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EVALUATION OF WOUND HEALING POTENTIAL OF HERBAL ETHOSOMAL GEL FORMULATION IN WISTAR RATS

[1]K. Lalitha*, [2]Md Rahmath Ali, [3]D.Pavani, [4]Dr. Satyabrata Bhanja

*[1,2,3]Sri Venkateshwara college of Pharmacy, Hyderabad-81, Telangana State, **INDIA***
*[4]Malla reddy college of Pharmacy, Maisammaguda, Dhulapally-500014, Telangana State, **INDIA***

Abstract

The objective of the present study was to evaluate the potential effect of Ethosomal formulation of ethanolic extract of *Tridax procumbens* on excision wounds in Wistar rats. Ethosomes with different concentrations of lecithin, cholesterol and ethanol along with herbal extract were prepared and optimized taking into regard their entrapment efficiencies. Three different formulations with concentrations 100,200,300 mgs of herbal extracts were prepared in the optimized ratio of ethosomes. Wound healing activity of formulated herbal gels was compared with the standard nitrofurazone cream, using experimentally induced excision wound model in albino wistar rats. The parameters, percentage wound contraction and period of epithilization were determined. From the results it has been found that formulated herbal gels showed significant epithelization period (*$p<0.01$) and the maximum was for F_3 formulation – 21.77 ± 0.86^a days and the percentage wound contraction on 21st day of the study was 99.81 ± 0.108 when compared with the standard showing a significant wound healing potential.

Keywords: *Tridax Procumbens,* Ethanol, Ethosomes, Wound healing.

Corresponding Author:

K. Lalitha

Assistant Professor,

Sri Venkateshwara college of Pharmacy,

*Hyderabad-81, Telangana State, **INDIA***

***E-mail:** kalakotalalitha@gmail.com*

***Phone:** +91-9246397071*

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INTRODUCTION

Wounds are injuries that result from the breaking or laceration of the skin or other body tissues. They include cuts, scrapes, scratches, and punctured skin. Wounds are still major problem in world or developing countries, because of poor hygienic conditions, often imposing severe complications and high cost for therapy [1]. Herbs as they contain several phytoconstituents possessing anti-inflammatory, anti-oxidant, anti-microbial properties show synergistic effect on wound healing process [1]. Though a number of antibiotics are available, increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive reagents of herbal origin. In India there are 2500 plants having medicinal values and nearly 6000 plants are used in traditional medicinal system. The objective of the present study was to explore the utility of plants for wound healing [2]. *Tridax procumbens* belong to Asteraceae family. In Indian traditional medicine Tridax is commonly used as anticoagulant, hair tonic, antifungal, insect repellant, diarrhea, dysentery and wound healing. The juice extracted from the leaves of the plant is applied directly on the wounds. The leaf sap of the plant applied topically cures sores and ulcers. It is used to treat liver disorders gastritis and heartburn. Usage of the herb in ethnobotany as internal medicine and its use as food for cattle in some areas of the world proves it non-toxic. It is used in the treatment of boils, blisters and cuts [3]. It contains a number of valuable constituents such as flavone glycoside, chromone glycoside, bithiophenes, flavonoid (Procumbenetin), sterols, terpenoids, lipids and polysaccharides [3].

The Significant pharmacological activities of *T.procumbens* are hypotensive, hepatoprotective, anti-inflammatory, antidiarrhoeal, wound-healing, insecticidal, leishmanicidal and hair growth promoting activities [4]. This herbal plant extract can be used as topical preparations such as creams, ointments and gels where they can be spread on the local wound area. The epidermal barrier, which is the main limiting factor of transdermal drug delivery systems is overcome by ethosomes [5].

Ethosome is a non invasive vehicle that enable drug to reach deep skin layers and/or the systemic circulation. Even after exhibiting the promising therapeutic effects, most of the phytochemical constituents fail to achieve bioavailability because of poor absorption [6].

Ethosome is a lipid based nanovesicle that is formulated of phospholipids, a high concentration of alcohols and water. It has a better ability to increase drug penetration. This ability is due to the presence of high concentration of ethanol, as ethanol is a penetration enhancer [7]. Therefore

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incorporation of these plant actives or extracts into vesicular carriers vastly improves their absorption and there by the bioavailability, because of the improved entrapment efficiency and skin penetration [8].

METHODS AND MATERIALS

COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACT:

The fresh weed of *T. procumbens* was collected in the flowering stage and shade dried and made in to fine powder. The plant was further authenticated at the Department of Botany, Yogi Vemana University, Kadapa. Powder of the plant was macerated with pure ethanol for 7 days in Soxhlet apparatus at room temperature. Later the ethanolic extract is filtered and the extract was concentrated under reduced pressure in rotary vacuum evaporator. The concentrate is placed in petriplate for drying to remove the traces of ethanol to obtain the concentrated extract and stored at cool temperature in a suitable container [9].

MONOGRAPHIC ANALYSIS OF THE HERB:

The monographic analyses of *T.procumbens* with regard to standard specifications according to the herbal pharmacopoeia of India, the tests were carried out as loss on drying, extractive values and ash values.

PRELIMINARYPHYTOCHEMICAL ANALYSIS:

The ethanolic extract of plant was screened for the presence of various phytoconstituents like alkaloids, carbohydrates, poly phenols, flavonoids, glycosides, tannins [7,10,11].

PREPARATION OF ETHOSOMES:

Accurately weighed measures of phospholipide and cholesterol are dispersed in water by stirring it on magnetic stirrer followed by heating at 40^{o}C.

Take 100 mg of extract, ethanol was added in a beaker. To this add propylene glycol with continuous stirring.

Lipid solution was added drop wise to the organic phase with continuous stirring on magnetic stirrer for 40 minutes to obtain ethosomes and stored at refrigerated temperatures until use. Ethosomal formulations were prepared using each two different concentrations of lecithin, cholesterol and ethanol.

Entrapment efficiencies of the formulations were determined and the formulation with highest entrapment efficiency was treated as optimized formulation and the formula of the same was fixed to make three different doses of ethosomes which were further incorporated into gel formulation.

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EVALUATION OF PREPARED ETHOSOMES:

Entrapment efficiency of *Tridax procumbens* ethosomal vesicles was determined and estimated using UV visible spectrophotometer at 214nm [12,15]. Entrapment efficiency can be determined from the formula:

$$EE\% = \frac{(\text{Total drug}) - (\text{free drug})}{\text{Total drug}} \times 100$$

FORMULATION OF GELS:

Gels were prepared by addition of gelling agent carbopol 934 to distilled water and allowed to soak overnight. Added triethanolamine drop by drop followed by addition of glycerol until desired and optimum viscosity was attained. To this the ethosomal solution was added and mixed thoroughly to obtain the transparent gel. methyl paraben was added as preservative and the gels were packed into vials and stored at 4-8^{0}C [6].

EVALUATION OF PREPARED GELS

PHYSICOCHEMICAL PROPERTIES

Physical properties such as appearance, colour, pH, viscosity, etc were evaluated [13]. The colour, appearance and the feel on application of different formulated gels was noted. The properties such as consistency, texture and skin irritation test were done. The pH was determined using digital pH meter.

SPREADABILITY

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) was placed on the ground slide. The gel was sandwiched between this slide and another glass slide, provided with the hook. Weight of 1 kg was placed on the top of the slide for 5 minutes to expel air and to provide a uniform film. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in seconds) taken by the top slide is noted [8]. A shorter interval indicates better spreadability.

Spreadability is calculated using the following formula:

$$S = \frac{M \times L}{T}$$

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.

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VISCOSITY

Viscosities of herbal gel formulations were determined by using Brookfield rotational viscometer at 5, 10, 20, 30 and 50 rpm using spindle no.64. Each reading was taken after equilibrium of the sample at the end of two minutes. The viscosity determination of samples was repeated three times [8].

STABILITY STUDIES

Accelerated stability testing was performed using standard procedure and recorded. The good manufacturing practices that take care of all aspects of preserving the integrity and properties of formulations throughout the shelf life need to be followed. These studies were performed as per ICH guidelines. Collapsible tubes filled with the formulations were stored at different temperatures and humidity conditions as per the protocol of the experimental design. Viz., 25^0 C ± 2^0C/ 60% ± 5% RH (Related Humidity), 30^0 C ± 2^0C/ 65% ± 5% RH, 40^0 C ± 2^0C/ 75% ± 5% RH, for a period of three months and observed for changes in appearance, pH, viscosity and spreadability.

ACUTE SKIN IRRITATION STUDY FOR TOPICAL FORMULATION:

Skin irritation test was performed following OECD guidelines 404. In this test total 9 rats were taken 3 in each group of weight between 120-140 gms. Hairs of rats were depilated with the aid of depilatories on both the side were one is for the test and the other for control. Test substance were applied which should adhere to the skin. The animals were examined for 14 days for the signs and symptoms of oedema and erythema [13].

WOUND HEALING ACTIVITY

ANIMALS

Wistar rats of either sex weighing about 110-120 g were obtained and quatantined. The animals were kept in animal house for acclimatization in the animal cages at room temperature of 22^oC + 2^oC, and fed with pelleted diet and water *ad libitum*. The experimental protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA(Committee for purpose of control and supervision of Experimental on animals) through its reference no: IAEC/SVCP/2016.

EXPERIMENTAL DESIGNS

Animals were divided in to five groups of 6 rats in each (n=6).

Group 1: Control with wound applied with simple gel base without drug.

Group 2: Test group with wound treated with F1 formulation gel.

Group 3: Test group with wound treated with F2 formulation gel.

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Group 4: Test group with wound treated with F3 formulation gel.

Group 5: Test group with wound treated with Nitrofurazone gel.

EXCISION WOUND

The wound procedure was carried out on anesthetized rats on the dorsal shaven portion. A wound is made by removing a circular portion of skin. During the surgical period all the items and materials must be sterilized. The formulations were applied topically on the wounds from 0 to 21 days, three times a day. Wounds were checked regularly and measured. Number of days required for falling of scab without any residue raw wound will give the period of epithelization [12,17]. The wound contractions were measured as the percentage reduction in the wounded area for every 4 – 5 days. The reduction in the wound size was calculated.

$$\%\text{Wound Contraction} = \frac{(\text{initial wound size}) - (\text{specific day wound size})}{(\text{initial wound size})} \times 100$$

STATISTICAL ANALYSIS

The results of the studies were expressed as **mean ± S.E.M.** Data analysis was done by one – way analysis of variance**.(ANOVA)**

RESULTS

The preliminary phytochemical evaluation of ethanolic extract of Tridax procumbens has revealed the presence of flavonoids, tannins, steroids in moderate qunantities and glycosides, saponins and alkaloids are also presents. The extractive, solubility and ash values are estimated by standard specifications of Herbal pharmacopoeia of India. The values are depicted in Table 1.

Table 1: Monographic analysis of *Tridax procumbens*

S.No	Parameters	%w/w (Mean)
1	Alcohol soluble extract value	5.8%
2	Acid insoluble ash	3.9%
3	Water soluble ash	4.1%
4	Total ash value	18.4%
5	Sulfated ash value	21.13%
6	Loss on drying	4.1%

n = 3; mean ± SD

Ethosomes of varying compositions of lipid, cholesterol and ethanol with the herbal extract were formulated and were entitled with formulation codes from E1-E6.the entrapment efficiency of ethosomes was calculated and observed to be in the range of 70.61-86.33%. the three ingredients

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in the composition of ethosomes influenced the entrapment efficiency(EE) and E6 showed highest EE, with the formula of ethosomes with highest EE i.e., E6, three concentration of gels with 100mg,200mg, 300mg of drug extract were prepared.

Table 2: Entrapment efficiency (%)

S.No.	Ethosomes combination code	Composition				Entrapment Efficiency (%)
		L(mg)	C(mg)	E(ml)	PG (ml)	
1	E1	100	20	10	3	71.41± 0.28
2	E2	200	20	10	3	73.62± 0.81
3	E3	200	30	10	3	70.61± 0.3
4	E4	200	40	10	3	74.79± 0.12
5	E5	200	30	20	3	83.47± 0.45
6	E6	200	30	30	3	86.33± 0.67

L – Lecithin; C – Cholesterol; E – Ethanol; PG – Propylene glycol

Entrapment efficiency is calculated using the formula. Ethosomal vesicles were subjected to evaluation and data was expressed in **mean ± S.E.M**; n= 3 readings.

The ethosomal topical gels formulated were evaluated for the physical parameters like colour, appearance, spreadability, pH and viscosity. Acute skin irritation test was conducted following OECD guidelines. There were no signs of oedema and erythema in the observation for 14 days which indicated the absence of skin toxicity.

Stability studies were conducted for a period of 3 months and the parameters inferred were appearance, pH, spreadability and viscosity. Results showed no significant changes in these parameters.

Table 3: Physical evaluation of formulated herbal gels

Formulation code	Appearance/consistency	Spreadability	Washability	Colour	Spreadability (g.cm/sec)	Viscosity (ps)	pH
F1	Smooth	Easy	Washable	Light green	31.8+0.5	3443	6.4
F2	Smooth	Easy	Washable	Dark green	30.1+0.8	3389	6.4
F3	Smooth	Easy	Washable	Dark green	32.4+0.6	3481	6.5

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Table 4: Stability studies of topical herbal gel formulations for a period of 3 months

Parameter	1st month			2nd month			3rd month		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
Colour	No change			No change			No change		
Odour	No change			No change			No change		
Appearance	Smooth and clear			Smooth and clear			Smooth and clear		
pH	No change			No change			6.4	6.4	6.4
Viscosity	No change			No change			No change		

The wound healing of formulated gel was evaluated using excision wound animal model. The wound area was determined after a continuous application of gels for a period of 21 days. on 4,8,12,16,and 21days the wound area is measured and %wound contraction was calculated using the formula. The formulated gels showed a significant increase in wound contraction with increase in drug concentration and significance (*p<0.01) was observed when compared to control group. The marketed standard showed 94.42±0.21% wound contraction after on 21st day with respect to initial wound size as 100% where as the F3 showed 99.81±0.1 % wound contraction and the control group showed 76.89±0.79 %. A significant increase in % wound contraction was also observed with increase in concentration of extract.F3 with 300mg extract concentration showed highest % wound contraction observed on 21st day of the test.

Table 5: Epithelisation periods of control, test and standard drugs

S.No	Name of the formulation	Epithelisation period (days)
1	Control	31.56±0.18
2	F1	21.61±0.63
3	F2	19.69±0.36
4	F3	17.25±0.58
5	Nitrofurazone	28.68±0.95

The data are expressed in Mean ± S.E.M; n=6 in each group; *p<0.01, significant compared to control and standard drug.

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Table 6: %wound contraction of control, test and standard drugs

No. of days	Simple gel control	F1	F2	F3	Reference standard
4	8.76±0.29	11.19±0.14	20.7±0.23	23.5±0.11	21.8±0.81
8	20.765±0.19	39.08±0.63	48.91±0.77	54.3±0.91	51.67±0.82
12	46.58±0.72	55.97±0.43	69.62±1.3	72.50±0.16	67.89±0.54
16	67.51±0.61	75.31±0.37	83.90±0.48	88.7±0.95	85.18±0.09
21	76.89±0.79	89.2±0.81	92.16±0.71	99.81±0.1	94.42±0.21

The data are expressed in Mean ± S.E.M; n=6 in each group; *p<0.01, significant compared to control and standard drug.

DISCUSSION

Wounds remain a challenging clinical problem with early and late complications presenting a frequent cause of morbidity and mortality. In the attempt to reduce the wound burden, effort is put in wound care with an emphasis on new therapeutic approaches and the continuing development of technologies for acute and long term wound management [11]. In the present study, the wound healing potential of ethnobiologically popular herbal drug, *Tridax procumbens* was enhanced and reframed in ethosomal novel drug delivery system with great advantages over traditional systems like avoidance of first pass metabolism, sustainable duration of action, improved pharmacological response and high patient compliance [14,15]. Wound healing involves a complex interaction between epidermal and dermal cells, the extracellular matrix, controlled angiogenesis and plasma-derived proteins, all co-ordinated by array of cytokines and growth factors [16]. The plant under investigation, *T. procumbens* has potential to promote wound healing in both normal and immune compromised rat models [4,17] a novel approach of incorporating the herbal extract in to ethosomes and formulation in to a topical gel has enhanced the potential of the herbal drug for the wound healing ability and henceforth utilized to the optimum.

CONCLUSION

Tridax procumbens has proved to have significant wound healing potential and it can be concluded that the healing efficiency of *Tridax procumbens* can be optimally utilized by improving the absorption and bioavailability of the drug with the ethosomal vesicles and the

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potential of the herbal drug can be tapped to the maximum when incorporated in to such drug delivery systems.

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CONFLICT OF INTEREST

The authors have no conflict of interest with anyone.

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