INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.10 | Issue 2 | Apr - Jun-2021
Journal Home page: www.ijpar.com

Research Study *Open Access*

Design, formulation and evaluation of a polyherbal gel for its wound healing activity

Raju Darla[1], Srisailam keshetti[2]

[1]*Assistant professor department of pharmacy,hits college of pharmacy, bogaram keesara Rangareddy district.501301,telangana state,india.*
[2]*Principal, University College of pharmaceutical sciences,satavahana University, karimnagar.505305 Telangana state, India.*

***Corresponding Author: Raju Darla**
Email: inform2rkjohnnydarla@gmail.com

ABSTRACT

The present study was aimed at designing, formulating and evaluating a polyherbal gel comprising of ethanolic extract of the leaves *Tridax procumbens* and petroleum ether extract of the leaves *Cassia occidentalis* have a great medicinal value for its wound and burn healing properties. The animals were divided into groups and were treated with polyherbal formulation, standard group and one served as control group.5% w/w betadine powder was used as reference standard. The period of epithelization in the excision wound mode was found to be 12 days. Different parameters like pH, viscosity, spreadability and stability were evaluated. The formulation showed good spreadability, good consistency, homogeneity, there was no change in the appearance, pH, and no phase separation noticed at the end of the stability studies. There was no evidence of skin irritation. This study has revealed that the poly herbal gel has shown the wound healing effect due to the synergistic activity of the phytoconstituents present in the extracts and may be used a potential herbal formulation for wound healing.

Keywords: *Tridax procumbens, Cassia occidentalis,* polyherbal formulation,excision wound method

INTRODUCTION

A wound has been defined as loss or breaking of cellular and anatomic or functional continuity of living tissue. The vast literature on wound healing is mainly focused on skin, which is the most susceptible organ in the body that interacts with the environment and therefore receives constant insult and damage.

Wound healing is a process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules that are later remodeled to form scar.

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and

growth factors. This dynamic process is classically divided into three overlapping phases – "inflammation, proliferation and remodeling". [1] There are various natural agents, which assist in wound healing process. This treatment provides fibrogenetic and concentration of collagen resulting in faster healing.

Gel is defined as semi solid preperation consisting of dispersion of small or large molecules in an aqueous liquid vehicle, rendered jelly like through the addition of gelling agent. These are the intermediate state of matter, consisting both solid and liquid components. The solid component comprises a three dimensional network of inter connected molecules or an aggregate that immobilizes the liquid in the continuous phase. Gels may be classified into two primary types:

a. Hydro gels
b. Organo gels

Gelling agents generally used are synthetic macro molecules (carbomer 934), cellulose derivatives (CMC, HPMC), and natural gums (Tragacanth). Carbomers, particularly high molecular weight, are water soluble polymers of acrylic acid cross linked with alkyl ethers of sucrose and/or pentaerythritol.Carbomer 910,934,934p, 940,941 and 1341 are used as gelling agents at concentrations of 0.5 to 2% in water.carbomer 940 yields highest viscosity i.e.40, 000-60,000 cp as a 5% aqueous dispersion.

Gels as well as their biocompatibility, increased duration of action with increased therapeutical efficiency due to the viscosity of the gel matrix and soft consistency (easy and safe administration at home by nonmedical persons) [1,2]

Topical application of gels at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments[3,4]

Material and Methods

Collection of plants

The leaves of *Tridax procumbens* and *Cassia occidentalis* were collected from pakal region in Warangal district of Telangana state, India.

Chemicals and reagents

Betadine powder (Win-Medicare Pvt Ltd), white petroleum jelly and white bees wax (LobaChemie)

Extraction

The collected plants *(Tridax procumbens and Cassia occidentalis)* were extracted by continuous hot percolation (Soxhletation). 50g of powdered leaves of the above four plants were defatted using petroleum ether and ethanol. The marc obtained from each of the powdered plant parts were successfully extracted separately with 250 ml of ethanol by using soxhlet apparatus. The extraction was carried out for 24 hours. After extraction, the solvents were distilled out; the concentrated residues were analyzed by chemical tests [2,6.]

Phytochemical analysis [5,6]

The methanolic extract obtained after soxhletation was subjected to various photochemical screening as per the standard procedure to reveals the presence of various active phytoconstituents.[5]

Table 1: Gel (Carbopol 934 p gel base) composition of gel 100ml

S.no.	Ingredients	Quantity(g)
1	Liquid paraffin	2.5
2	White petroleum jelly	3.5
3	Bioactive (extract)	1.5
4	Isopropyl alcohol	10 ml
5	Propylene glycol	10 ml
6	Carbopol 934 p	1
7	Sodium hydroxide 10% solution for ph adjustment	q.s
8	Distilled water	q.s

Procedure

Required quantity of distilled water was taken in a beaker and heated, suspended Carbopol 934 p by adding slowly under homogenizer type stirrer with fast stirring (carbopol suspension). A solution of isopropyl alchohol, extract and propylene glycol was prepared. Slowly added this solution to carbopol suspension. White petroleum jelly was melted and mixed with liquid paraffin and added to the carbopol jelly. Mixed well. Sodium hydroxide 10% solution was added to adjust the ph to 7.0.The preservatives were added and mixed well. Adjusted

the final volume with distilled water and mixed well.

Evaluation of gel: The formulated gel formulation was subjected to evaluation of following parameters[7].

Physical observation: Physical parameters such as color, appearance and feeling on application were recorded. All formulations were observed visually for their clarity and color.

Physicochemical parameters[7]: Preliminary evaluation of formulations at different concentrations was carried out as follows:

Color and odour: Color and odour was examined by visual examination.

Loss on drying: Loss on drying was determined by placing ointment in petridish on water bath and dried for 105^0C.

pH: The pH of various formulations was determined by using digital pH meter. One gram of ointment was dissolved in 100 ml of distilled water and stored for two hours.

Viscosity: Viscosity of formulation was measured by using brookfield viscometer.

Determination of viscosity by using brookfield viscometer (DV-II + pro)

Procedure

Required formulation was taken in a beaker around (50-200g/ml).suitable spindle (No.61, 62, 63 or 64) was fixed to the below spring of the viscometer. Set the speed from (3,6,9,12,16,30,45 and 60) note the obtained Cps value every time set the speed and on the motor, note the Cps value with increase in speed of rotation we will observed the decrease in viscosity i.e,Cps values.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the ointments readily spreads on application to skin or affected part. A special apparatus has been designed by multimer to study the spreadability of formulations. The spreadability was expressed in terms of times in seconds taken by two slides to slip off from ointment and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, result the better spreadability. Spreadability was calculated by using the formula.

$$S = (M.L/T)$$

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a petridish and allowed to set. A hole was bored at the centre of the petridish and the prepared formulation was placed in it. The time taken for the ointment to get diffused was noted.

Skin irritation study[8]

Healthy rabbits were selected and were shaved in two different areas of the dorsal side, each about 500 mm^2.The rabbit was kept in rabbit holder and the first area was kept as control, to which gel was applied, the second area was treated with polyherbal gel. After 4hrs the skin was observed and compared with the control.

Stability studies

The stability studies were carried out for the prepared formulations at different temperature conditions (4^o C, 25^o C, 37^o C and 45^oC) for 3 months.

Standard used: 5% w/w betadine powder.

Development of topical herbal formulation

In the present investigation, petroleum ether extract of *Cassia occidentalis* and ethanolic extract of *Tridax procumbens* were used for the development of topical formulations. Accelerated stability studies of formulation were performed for nature, color, odour, texture, pH, phase separation, consistency and speadability and trace of gritty particles.

Formulation I- ethanolic extract of *Tridax procumbens*

Formulation II-petroleum ether extract of *Cassia occidentalis*

Formulation III-Standard

Formulation IV-Herbal drug combination

Results and Discussion

STUDY DESIGN

Excision method

Screening of wound healing activity was performed by excision wound model. Adult albino rats of either sex weighing between 130-180 g were used in this study. Animals are divided into 15 groups, each containing 18 animals. They are depilated at the desired site and wound was performed light ether anaesthesia. A circular wound of approximately 2.5 cm diameter was impressed on the skin from the demarked area. The skin was excised to get a wound measuring approximately 500 mm^2. After achieving full haemostasis by blotting the wound with cotton swabs soaked in saline, the animals were placed in their individual cages.

The animals were treated daily as follows, from 1-12 th post- wound day.

Group I- treated with control (gel)

Group-II- treated with standard (betadine powder)

Group III- treated with 5% gel formulation of petroleum ether extract of *Cassiaoccidentalis.*

Group IV- treated with 5% gel formulation of ethanolic extract of *Tridax procumbens.*

Group V- treated with 5% gel formulation (herbal drug combination) of petroleum ether extract of *Cassiaoccidentalis* and ethanolic extract of *Tridax procumbens.*

The wound contraction rate was monitored by planimetric measurement of wound area of each animal on 1st, 4th, 8th, and 12th post wounding day. This was achieving by tracing the wound area on a graph paper. Reduction in wound area was expressed as a percentage of the original wound size. The results were expressed by calculating the mean and standard error using student's *t* test.

Physical characteristics of extracts by Soxhlation

Table 2 :

Crude drugs	Extract	Nature	Colour	Odour	Taste
Cassia occidentalis	Petroleum ether	Semi solid	Black	Characteristic	Mucilagenous, Bitter
	Ethyl acetate	Semi solid	Black	Characteristic	Mucilagenous,Bitter
	Chloroform	Solid	Black	Characteristic	Mucilagenous,Bitter
	Ethanol	Solid	Brown	Characteristic	Mucilagenous,Bitter
	Aqueous	Solid	Brown	Characteristic	Mucilagenous,Bitter
Tridax procumbens	Petroleum ether	Solid	Green	Pungent	Bitter
	Ethyl acetate	Semi solid	Black	Pungent	Bitter
	Chloroform	Semi solid	Black	Pungent	Bitter
	Ethanol	Semi solid	Black	Pungent	Bitter
	Aqueous	Semi solid	Black	Pungent	Bitter

Physicochemical parameters[7]

Table 2:

Parameter	Gel formulation			
	Formulation I- ethanolic extract of *Tridax procumbens*	Formulation II- petroleum ether extract of *Cassia occidentalis*	Formulation III- Standard	Formulation IV Herbal drug combination
Nature	Semi solid	Semi solid	Semi solid	Semi solid
Color	black	Black	Pungent	Black
Odour	Pungent	Pungent	Pungent	Characteristic
pH	6.52	7.02	6.55	6.32
Phase separation	No	No	No	No
Spreadability(dynes/ cm^2	86	52	52	121
Trace of gritty particles	No	No	No	No

Viscosity

Table 3:

Crude drug	Dosage form	10 rpm	20 rpm	50 rpm	100rpm
Tridax procumbens	Gel	34	32	27	21
Cassia occidentalis	Gel	247	115	104	61
Standard	Gel	120	15	4.51	3.82

Effect of gel formulation of 5% (w/w) petroleum ether extract of *Cassia occidentalis* on percentage reduction in wound size

Table 4:

		Day 1	Day 4	Day 8	Day 12
Gel formulation	Control	435± 11.4	378 ± 9.6	290± 8.46	238 ± 0.2
	Treated	359 ± 6.7	295± 15.8	156 ± 2.4	35 ± 0.56
	Standard	480± 25	314± 36.1	126 ± 11.2	0 ± 0.00

(Values are mean + SD from 6 readings each).

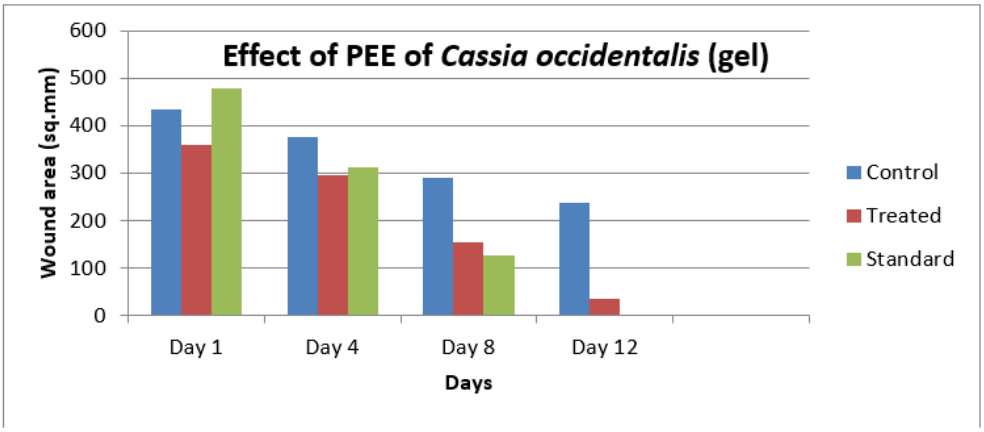

Fig 1

Effect of gel formulation of 5% (w/w) ethanol extract of *Tridax procumbens* on percentage reduction in wound size.

Table 5

		Day 1	Day 4	Day 8	Day 12
Gel formulation	Control	410 ±16.4	340 ± 23.4	210 ± 2.6	168 ± 0.8
	Treated	480±23.8	356 ± 33	208 ± 28.3	97 ± 23
	Standard	420± 20.4	304± 14.3	118± 11.2	0± 0.0

(Values are mean + SD from 6 readings each).

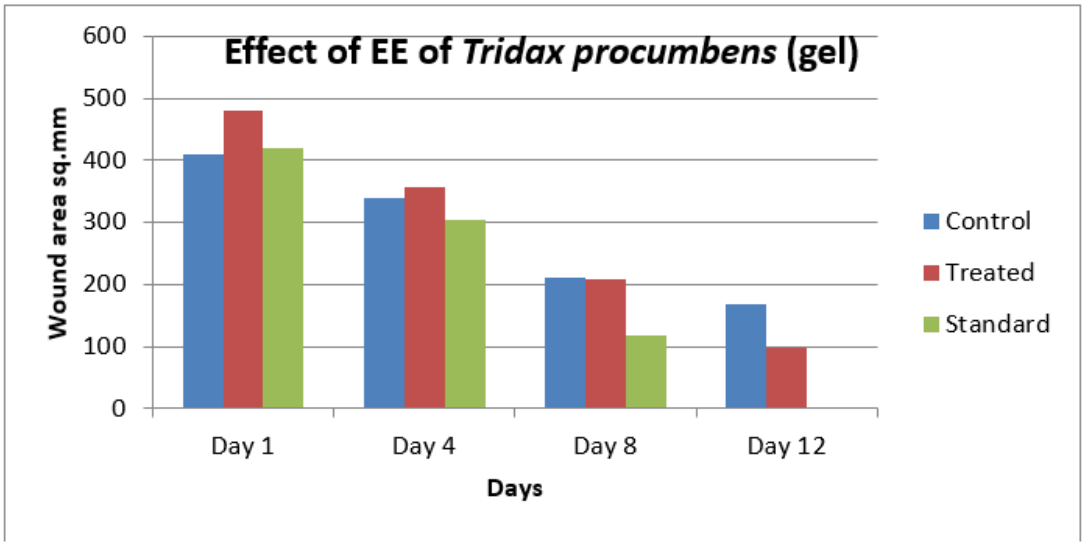

Fig 2

Effect of gel formulation of 5% (w/w) herbal drug combination (ethanolic extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis*) on percentage reduction in wound size

Table 6

		Day 1	Day 4	Day 8	Day 12
Gel formulation Herbal Drug Combination	Control	448± 34.2	386 ±31.50	273 ± 24.3	104 ± 2.3
	Treated	512± 26.9	394± 2.4	186 ± 11	20.8 ± 1.54
	Standard	482± 37.3	72 ± 18.9	194 ± 8.64	00 ± 0.0

(Values are mean + SD from 6 readings each).

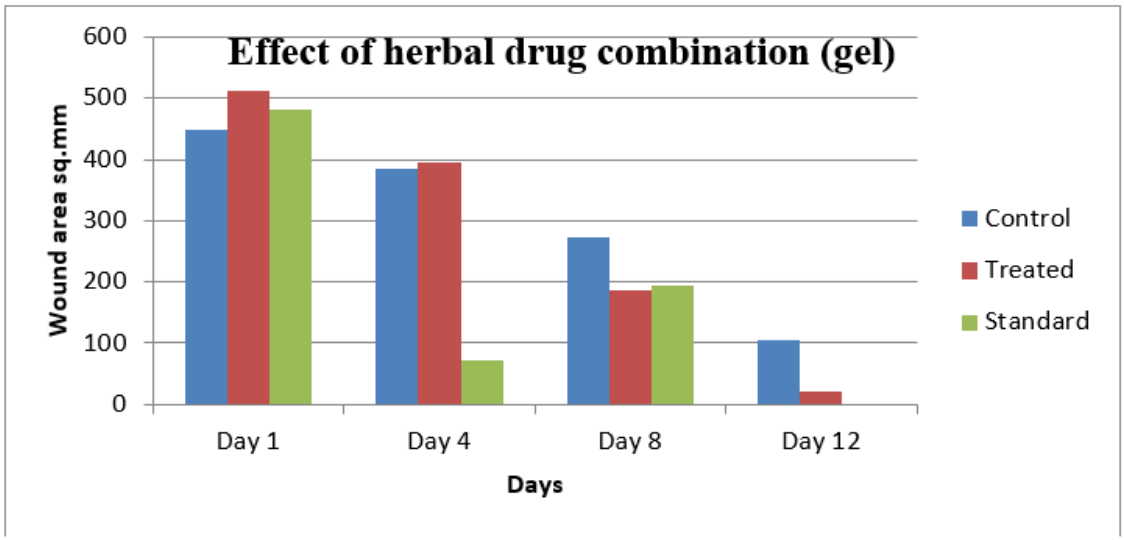

Fig 3

Excision wound model

Formulation:5% (w/w) petroleum ether extract of *Cassia occidentalis* gel

The percentage reduction in wound was 435± 11.4, 378 ± 9.6, 290± 8.46, 238 ± 0.2mm^2 as measured on the 1st, 4th,8th and 12th day respectively in the control group and 359 ± 6.7, 295± 15.8, 156 ± 2.4, 35 ± 0.56 mm^2 respectively in the treated group. The percentage reduction in wound size was significantly increased in 5% w/w gel of petroleum ether extract of *Cassia occidentalis* treated group compared to the control group on the 12th day.

Formulation: 5% (w/w) ethanol extract of *Tridax procumbens* gel

The percentage reduction in wound was 410 ±16.4, 340 ± 23.4, 210 ± 2.6,168 ± 0.8 mm^2 as measured on the 1st, 4th,8thand 12th respectively in the control group and 480±23.8, 356 ± 33, 208 ± 28.3, 97 ± 23 mm^2 respectively in the treated group. The percentage reduction in wound size was significantly increased in 5% w/w gel ethanolic extract of *Tridax procumbens* treated group compared to the control group on the 12th day.

Formulation: 5% (w/w) herbal drug combination (ethanolic extract of *Tridax procumbens* and petroleum ether extract of *Cassia occidentalis*)

The percentage reduction in wound are was 448± 34.2, 386 ± 31.50, 273 ± 24.3, 104 ± 2.3 mm^2 as measured on the 1 st,4,th 8th and 12th day respectively in the control group and 512± 26.9, 394± 2.4, 186 ± 11, 20.8 ± 1.54 mm^2 respectively in the treated group. The percentage reduction in wound size was significantly increased in 5% w/w gel (ethanolic extract of *Tridaxprocumbens* and petroleum ether extract of *Cassia occidentalis*) treated group compared to the control group on the 12th day.

CONCLUSION

The formulation promotes wound-healing activity. It showed remarkable wound healing activity and it may be suggested for treating various types of wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity.

REFERENCES

1. Howes.E.L., Harvey, S.G., andHewitt, C., surgery, 1939, 38,934.
2. Adamsons.R.J, and Kahan, S.A., Surgery Gynecol Obstat., 1970,130,837.
3. Loganathan V, Manimaran S, Jaswanth A, Sulaiman A, Shivaprasadha RMV, Senthil Kumar B, et al.The Effects of Polymers And Permeation Enhancers On Releases Of Flurbiprofen From Gel Formulations Ind.J.pharm Sci 2001;63(3):200□4.
4. Libermann HA, Rieger MM, Banker GS. Pharm. dosage form Disperse systems. 1987; 2:506.
5. VadnereG.,Somani R.,Sighai A.K.2006.Antiasthmatic potential of aqueous extract of *Cassia occidentalis*.Plant Medica,72:297
6. Adamu H.M., Abayeh O.J., Ibo N.N.E.U., Kafu S.E.2006.Antifungal activity of extracts of some *Cassia, Detarium* and *Ziziphus* species against dermatophytes. Natural Product Radiance, 5:357-360.
7. 7.Gupta A, Formulation and Evaluation of Topical Gel of Diclofenac Sodium Using Different Polymers, Drug Invention Today, **2(5)**, 250-253 (2010).
8. Raja shree, PH; Vidya, V; Merin, C; Jincy, E and Ranjith, S (2012), "Formulation and evaluation of antiseptic polyherbal ointment", *Int J Pharm Life Sci*, Vol 3 (10).