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Herbal Wound Healing Cream for Chronic Wounds in Wildlife

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Abstract
Traditional ethnoveterinary medicine has developed over generations within various parts of world even before the era of modern medicine. Modern day problems of emergence of multidrug resistant organisms and a decrease in newer antibiotics has been also a primary factor for Veterinarians to revisit the ancient healing methods by using traditional and alternative medicine in wound management. Several studies using herbal and traditional medicine from different countries have been documented in wound care management in animals.

Keywords: Wound healing, Medicinal plants, Animal care

Introduction

Herbal plants have been found to contain numerous pharmacologically-active ingredients and every herb may have its own unique combination and properties for its applicability for medicinal purpose. There are many herbs which contain ingredients with pharmacological actions which include anthelmintic, anti-catarrhal, anti-emetic, anti-inflammatory, antilithic, antibacterial, antifungal, antispasmodic, laxative, astringent, bitter, purgative, diuretic, ecbolic, emollient, expectorant, galactagogue, hepatic, styptic etc. However, a particular herbal plant may contain only one or some of these actions in varying degree of combination. It is the decision of physician whether a single or combination of some herbs is to be used for treatment which depends on the spread of activity of each herb and whether or not it supplies the necessary spectrum of action in the body of the animal.

Wounds are found to have originated as a result of the physical injury which leads to breach in continuity of the skin. However, an appropriate method for healing of the wound is very much essential for the restoration of the disrupted anatomical continuity and disturbed functional status of the skin to arrive at appropriate time (Meenakshi *et al*., 2006) because wound is one of the major obstacles to the establishment of infections by bacterial pathogens in the internal tissues which can have fatal consequences (Giacomette *et al*., 2000). Wound healing phenomenon has been found to involve a complex series of interaction between different cell types, cytokine mediators and the extracellular matrix. The different phases of normal wound healing include haemostasis, inflammation, proliferation and remodeling with one stage intermingling with other in different types of wounds (Douglas and Alan, 2003).

The problem in wound healing continues to cause significant morbidity and mortality despite of having many recent advances in understanding its basic principles (Peacock and Cohen, 1990). Veterinary practitioners often encounter animals with traumatic wounds that are infected, too large to close immediately or both. The management of wounds depends on the stage of wound healing and can include irrigation, mechanical and chemical debridement, use of antiseptics and antimicrobials, adherent and non- adherent dressings, and miscellaneous topical applications. Extensive research has been carried out in this field and many topically applied agents have been used to treat open wounds, but most products investigated in domestic animals either do not affect wound healing or inhibit rather than enhance it (Swaim *et al*., 1993). Therefore, products selected to create a healing environment must be chosen thoughtfully and scientific rationale must support their use. In rural areas of developing countries wounds and dermatological conditions constitute one of the five most common reasons for people seeking medical care (Ryan, 1992).

Several studies using herbal and traditional medicine from different countries have been documented in wound care management in animals. It can be concluded that scientific evidences and clinical trials conducted using traditional and alternative medicine in wound therapy for animals holds good promise in the future.

Thus, the herbal wound healing cream is the much-needed essential ingredient for wildlife. If the present modern multi-drug is economically replaced with herbs, it will be a game changer.

Materials And Methods

Sample collection

Fresh leaves of *Cassia tora, Cassia alata, Lantana camera* and *Hemigraphis alternata* were collected from State Forest Research Institute, Kolapakkam. After authentication, the leaves were shade dried (25°C) for a week, pulverized separately in a mechanical grinder, passed through a 40-mesh sieve and stored in well closed container till further use.

Preparation of extraction

The dry leaves were pulverized mechanically and 100 g of the powder was weighed and extraction was done by cold maceration in different solvents such as hexane, chloroform, ethyl acetate, petroleum ether, ethanol and methanol for 72 hours. The macerate was filtered and the extract was concentrated using a rotary evaporator to obtain a viscous solid. This was then left in a dark place to evaporate the remaining solvents.

Preliminary phytochemical analysis

Preliminary analysis was carried out on the plant extract to identify the useful constituents like alkaloids, flavonoids, saponins, tannins, phenols and terpenoids using standard methods.

Formulation and preparation of cream

The required quantity of plant extract of different concentration was mixed to the above mixture. All the ingredients were mixed properly and with continuous stirring. The same method was followed for the preparation of control sample that is cream without drug. Prepared cream was filled in collapsible container and stored at a cool and dry place. Physical parameters such as colour, appearance, and feeling on application were recorded.

Table 1: Formulation of cream

Sl. No	Ingredients	Concentration of solvents extracts				
		F1	F2	F3	F4	Control
1.	Bees wax	3.2 g	3.2 g	3.2 g	3.2 g	3.2 g
2.	Borax	0.16 g	0.16 g	0.16 g	0.16 g	0.16 g
3.	Methyl Paraben	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g
4.	Liquid Paraffin	10 g	10 g	10 g	10 g	10 g
5.	Crude Drug	75 mg	100 mg	150 mg	200 mg	-

Evaluation of topical cream formulation

Physical parameters such as color, appearance, homogeneity and grittiness were inspected through visual inspection.

Measurement of pH

The pH of the cream was measured by using pH meter. One gram of cream was dissolved in 100 ml distilled water and stored for two hours. The measurement of each formulation was done in triplicate and average values are calculated.

Spreadability

The cream was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. 1 kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the cream between the slides. Excess of the cream was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability.

Spreadability was calculated using the following formula:

$$S = M \times L / T \quad (1)$$

Where, S = Spreadability, M = weight in the pan (tied to the upper slide), L = length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Antimicrobial activity for the herbal drug

The antimicrobial activity of different concentration of herbal drug and formulated creams were carried out using the technique of zone of inhibition in disc method, employing *Escherichia coli* as test organism.

***In vivo* studies**

The study was conducted after obtaining approval from the institutional animal ethical committee. Wister albino rats of 200-250g were used in the study. The predetermined area for the wound infliction at the back of the animal for surgery is prepared by removing the hairs with depilatory cream. The animals were anesthetized using anesthetic ether by open mask method and placed on the operation table in its natural position.

Skin excision wounds of 1cm x 1cm was created using a punch biopsy needle, and a depth of about 1mm on the dorsal aspect of the thoracolumbar region of the rats, which include a standard group treated with marketed herbal wound healing gel, control group is treated with Carbopol base gel, the treatment group is treated with the optimized formulation. The animals are maintained under standard husbandry conditions and on a uniform diet and managed throughout the experimental period. Animals are closely observed for any infection; those who show signs of infection are separated and excluded from the study. They are periodically weighed before and after the experiments.

Excision wound model

Excision wounds are inflicted on the dorsal thoracic region 1–1.5 cm away from the vertebral column on either side and 5 cm away from the ear. An area of about 1 sq. cm was marked with a marker circularly on the shaven back of the animals. The marked area was excised with full thickness with surgical sterile blade and scissors. The respective therapeutic treatment is administered topically to the animals of respective groups until complete epithelialization starting from the day of operation. Collagen estimation, percentage wound contraction, and period of epithelialization parameters are studied.

Percentage wound contraction

The progressive reduction in the wound area is monitored planimetrically by tracing the raw wound boundaries and the wound area recorded is measured using a graph paper on every 4 days interval. The period of epithelialization is expressed as the number of days required for falling of the eschar (dead-tissue remnants) without any residual raw wound is considered as the end point of complete epithelialization.

$$\text{Percentage} = \frac{A.O - A.D}{A.O} \times 100$$

Where, A – O = wound area on day zero,
A – D = wound on corresponding days.
The number of days for complete closure was noted & the scar shape and area were traced and measured on complete closure.

Measurement of wound area

The dynamic change in the wound area was monitored by camera on fore ordained days (4, 8, 12, 16 and 20 days). Later in which, wound area was measured by tracing the wound on a millimeter scale graph paper.

Period of Epithelisation

Falling of scab deserting no raw wound behind was taken as end point of complete epithelisation and the days required for this was taken as period of epithelisation.

Measurement of wound index

Wound index was measured daily with an arbitrary scoring system i.e., “0” for complete healing, “1” for incomplete but healthy healing, “2” for delayed but healthy healing, “3” for healing has not yet been started but environment is healthy, “4” for formulation of pus evidence of necrosis.

Histopathological studies

The regenerated tissue previously collected and preserved in 10% buffered formalin was used for histopathological studies.

Preparation of histological studies

The tissue was removed from buffered formalin after a day or two, dehydrated in ascending grades of alcohol, cleared in chloroform, embedded in paraffin using tissue processor and cut with rotary microtone, getting sections of 3 to 5 micrometer in thickness. The section was dewaxed by xylene and the xylene was removed by descending grades of alcohol to facilitate staining procedure.

The Histopathological changes in the regenerated tissue, which took place during the wound contraction or healing phase in both the test, and control, was observed for "epithelisation, fibroblast, collagen, cell infiltration (inflammation) and neovascularization". These parameters were qualitatively assessed and micro photographed under "400X" magnification.

Results and Discussion

Extraction of plant material

The simple solvent extraction method was used for the preparation of the extract. The percentage yield of *Cassia tora, Cassia alata, Lantana camera* and *Hemigraphis alternate* in hexane and methanolic extract were found to be higher when compared to all other solvents such as chloroform and acetone (Table 3).

Table 3: Percentage yield of the solvent extracts

Sl. No	Name of the plant	Percentage yield (gm)			
		Hexane	Chloroform	Acetone	Methanol
1.	*Cassia tora*	10.1 gm	7.8 gm	5.6 gm	10.1 gm
2.	*Cassia alata*	8.5 gm	6.5 gm	4.3 gm	8.2 gm
3.	*Lantana camera*	8.01 gm	6.3 gm	4.5 gm	7.5 gm
4.	*Hemigraphis alternate*	12 gm	10 gm	8.2 gm	11.5 gm

Organoleptic properties

The organoleptic properties of the plant extracts are mentioned in the Table 4.

Table 4: Organoleptic properties of the plant extracts

Sl. No	Name of the plant	Description			Ethanolic extract
		Colour	Odour	Taste	
1.	*Cassia tora*	Greenish black	characteristic	Bitter	Light green
2.	*Cassia alata*	Greenish black	characteristic	Bitter	Light green
3.	*Lantana camera*	Greenish black	characteristic	Bitter	Light green
4.	*Hemigraphis alternate*	Purple black	characteristic	Bitter	Light purple

Preliminary phytochemical analysis

Preliminary phytochemical studies of *Cassia tora, cassia alata, Lantana camera* and *Hemigraphis alternate* reveal the presence of alkaloids, carbohydrates, cardiac glycosides, tannins, saponins, phenols

and flavonoids. The results obtained were summarized in the Table 5.

Table 5: Phytochemical analysis

Tests	*Cassia tora*				*Cassia alata*				*Lantana camera*				*Hemigraphis alter-nate*			
	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol
Carbohydrates	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+
Tannin	+	-	+	+	+	-	-	+	+	-	+	+	+	-	+	+
Saponin	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-
Flavanoids	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-
Quinones	+	-	+	+	+	+	+	+	-	-	+	-	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Cardia Glyco-sides	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-
Coumarin	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+
Steroids	-	+	+	-	-	+	+	-	-	+	+	-	+	+	+	+
Phytosteroids	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Phlabotannin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = *Present*; **-** = *Absent*

Evaluation of topical cream

Physical evaluation

The physical parameters such as appearance (Figure 1), homogeneity and grittiness were checked by visual examination and the results are given in the Table 6.

Table 6: Physical evaluation of prepared cream formulations

Sl. No	Formulations	Appearance	Homogeneity	Grittiness
1.	F1	Light green	Good	Nil
2.	F2	Light green	Good	Nil
3.	F3	Milky purple	Good	Nil
4.	F4	Green purple	Good	Nil
5.	Control	White	Good	Nil

Figure 1: Appearance of topical creams

Measurement of pH

The pH of various cream formulations was determined by using digital pH meter. All the formulations showed 6.5 to 6.7.

Determination of Spreadability of the formulations

It was observed that the cream belongs to the semi fluid category and observed that increase in concentration of plant extract decreases the spreadability of the formulation.

Antibacterial Activity

The antibacterial activity of all the herbal drugs and formulated creams were carried out using *E. coli* (Table 9 and Table 10). The results were found to be encouraging. The negative control samples did not show any zone of inhibition (Figure 2 and Figure 3).

Table 9: Antibacterial activity of the herbal drugs

Concentration	Zone of Inhibition in *E. coli* (mm)															
	Cassia tora				*Cassia alata*				*Lantana camera*				*Hemigraphis alternate*			
	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol	Hexane	Chloroform	Acetone	Methanol
50 mg	5.6	3.9	3.4	5.5	3.4	3.0	2.1	4.2	3.2	2.9	3.2	4.8	6.2	4.4	2.1	5.4
75mg	6.5	4.4	4.0	6.1	6	3.9	3.0	5.4	4.5	3.5	4.1	3.3	7.0	0.1	3.2	6.0
100 mg	7.2	5.0	5.9	7.0	7.0	4.5	4.1	6.4	6	4.0	5.2	4.1	8.0	6.8	4.3	6.9
+ve control	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
-ve control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2: Antibacterial study in herbal drugs

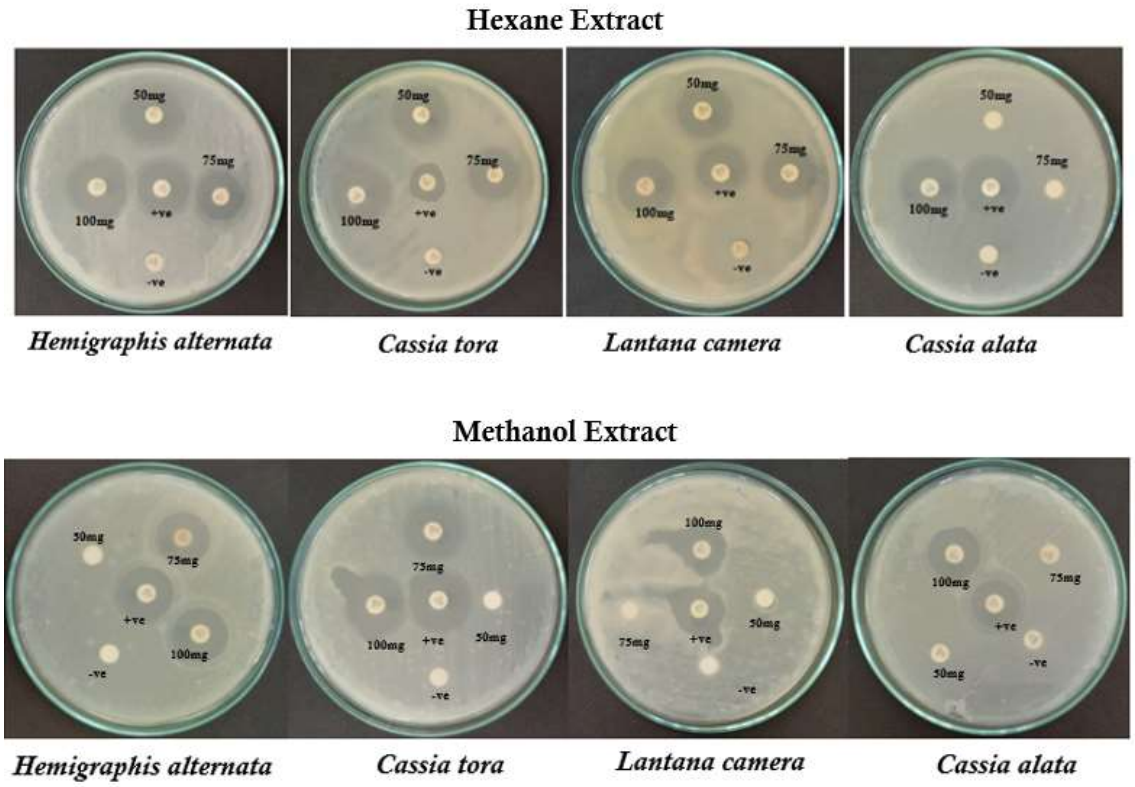
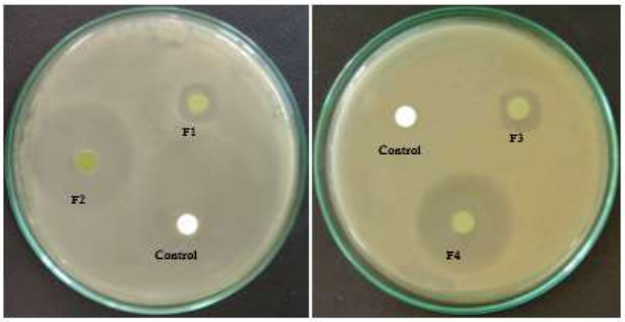


Table 10: Antibacterial activity of the cream formulations

Sl. No	Formulation	Zone of Inhibition in *E. coli* (mm)
1.	F1	1.2
2.	F2	15
3.	F3	1.5
4.	F4	25
5.	Control	0

Figure 3: Antibacterial study in cream formulations



Percentage Wound Contraction for the optimized formulation

The reports of the wound contraction studies in rats are reported in Table 11 and their profiles are given in the Figure 4. The results indicate that the rate of wound contraction was maximum on the 12th day in

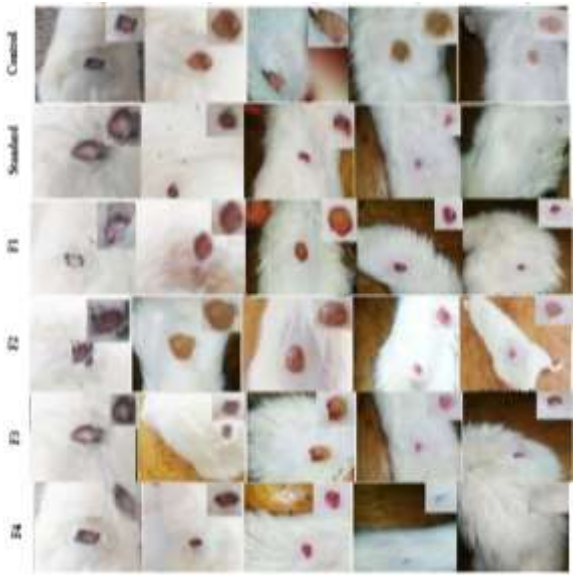
the treated group. Wound contraction studies depicted that Formulation F4 showed the maximum wound contraction when compared with that of standard. Since the wound contraction ceases after 16 days, the treatment was undertaken only for 16 days. The scars left behind F4 and standard were very light, but in F1, F2, F3 and control the scars left behind were very thick.

Table 11: Excision wound studies showing percentage reduction in wound size

% Contraction (Mean ± S.E.M)						
Days	**Control**	**Standard**	**F1**	**F2**	**F3**	**F4**
4	15.60	20.58	15.92	17.28	16.19	19.21
8	39.42	52.41	42.13	48.25	46.75	51.94
12	56.31	76.34	65.42	75.21	72.63	83.12
16	72.14	93.57	88.13	90.63	92.45	94.36

S.E.M* = Standard Error mean, n=6

Figure 4: Wound contraction profile



Period of Epithelialization

The values of period of epithelisation for the control and the treated groups are depicted in Table 12.

Table 12: Period of epithelisation

Sl. No	Groups	Epithelialization Period (Days)
1.	Control	22.03±0.92
2.	F4	13.45±1.23
3.	Standard	15.23±0.72

S.D* = Standard Deviation, n=6

Wound Index

Wound index was measured daily with an arbitrary scoring system and reported in Table 13.

Table 13: Wound Index

Sl. No	Groups	Wound Index
1.	Control	2.92±0.22
2.	F4	1.55±0.77
3.	Standard	1.75±0.55

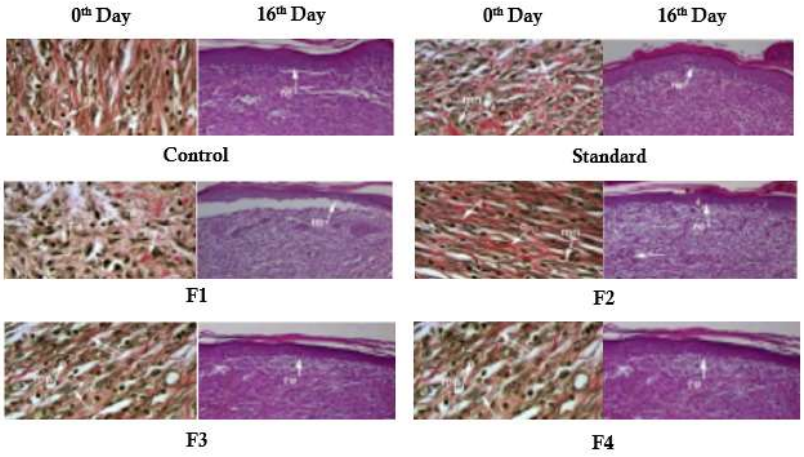
S.D* = Standard Deviation, n=6

Histopathological Studies

Microscopical examination of the sections prepared from the wounds of normal, standard, control and treated are depicted as follows,

1. Normal – The tissue is composed of dense collagen fibres, fibroblasts with round to oval nuclei and blood vessels. Epitheialization is not seen in the given section.
2. Control – The tissue shows densely inflamed connective tissue with chronic inflammatory cells interrupted between the collagen fibres, this shows the incomplete healing of wound. Many thin-walled blood vessels are seen.
3. Standard – The tissue shows dense fibrous connective tissue with fibroblasts and collagen fibres.
4. F4 – The tissue shows dense fibrous tissue with fibroblasts and collagen fibres which is more than that of the standard. The characters are almost to that of the normal (Figure 5).

Figure 5: Histology of regenerated tissue as on 16th day at 400X magnification



Conclusion

In this study, we have found that phytoconstituents present in the plant materials are responsible for wound healing. *In-vivo* wound healing results reveal that Formulation 4 showed enhancement in 94.36% wound contraction and test as compared to Positive control (93.57%), Control (72.14%), Formulation 1 (88.13%), Formulation 2 (90.63%) and Formulation 3 (92.45%). Based on these findings, it is recommended that many therapeutic approaches may be employed concurrently in managing wounds, especially chronic wound injuries, to speed up the healing process and prevent complications. Good production standards and regulatory regulations are equally essential to increase practitioners' use of phytotherapy and encourage its incorporation into national health systems.

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