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FORMULATION AND EVALUATION OF WOUND HEALING ACTIVITY OF POLYHERBAL GEL

Vinitha Edula[1(*)], Malothu Nagulu[2], Papagatla. Poli Reddy[3]

[1]Assistant professor, Department of Pharmacology, Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India, 500084
[2]Principal and Head, Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India, 500084
[3]Associate Professor, Department of Pharmacology, Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India, 500084

*Corresponding Author Email: vinita.eedula@gmail.com

ABSTRACT

The present study is designed with the aim to formulate and evaluate the polyherbal gels for wound healing activity. The polyherbals selected are Piper betle, Curcuma longa, Aloe vera and Thymus vulgaris. The carbopol 934 gel formulations containing varied concentrations of ethanolic extract of the above-mentioned herbs were formulated and their wound healing activity was studied on experimentally induced wounds in wistar rats. The prepared gels were evaluated for physical appearance, pH; spreadability and skin irritation tests. It was inferred from the results that the formulated gels were good in appearance and homogeneity. The values of spreadability indicated that these polyherbal gels were easily spreadable by small amount of shear and also free from erythema and oedema. Formulations containing 1%, 2% and 4% (G1, G2 and G3) herbal extracts were applied topically once daily to open wounds and compared with control animals. The tensile strength of wounds treated with G3 gel raised and it may be due to increase in collagen synthesis. Wound tissues were examined on days 4, 8, 12 and 16 to estimate DNA, total protein, total collagen, hexosamine and uronic acid. It is inferred that in G3 gel treated groups the amount of protein, hexosamine, collagen and uronic acid increased from day 4 to day 12 and thereafter there was a gradual decrease until the 16th day. Flavonoids, triterpenoids and tannins present in the polyherbal gel and their synergistic action may be responsible for better wound healing activity. The treated wounds showed a faster rate of wound contraction compared with controls and the wound contractions increased with an increase in herbal extract concentration.

KEY WORDS

Polyherbal gel, Piper betle, Curcuma longa, Aloe vera and Thymus vulgaris.

INTRODUCTION

Wound is a physical or thermal injury that results in laceration or breaking of epithelial integrity of skin (Hashemi et al., 2015). Wound healing is a complex and multifaceted biological process including four different overlapping stages namely haemostasis, inflammation, proliferation and re-modelling (Orsted et al., 2004). Numerous growth factors, cytokines and intricate network of blood cells are involved to restore the normal condition of injured skin (Branski et al., 2008). Chronic wounds affect 5.7 million patients annually causing both physiological and mental trauma (Frykberg et al., 2015; Babu et al., 2002). Irrational use of antibiotics, over the counter drugs and environmental pollution are the main culprits for the development of bacterial resistance thereby hindering

the process of wound healing. This necessitated the paradigm shift from allopathic medicine usage to plant medicine. Poly herbal formulations are better compared to pure isolated chemical alone because polyherbals contain various phytoconstituents that possess anti-inflammatory, antioxidant, antimicrobial properties and show synergistic effect on wound healing process. Hence phytoherbals show better safety, efficacy and paucity of adverse reaction. Hence systematic scientific investigation is utmost important to explore the pharmacological activities of herbal medicines and to elucidate the claims made about them in traditional medicines. Worldwide more than 80% of the population depends on folklore medicine for the cure of various skin ailments (Clark et al., 1991) and more than 70% of the pharma products for wound healing are from plant origin (Biswas et al., 2003).

Present investigation is carried out on following herbal extracts: Curcuma longa, Thymus vulgaris, Piper betle, Aloe barbadensis Miller. These herbs are reported for anti-bacterial, immunomodulatory, antioxidant and anti-inflammatory properties which are complementary to wound healing. The present study is an effort to formulate and investigate wound healing ability of polyherbal topical gel formulation. Gel formulation is selected because it results in faster drug release directly to targeted site independent of water solubility of drug compared to ointments and creams.

MATERIALS AND METHODS

Collection, identification and authentication of plants

Plant material of *Piper betle*, *Thymus vulgaris* were collected in the month of January- February from the region of Nalgonda. Aloe vera (Aloe barbadensis) was collected from medicinal garden of Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda. Curcuma longa were procured from local market, Nalgonda. All the plant materials were identified and authenticated by pharmacognosist- Karnati. Sushma, Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda and a voucher specimen (No:SRTIPS/COG/2346) was deposited in department of Pharmacognosy. All the collected plant material except Aloe vera was shade dried thereafter reduced to powder form. In order to obtain the gel from Aloe vera the traditional hand-filleting method was selected.

Preparation of Plant extract

All the materials (25g) were extracted in a Soxhlet apparatus with 250 ml of Ethanol (60 – 80ºC) for 18 hours. The extracts was filtered and dried.

Drugs and Reagents

Carbopol 934, Ethanol, Propyl paraben, Methylparaben, Propylene glycol and Triethanolamine were procured from S.D fine chemicals Pvt LTD India.

Preparation of Topical Formulations

Carbopol 934 was soaked in water for 24hours and the selected herbal extracts were incorporated into carbopol gel base in three different concentrations. Compositions of gel formulations are depicted in Table 1.

Table No. 1 Compositions of gel formulations

Ingredients	Formulation		
	G1	G2	G3
Piper betle extract (%w/w)	1	2	4
Thymus vulgaris extract (%w/w)	1	2	4
Curcuma longa extract (%w/w)	1	2	4
Aloe vera extract (%w/w)	1	2	4
Camphor extract (%w/w)	1	2	4
Carbopol (%)	1	1	1
Propyl paraben (%)	0.02	0.02	0.02
Methyl paraben (%)	0.2	0.2	0.2
Propylene glycol (%)	4	4	4
Ethanol (%)	3	3	3
Water	Upto 100ml	Upto 100ml	Upto 100ml
Triethanolamine	q. s. to neutralize the gel base	q. s. to neutralize the gel base	q. s. to neutralize the gel base

***In vivo* pharmacological study**

Experimental animals

Male *Wistar rats,* weighing 200–250 g was used in the study and fed with standard laboratory pellet diet; Provimi limited (India), provided water ad libitum and were maintained at 23–25◦C, 35 to 60% humidity, and 12 h light/dark cycle. The rats were acclimatized to the laboratory conditions for a period of 7 days prior to experiment. The experimental protocol (SRTIPS/FM/1468/PO/a/11/CPCSEA/ 118/2017) was duly approved by institutional animal ethics committee (IAEC).

Acute dermal toxicity studies

As per OECD guidelines no.402 (OECD guidelines., 1987) dermal toxicity studies were carried out. Polyherbal gel with highest concentration (4%w/w) was applied topically on shaved skin of rats and observed for signs of erythema.

Animal grouping

The wistar rats were divided into five groups of six animals each.

Group I: Control treated with blank gel

Group II: Standard treated with Povidone iodine

Group III: treated with polyherbal gel formulation G1 (1%w/w)

Group IV: treated with polyherbal gel formulation G2 (2%w/w)

Group V: treated with polyherbal gel formulation G3 (4%w/w)

Excision wound model

The animals in each group were anaesthetized with ether. Depilations of the rats on dorsal side were performed. From shaved area 100mm^2 skin was excised using a sterile surgical scissors (Saha et al., 1997). The povidone iodine (standard), prepared gel formulations G1, G2 and G3 were applied topically once daily for groups II, III, IV, V animals respectively until the wound healed completely. Wound contraction studies and biochemical parameters estimations were carried out.

Incision wound model

The animals in each group were anaesthetized with ether and one paravertebral incision of 4 cm length was made through the skin on the depilated dorsal side till the cutaneous muscle. After incision was made the parted skin was kept together and stitched with surgical silk thread (No.000) at 1 cm intervals using curved needle (No.11). Wound closure was made by tightening the edges of the thread and was left undressed. G1, G2 and G3 gels and reference standard were applied once daily topically to their respective groups. The sutures were removed on the 9th day. Tensile strength of the cured skin was measured using tenisiometer (Hemalatha et al., 2001).

EVALUATION OF GEL

***p*H**

1.0 g gel was weighed and dispersed in 100 ml water. Using digital pH meter, the *p*H of the dispersion was calculated. The *p*H meter was calibrated before use with standard buffer solution at 4.0, 7.0 and 9.0. The readings of *p*H were done in triplicate and average values were calculated.

Spreadability

One of the important criteria for a topical formulation is that it should possess good spreadability. It is the term used to denote the extent of area to which formulation readily spreads when applied to skin or affected part. The therapeutic efficacy of a formulation depends upon its spreading value. To determine the spreadability of gel formulations, 0.5 g of gel was placed in a circle of 1 cm diameter pre-marked on a glass plate of 20 × 20 cm, over which a second glass plate was placed. A weight of 500 g allowed resting on the upper glass plate for 5 min. The increase in the diameter due to gel spreading was noted.

Homogeneity

The developed formulations G1, G2, G3 were tested for homogeneity by visual inspection after the gel had been filled in the container. They were tested for the appearance of gel and presence of any aggregates in gel.

EVALUATION OF WOUND HEALING PARAMETERS

Measurement of wound contraction

The periphery of the excision wound was traced on every 4th day on a transparent paper, till the wound got healed. Wound area was calculated by retracing the wound on a millimetre scale graph paper. The evaluated surface area was used to determine the percentage of wound contraction (Sadaf et al., 2006).

Epithelialization period

The wound was observed for complete epithelialization. It was calculated in days from wounding day (0th day) till the Escher totally gets separated itself with the raw wound left behind.

Tensile strength of incision wound model

The degree of wound healing is directly proportional to the increase in tensile strength of the tissue. Tensile strength was measured on the 10th day.

Biochemical estimations

Protein estimation was done by the method of Lowry (Lowry et al., 1951). DNA estimation was done by the method of Burton (Burton et al., 1956). An uronic acid level was measured by the method of Schiller (Schiller et al., 1961). Hexosamine content and collagen levels were estimated by Woessner (Woessner et al., 1979) method.

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of ethanolic extract of *Curcuma longa* revealed the presence of flavonoids, terpenoids, phenols, steroids and tannins. *Thymus vulgaris* showed the presence of flavonoids, phenols and terpenoids. *Piper betle* showed the presence of tannins, anthraquinones, flavonoids, terpenoids, cardiac glycosides and alkaloids. *Aloe barbadensis* revealed the presence of flavonoids, terpenoids, tannins and reducing sugars.

Evaluation of gel

The *p*H was found to be 6.2 ± 0.5, 6.3 ± 0.5 and 6.4 ± 0.5 for G1, G2 and G3 gel which was near to the neutral *p*H, thus the formulations can be used without the risk of skin irritancy. By this we can infer that the selected ingredients for gel formulation did not alter the *p*H of the formulation. The spreadability of gel formulations was found to decrease with increasing the concentration of gelling agent. The values of spreadability for G1, G2 and G3 gel was found out to be 8.4, 8.5, 8.6cm indicating that the gel is easily spreadable by small amount of shear. The results concluded that the formulation can be applied easily without being runoff. This assures that the formulation maintains a good wet contact time when applied to the targeted site. All the three prepared gel formulations were good in appearance and homogeneity.

Wound contraction studies

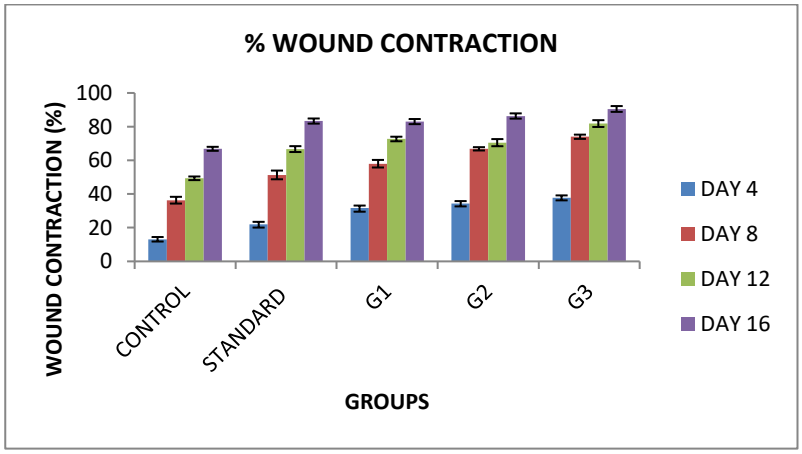
Reduction in wound area of different groups on days 4, 8, 12 and 16th days using excision wound model was calculated, compared and depicted in Table 2 and Fig.1. Least rate of wound healing is observed in control groups and faster wound healing was seen in group treated with G3 gel.

Table No. 2 The Effect of Formulations on Excision Wound Healing in Rats

TIME (DAYS)	% WOUND CONTRACTION				
	CONTROL	STANDARD	G1	G2	G3
4	13.00± 1.21	22.00±1.93*	31.67±2.17*	34.33± 1.68*	37.67±1.47*
8	36.33± 2.01	51.33±2.59*	58.00±2.25*	68.83±0.98*	74.00±1.26*
12	49.33± 1.08	66.67±1.74*	72.67±1.35*	70.50±2.12*	81.83±2.02*
16	66.83±1.19	83.33±1.52*	83.00±1.52*	86.33±1.56*	90.50±1.72*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

FIGURE 1: The Effect of Formulations on Excision Wound Healing in Rats



Epithelialization period

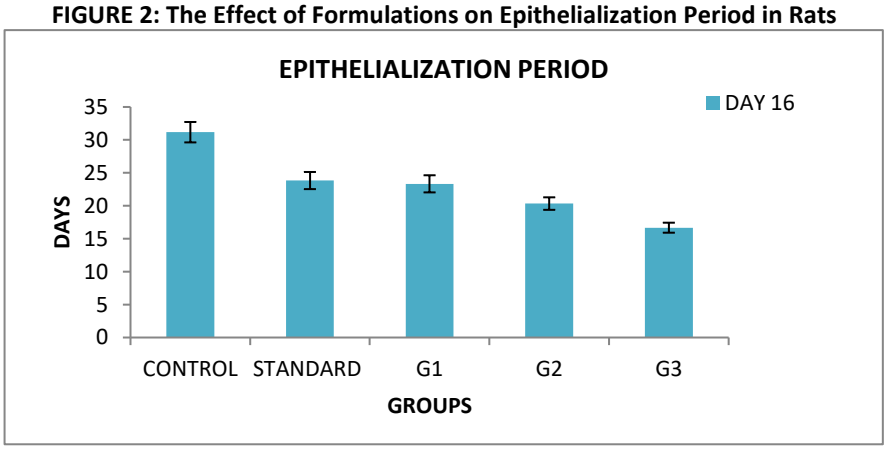
The epithelialization was observed from the first day. The epithelialization time was found to be lesser in group treated with G3 gel when compared with the other three groups treated with G1 gel, G2 gel and povidone iodine (Table 3 and Fig. 2).

Table No. 3 The Effect of Formulations on Epithelialization period in Rats

GROUPS	PERIOD OF EPITHELIALIZATION
CONTROL	31.17± 1.55
STANDARD	23.83±1.30*
G1	23.33±1.30*
G2	20.33±0.95*
G3	16.67±0.76*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

FIGURE 2: The Effect of Formulations on Epithelialization Period in Rats



Measurement of tensile strength

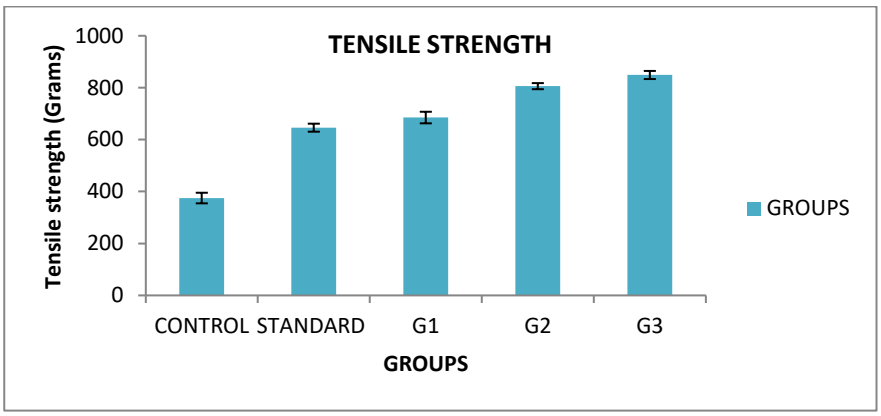
Comparison of the tensile strength of the healed skin of the rats in different groups is shown in Table 4 and Fig. 3. Minimum tensile strength was noticed in control group which is left untreated. Among the tensile strength of the tissues treated with gels; Reference standard and 1% w/w G1 gel exerted more or less the same strength. The groups treated with 4% w/w G3 gel have the highest tensile strength. It is inferred that G3 gel possesses excellent wound healing property.

Table No. 4 The Effect of Formulations on Tensile strength in Rats

GROUPS	TENSILE STRENGTH
CONTROL	375±20.4
STANDARD	646±15.4*
G1	685±22.3*
G2	806±11.7*
G3	849±15.6*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

FIGURE 3: The Effect of Formulations on Tensile strength in Rats



Biochemical estimations

The total protein, DNA levels, hexosamine and total collagen content in the granulation tissues of control and treated wounds are depicted in Tables: 5, 6, 7 and 8 and Figs: 4, 5, 6 and 7 respectively. G3 gel treatment significantly increased the DNA content from day 4 to day 12 and decreased on day 16. Increased protein levels, hexosamine and collagen from day 4 to day 12 were observed in G3 gel treated groups. There after there is a gradual decrease until the 16th day.

Table No. 5 The Effect of Formulations on Total protein levels in Rats

TIME (DAYS)	TOTAL PROTEIN mg/100mg WET TISSUE				
	CONTROL	STANDARD	G1	G2	G3
4	4.53±0.15	6.17±0.08*	6.56±0.17*	6.95±0.09*	7.21±0.10*
8	5.03±0.18	8.51±0.11*	8.08±0.27*	8.65±0.14*	9.16±0.13*
12	6.73±0.24	8.83±0.10*	9.01±0.13*	9.46±0.14*	9.56±0.13*
16	6.66±0.07	5.56±0.11*	5.35±0.11*	4.80±0.17*	4.47±0.20*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

Table No. 6 The Effect of Formulations on DNA levels in Rats

TIME (DAYS)	DNA mg/100mg WET TISSUE				
	CONTROL	STANDARD	G1	G2	G3
4	1.46±0.11	2.40±0.07*	2.68±0.09*	3.21±0.05*	3.23±0.07*
8	5.83±0.10	6.50±0.09*	6.64±0.10*	6.68±0.10*	7.25±0.09*
12	4.00±0.12	7.66±0.11*	8.38±0.12*	8.44±0.10*	8.57±0.12*
16	3.61±0.05	2.64±0.14*	3.13±0.05*	2.68±0.07*	2.31±0.06*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

Table No. 7 The Effect of Formulations on Hexosamine levels in Rats

TIME (DAYS)	HEXOSAMINE µg/100mg DRY TISSUE				
	CONTROL	STANDARD	G1	G2	G3
4	773±14.5	877±13.3*	928±12.2*	949±13.8*	954±14.2*
8	570±20.2	895±13.4*	951±13.7*	951±13.3*	962±12.0*
12	376±18.3	930±12.5*	962±12.9*	944±16.5*	964±14.4*
16	388±10.7	306±14.9*	281±18.3*	261±19.0*	206±19.6*

Data was expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnett's test, P<0.01* when compared to control group.

Table No.8 The Effect of Formulations on Total Collagen levels in Rats

TIME (DAYS)	TOTAL COLLAGEN mg/100mg DRY TISSUE				
	CONTROL	STANDARD	G1	G2	G3
4	2.40±0.18	3.43±0.16^{c}	3.58±0.23^{ns}	3.56±0.15^{b}	3.79±0.11^{a}
8	4.48±0.11	5.53±0.15^{c}	5.96±0.14^{ns}	6.33±0.15^{b}	7.03±0.25^{a}
12	5.60±0.16	7.64±0.16^{c}	8.02±0.14^{ns}	8.55±0.17^{b}	8.81±0.15^{a}
16	3.52±0.11	2.83±0.22^{c}	2.95±0.06^{ns}	2.73±0.20^{b}	2.41±0.21^{a}

Data was expressed as mean ± S.E.M. and Superscript letters represents the statistical significance done by ANOVA, followed by followed by Dunnett's test.

[a] P<0.01 indicates comparison of control to G3 group.

[b] P<0.05 indicates comparison of control to G2 group.

[c] P<0.05 indicates comparison of control to standard group.

[ns] indicates non-significance of control to G1 group.

FIGURE 4: The Effect of Formulations on Total protein levels in Rats

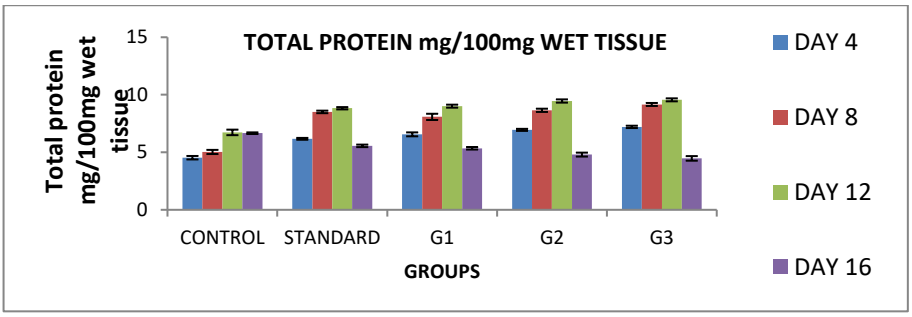


FIGURE 5: The Effect of Formulations on DNA levels in Rats

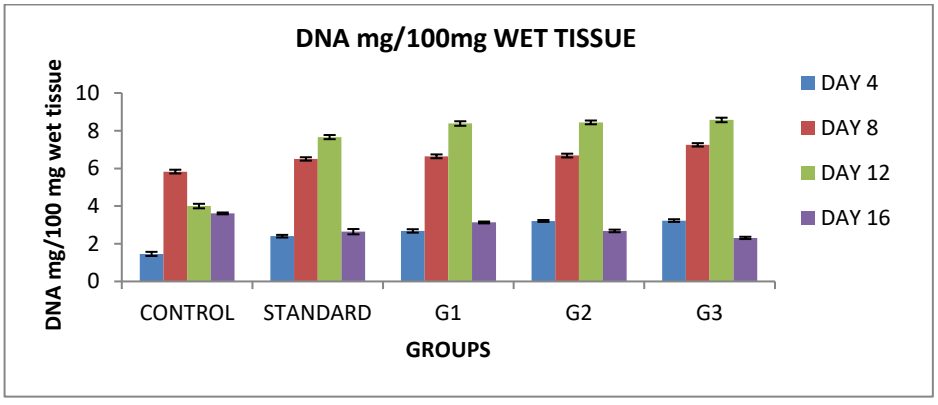


FIGURE 6: The Effect of Formulations on Hexosamine levels in Rats

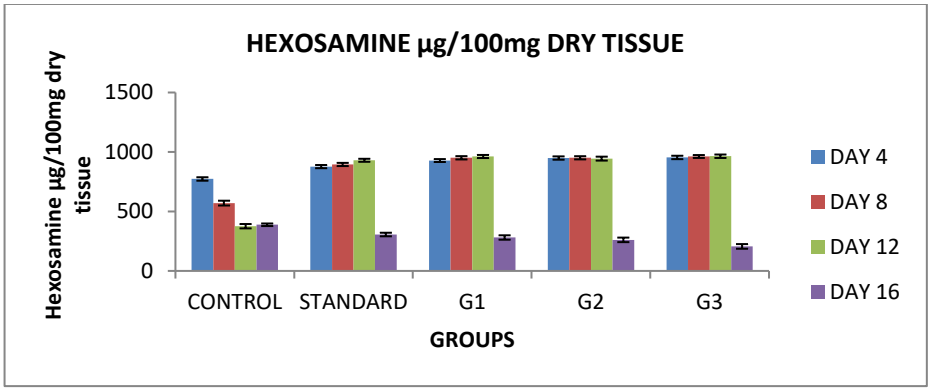
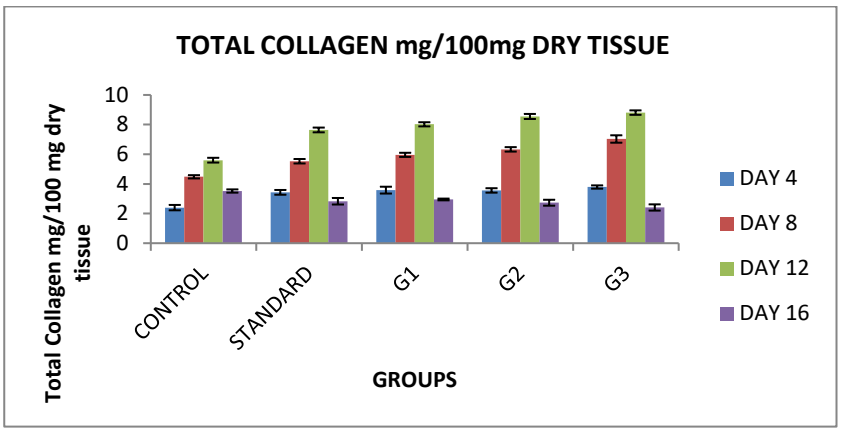


FIGURE 7: The Effect of Formulations on Total Collagen levels in Rats



DISCUSSION

Wound healing is a complex, multifactorial process differs pathologically and making it difficult to understand the underlying mechanism. Perfect wound healing is demarcated with complete closure of wounds in less span of time without any adverse effect. In the present investigation it was observed that polyherbal gel (G3 formulation) on topical application showed potent wound healing property, indicating that it enhances various phases of healing process. The potent wound healing property of G3 gel may be due to the synergistic activity of phytochemical constituents such as flavonoids (Tsuchiya et al., 1996), triterpenoids (Scortichini et al., 1991) and tannins (Rane et al., 2003). Phytoconstituents such as flavonoids, triterpenoids possess free radical scavenging property (Tran et al., 1997), modulate the immune system which is complementary to wound healing process.

Wound healing is a natural and inbuilt process but delayed by oxidative stress, diabetes mellitus and by microbial infection (Bodeker et al., 1998). Wound healing process involves cell proliferation, inflammation supression and contraction of the collagen (Houghton et al., 2005). Antioxidant activity helps in the release of oxygen radicals, reduces oxidative stress thus controlling microbial infection and clearing the wound fibrin matrix, thus enhancing the healing process (Grinnell et al., 1994). G3 formulation shown better wound healing activity and it may be due to antioxidant activity of flavonoids.

Wound healing process involves several steps such as hemostasis, coagulation, inflammation, granulation tissue formation, matrix formation, connective tissue remodeling, collagenization and acquisition of wound strength (Suresh reddy et al., 2002). In excision wound, all three phases coexist. The results of the excision wound model manifest that the percentage of wound contraction is 83.33±1.52% in standard treated group on the 16th day and in control it is 66.83±1.19%; whereas it is 90.50±1.72% in G3 formulation treated group. Wound contraction on the 16th day itself is increased in G3 when compared with control and standard groups.

Re-epithelialization is a process of epidermal restoration and it involves proliferation and migration of keratinocytes. Dermal and epidermal regeneration in G3 gel treated rats is increased indicating that the gel had a constructive effect towards cellular proliferation, granular tissue formation and epithelialization.

The present investigation confirmed that the rate of wound contraction was higher and epithelialisation period was shorter in G3 gel treated rats. In conclusion, topical administration of G3 gel accelerated scar formation and promotes various phases of wound healing such as collagen synthesis, wound contraction and epithelialization.

The evaluation of the biochemical parameters revealed the amplified DNA content in the treated wounds, indicating cellular hyperplasia. Increase in the total protein content represents the active synthesis and deposition of matrix proteins in the granulation tissues. In incision wound model the tensile strength of the tissues was measured on 10^{th} day and in control group it was 375±20.4%, and in standard treated group was 646±15.4% while in G3 gel treated group it was 849±15.6%. From the above results it is inferred that the tensile strength was almost tripled in G3 treated rats in comparison with control group and this augment in tensile strength of G3 treated group may be due to

the increase in collagen, hexosamine content and stabilization of the fibres by increased protein concentration (Shetty et al., 2008; Udupa et al., 1995). New extracellular matrix synthesis is improved by the matrix molecules such as hexosamine and uronic acid. The collagen synthesized gets deposited at the wound site and undergoes cross linking to form fibres. Collagen not only offers strength and integrity to the tissue matrix but also plays an imperative role in homeostasis and in epithelialization at the later phase of healing.

Hydroxyproline, the major constituent of collagen serves as a marker of collagen synthesis at the wound site. The amount of collagen content in granulation tissues of control and experimental induced wounds was measured suggesting that G3 formulation enhanced collagen synthesis and deposition and it is due to increased cell division. Hexosamine helps in proper deposition and alignment of collagen. When compared with control, G3 gel treated rat's depicted increased hexosamine levels until day 8 post wounding and declined thereafter. Similar tendency was reported in previous studies that there is an increase in the levels of these parameters during the early phases of wound healing, following which normal levels are restored (Dunphy et al., 1956).

CONCLUSION

It can be concluded from the present findings that polyherbal G3gel could be efficiently used as a wound-healing agent. Wound contraction, increased tensile strength activity, increased hexosamine levels and collagen synthesis support this. Wound healing activity increased with an increase in herbal extract concentration (G3 high concentration 4% w/w). Further studies have to be emphasized on isolating the bioactive molecule responsible for wound healing activity.

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

REFERENCES

1. Hashemi SA., Madani SA., Abediankenari S. The review on properties of Aloe vera in healing of cutaneous wounds. Biomed. Res. Int, 2015, 714216.
2. Orsted HL, Keast D, Forest-Lalande L, Medie MF. Basic principles of wound healing. Wound Care Canada, 9, 2004, 4–12.
3. Branski LK, Gauglitz GG, Herndon DN, et al. A review of gene and stem cell therapy in cutaneous wound healing. Burns, 35(2), 2008, 171-80.
4. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle), 4 (9), 2015, 560-582.
5. Babu M, Gnanamani A, Radhakrishan N, and Priya K. Healing potential of Datura alba on burn wounds in albino rats. J. Ethnopharmacology, 83(3), 2002, 193-199.
6. Clark RAF. Cutaneous wound repairs. In: Goldsmith LA editors. Physiology: Biochemistry and Molecular Biology of skin. New York: Oxford University Press, 1991, 576.
7. Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: a review. Int J Low Extrem Wounds, 2(1), 2003, 25-39.
8. OECD. Guidelines for the Testing of Chemicals/Section 4: health effects: Acute Dermal Toxicity Test No. 402, 1987.
9. Saha K, Mukherjee PK, Das J, Pal M, Saha BP. Wound healing activity of Leucas lavandulaefolia Rees. J Ethnopharmacol, 56(2), 1997, 139–44.
10. Hemalatha S, Subramanian N, Ravichandran V, Chinnaswamy K. Wound healing activity of Indigofera ennaphylla Linn.. Indian J Pharm Sci, 63, 2001, 331–3.
11. Sadaf F, Saleem R, Ahmed M, Ahmed SI, Navaid-ul-zafer. Healing potential of cream containing extract of Sphaeranthus indicus on dermal wounds in guinea pigs. J Ethnopharmacol,107(2), 2006, 161–3.
12. Lowry OH, Rosebrough NJ, Farr Al Randall RT. Protein measurement with the folin phenol reagent. J Biol Chem, 193(1), 1951, 265–76.
13. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J, 62(2), 1956, 315–323.
14. Schiller S, Slover G, Dorfman AA. A new perspective with particular reference to ascorbic acid deficiency. J Biol Chem, 36, 1961, 983–5.
15. Woessner JR. The determination of hydroxyl proline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys, 93, 1979, 440–7.
16. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin resistant Staphylococcus aureus. J Ethnopharmacol, 50(1), 1996, 27–34.
17. Scortichini M, Pia Roosi MJ. Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards Erwinia amylovora. J Appl Bacteriol, 71, 1991, 109–12.
18. Rane M, Madhura, Mengi A, Shusma. Comparative effect of oral administration and topical application of alcoholic extract of Terminalia arjuna bark on incision and excision wounds in rats. Fitoterapia, 74(6), 2003, 553–8.
19. Tran VH. Antioxidant and free radical scavenging effects of some polyphenolic extracts from medicinal plants on wound

healing. In: Abstract, PAN/Asian European Tissue Repair Society Post-satellite Meeting, 1997, 2–3.

20. Bodeker G, Hughes MA. Wound healing, traditional treatments and research policy. Plants for food and medicine. Kew, London: Royal Botanic Gardens, 1998, 245–359.
21. Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. In vitro tests and ethnopharmacological investigations: wound healing as an example. J Ethno-pharmaco, 100 (1-2), 2005, 100–7.
22. Grinnell F, Zhu MF. Identification of neutrophil elastase as the proteinase in burn wound fluid responsible for degradation of fibronectin. J Invest Dermatol, 103 (2), 1994,155–61.
23. Suresh Reddy J, Rajeswara Rao P, Mada Reddy S. Wound healing effects of Heliotropium indicum, Plumbago zeylanicum and Acalypha indica in rats. J Ethnopharmacol, 79(2), 2002, 249–51.
24. Shetty S, Udupa S, Udupa L. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of Ocimum sanctum Linn. in rats. Evid Based Complement Altern Med, 5(1), 2008, 95–101.
25. Udupa AL, Kulkarni DR, Udupa SL. Effect of Tridax procumbans extracts on wound healing. Int J Pharmacogn, 33(1), 1995, 37–40.
26. Dunphy JE, Udupa KN. Wound healing. A new perspective with particular reference to ascorbic acid deficiency. Ann Surg, 144(3), 1956, 304–16.

***Corresponding Author:**
Vinitha Edula
Email: vinita.eedula@gmail.com