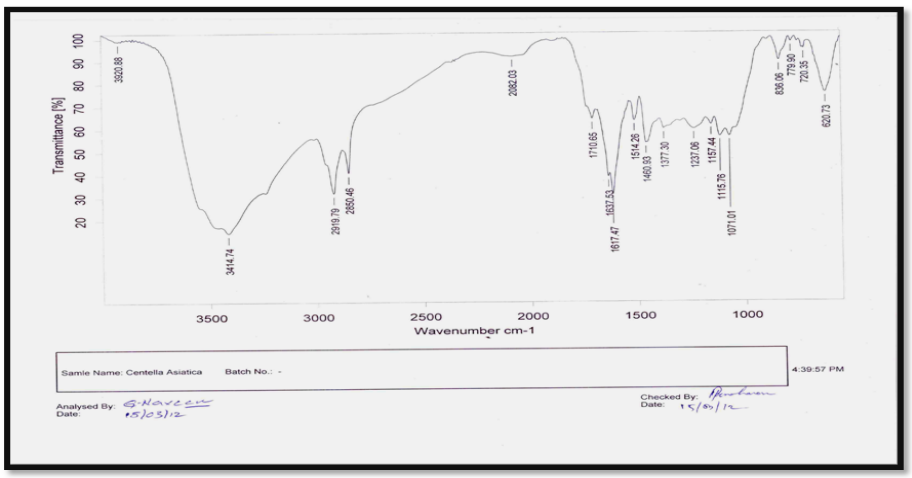

Fig. 3: FT-IR Spectrum of centella asiatica.

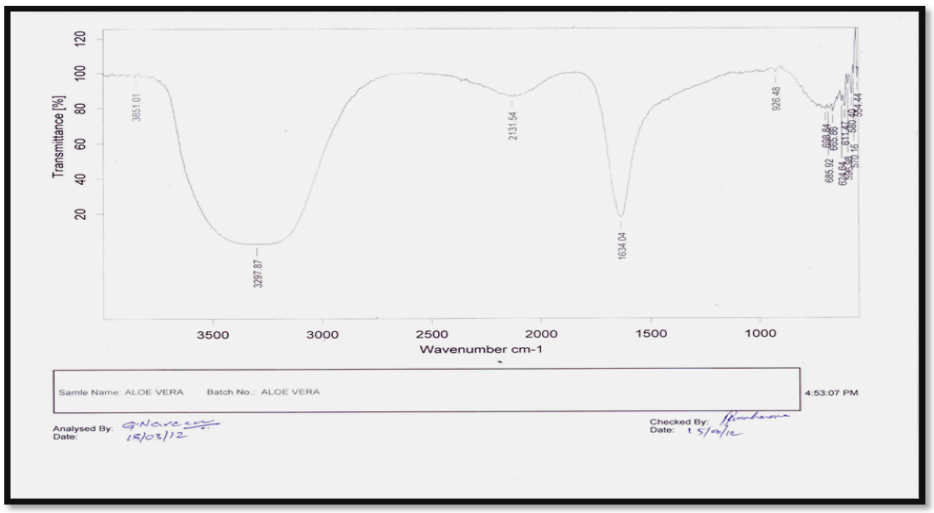

Fig. 4: FT-IR Spectrum of Aloe vera.

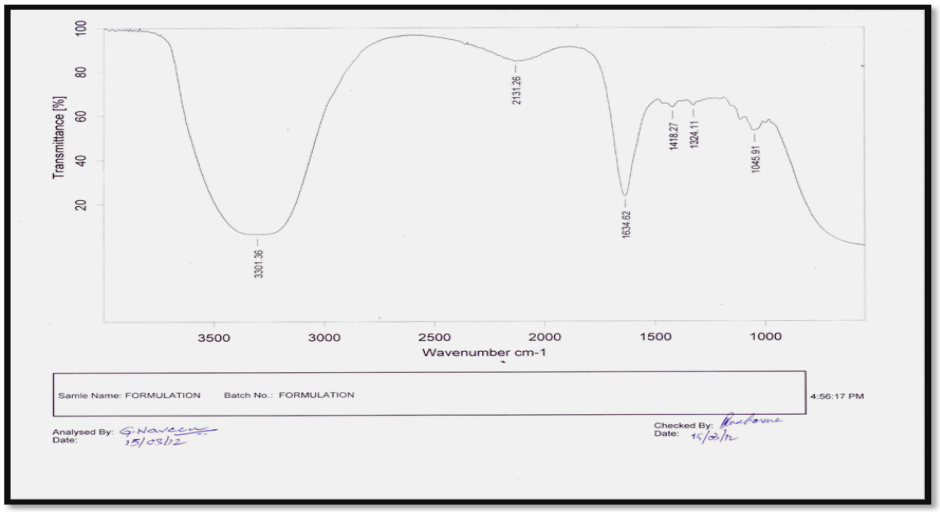

Fig 5: FT-IR Spectrum of herbal hydrogel (HHF-1).

Fig. 6: Photographic image of prepared herbal hydrogel film.

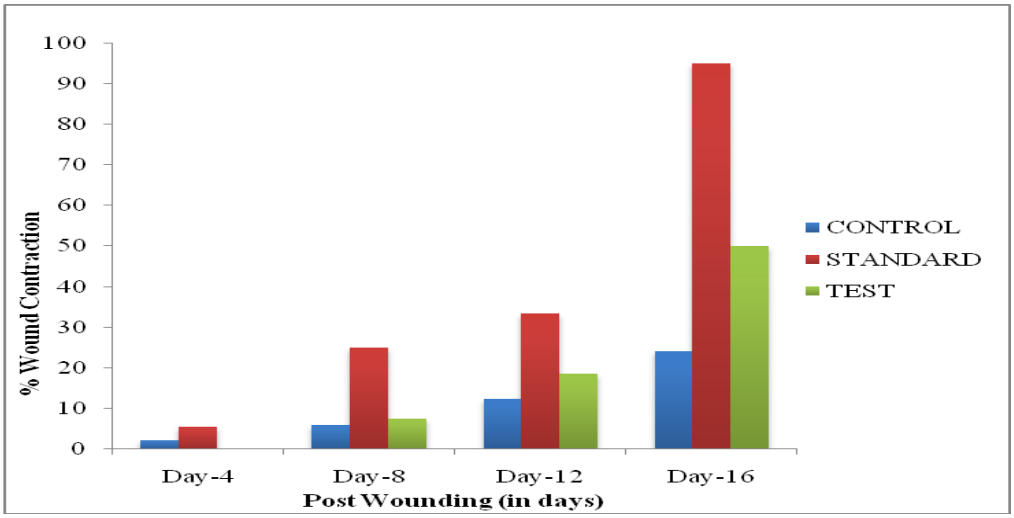

Fig. 7: Comparative Graphical representation of Control, Standard and Test formulation on wound healing.

Fig. A)

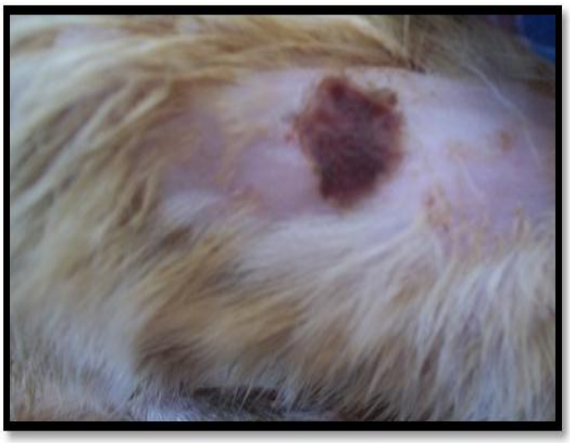

Fig. B)

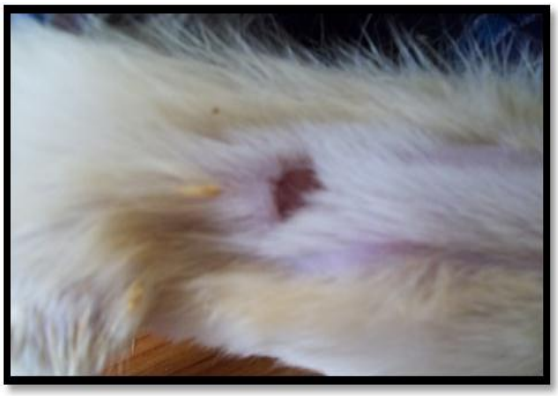

Fig. D)

Fig. C)

Fig. 8: Wound healing activity of Herbal hydrogel formulation HHF-2 on A) Zero post wounding day, B) Eighth post wounding day C) Twelveth post wounding day D) Sixteenth post wounding day.

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