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Research Article

Formulation Development and Evaluation of New Polyherbal Gel Formulations for Their Wound Healing Activity in Rat

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Abstract

Synoptic studies on natural products have been conducted as a result of the lack of a potential remedy and associated flaw in allopathic medicines, as these items have been found to be less harmful and more affordable. The identification and assessment of therapeutic potential in pharmaceuticals led to the development of novel, affordable medications for the treatment of a variety of ailments, including chronic wounds. To determine if plant extracts can effectively cure wounds, in-vitro cell scratch testing is a practical and affordable method. Traditional herbal remedies for treating wounds include Azadirachta indica (A. indica) and Aloe barbadensis miller (Aloe vera, *A. vera*). The goal of the current study was to assess the ability of a polyherbal formulation (A. vera and A. indica) to promote wound healing in Wistar albino rats utilising excision, incision, and dead space wound models. A. vera and A. indica extracts were used in varied concentrations to create formulations PHF1 through PHF5. Utilising criteria like physical appearance, pH, extrudability, viscosity, spreadability, homogeneity, grittiness, and stability study, the generated polyherbal gel formulation was assessed. On days 3, 6, 9, 12, 15 and 21, as well as day 24, wound healing was observed along with the percentage of wound contraction, epithelialization period, hydroxyproline content, tensile strength, granuloma weight, and protein content (dead space wound models). In an excision wound model, polyherbal gel PHF4 demonstrated percent wound contraction in 12 days, and Groups II and IV saw considerably (P 0.01) shorter epithelialization times. In incision wound models, the animal group treated with polyherbal gel PHF4 demonstrated significantly higher levels of hydroxyproline than all other formulations as well as significantly higher tensile strength. PHF4 demonstrated the best efficacy in dead space wound models compared to other extracts and was significantly different from the treatment group in terms of granuloma weight and protein content. It can be said that poly herbal formulations have wound healing properties, possibly as a result of their epithelization, supporting the traditional assertion that they can be used to treat many human wound types.

Keywords: Wound healing, *Azadirachta indica, Aloe barbadensis,* Polyherbal formulation, Excision, Incision, Dead space wound models

INTRODUCTION

The process of cellular and metabolic reactions that are triggered in response to an injury to restore the function and integrity of damaged tissues is known as wound healing[1]. 4 overlapping phases of re-epithelialization, which includes the proliferation, migration, and differentiation of epithelial cells of the epidermis (3–12 days), collagen deposition and remodelling within the dermis (3-6 mo), and inflammation all occur as part of continuous cell-cell and cell-matrix interactions during wound healing[2]. Tumour necrosis factor alpha (TNF) and the interleukin (IL) family, growth factors, platelet-derived growth factors, epidermal growth factor, insulin-like growth factor (IGF-1), fibroblast growth factor, vascular endothelial growth factor (VEGF), granulocyte macrophage colony stimulating factor (GM CSF), and connective tissue growth factor (CTGF) all play important roles in wound healing[3-5]. Traditional (herbal and animal-derived substances, living organisms, silver-based treatments, and traditional dressings) and modern (grafts, modern dressings, bioengineered skin substitutes, and cell/growth factor therapies) are the two primary groups into which wound healing therapies can be divided. Different levels of clinical acceptance, efficacy, and side effects exist for these treatments[6]. Due to their efficiency and safety, herbal medications are the most widely used traditional medicines for the treatment of skin wounds[7]. The scientific literature is replete with information on the antibacterial and wound healing properties of herbs, including coagulation, inflammation, fibroplasia, epithelialization, collagenation, and wound contraction[8,9]. Additionally, a fast wound healing period is linked to a low risk of infection, complications, and expense. Due to the wide range of physiologically active substances that plants produce, it seems that herbal remedies can be utilised effectively as wound healing stimulators. A examination of the literature reveals that no systematic approach has been used to study how these two plants' leaves can treat wounds. Three models were used in the current investigation to assess the topical wound-healing abilities of a

polyherbal formulation in comparison to povidion-iodine ointment in wistar albino rats.

MATERIALS AND METHODS

Plant material

In the period from January to April 2019, the leaves of Azadirachta indica and Aloe barbadensis were gathered in the Bhopal region of Madhya Pradesh, India. Dr. Saba Naaz, a botanist at the Saifia College of Science in Bhopal, carried out the plant's identification and authentication. For future use, a voucher specimen with the numbers 245/Saif./Sci./Clg/Bpl and 246/Saif./Sci./Clg/Bpl was stored in the botany department at Saifia College of Science in Bhopal for A. vera and A. indica, respectively. A chopper was used to clean and slice the gathered A. vera leaves into small pieces. After that, it was dried for 16 hours using a solar dryer. A crushing device was used to break up the dry bulk, and it was then sieved (200 mesh). Finally, sodium meta bisulphite (0.1%) was added to it while triturating. The final output was the desired A. vera powder, which was brown in colour. A. indica leaves were initially cut off from the body of the plant and rinsed with distilled water, dried in the shade, homogenised into a fine powder, and then packaged in airtight containers.

Chemical reagents

The HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this study.The investigation only employed analytical-grade compounds.

Preparation of extracts

dried by air For 4-5 hours, ethanol was extracted from coarsely ground plant materials of (A. vera and A. indica) using a soxhlet equipment. By using a rotary flash evaporator to concentrate all the extracts under low pressure, they were then all air-dried[10].

Preparation of polyherbal gel containing plant extract

The gel formulation comprising plant extracts was created by combining A. vera and A. indica extract in gel base to produce PHF1 through PHF5, and it was then assessed using the parameters stated before (Tables 1 and 2). The amounts of the ingredients for the gel shown in Table 2 In a beaker with around 25 ml of water, methyl paraben sodium (50 mg), glycerine (5 ml), and polyethylene glycol (1ml) were dissolved. Using a mechanical stirrer, the dissolved mucilaginous components were quickly stirred. Then, while stirring, Carbopol 934 (1gm) and PVP (25 mg) were gradually added to the beaker containing the liquid mixture described above. Triethanolamine (1ml) was slowly added to the gel solution containing plant extract while stirring to act as gelling agents, resulting in a translucent gel structure with the highest viscosity. Finally, the gel from the plant extract was put into a tube made of collapsible aluminium and marked.

Table 1: Composition of gel base

Formulation	Carbopol 934 (g)	PVP (mg)	Methyl paraben Sodium (mg)	Glycerine (ml)	PEG (ml)	Triethanolamine (ml)
Gel base	1.0	25	50	5	1	1

Table 2: Formulation preparation of gel with *A. vera* and *A. indica* extract

Formulation	PHF1	PHF2	PHF3	PHF4	PHF5
Neem extract (g)	30	20	15	10	0
Aloe vera gel (g)	0	10	15	20	30
Gel base (g)	70	70	70	70	70

Evaluation of base gel formulation

Physical appearance: The physical attributes of the created gel base, such as colour, look, and feel upon application, were visually examined.

pH determination: Using a digital pH metre, the pH of the gel base was determined. In 20 ml of distilled water, 0.5 grammes of gel base were dissolved and left to stand for two hours. pH was measured by fully dipping pH electrodes into the mixtures. Each formulation's pH was measured three times, with the average readings being computed.

Extrudability determination: Metal tubes with foldable ends were filled with the gel base. The extrudability of the formulations was assessed by pressing the tubes with the same amount of pressure using the fingers. The weight in grammes needed to extrude a 0.5 cm gel ribbon in 10 seconds was used to gauge the formulation's extrudability.

Viscosity determination: A Brook field viscometer was used to gauge the gel base's viscosity. The wide mouth jar was separately filled with an adequate amount of gel base, which should be enough to allow dipping the spindle. The spindle's RPM was changed to 2.5 RPM. The formulas' viscosities were noted.

Spreadability: Spreadability is the region over which gel spreads easily when applied to skin or a skin-affected area. The spreadability of a formulation affects its medicinal effectiveness as well. The spreadability of a formulation is measured in terms of how long it takes two slides put between them to separate from a gel base under the influence of a specific load. Better spreadability is achieved with shorter gap times between two slides. With the use of the formula, it is calculated.

$$S = M * L / T$$

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

Homogeneity: The container has been filled with the gel base compositions, and visual inspection was used to check the homogeneity of each created gel. They had examinations to check for lumps, flocculates, and aggregates.

Grittiness: Under a light microscope, the gel base compositions were examined microscopically to see whether

any detectable particle matter was present. The preparation must be devoid of particles, and any topical preparation's gritiness can be tested.

Pharmacological screening

Animals

Healthy albino wistar rats of either sex, weighing 180 to 230 grammes, were selected for the investigation. There were seven groups, each with six animals. The animals were kept in temperature ranges of 18 to 20°C with light and day cycles of 12:12 hours. They were housed during the trial in a spacious, spotless cage. Animals had full access to water and a regular pellet meal up to the study's completion. The animal studies were approved by the Institutional Animal Ethics Committee of the PBRI, Bhopal (Reg. No. 1824/PO/RcBi/S/15/CPCSEA), which was formed for the management and oversight of experimental animals. The protocol's approval code was PBRI/IAEC/10-09-22/013.

Acute toxicity studies of *A. vera and A. indica* extracts as per OECD guideline: The goal of this study was to determine the toxicological profile of the test substance when administered orally once (single dosage) to the test system (rats) and to monitor the vital signs for 14 days. The study used extracts of A. vera and A. indica. This study will provide details on the potential health risks that could result from a single oral intake of plant extracts. According to OECD guidelines, acute toxicity studies of A. vera and A. indica extracts were carried out in Albino rat dose levels of 50, 300, and 2000 mg/kg. The treated animals behaved normally and showed no discernible alterations in their behaviour patterns. The presence of tremors, convulsions, exophthalmos, salivation, diarrhoea, lethargy and other clinical symptoms was also absent. The mean body weights between the treated groups and the control group did not differ significantly, and the rats gained typical amounts of weight while participating in the experiment. Following a single oral administration of all extracts at the chosen dose levels throughout the test period, no fatal effects or animal mortality were noticed. After 14 days, the animals were tested for long-term toxicity.

Wound healing activity

A group of animals: The effectiveness of the herbal gel formulation's ethanol water extract for wound healing was assessed using the excision, incision, and dead space wound models. In order to create excision, incision, and dead space wound models, the wistar rats were separated into a number of groups, each group consisting of six animals. Each animal received a daily topically administered 5% and 10% extract-containing formulation gel.

Group I: The animals of received gel base (control)

Group II: Treated group with a marketed povidone formulation

Group III: Polyherbal gel PHF1

Group IV: Polyherbal gel PHF2

Group V: Polyherbal gel PHF3

Group IV: Polyherbal gel PHF4

Group V: Polyherbal gel PHF5

Excision wound model: Before creating wounds, the animals were divided into groups of six and given an open mask anaesthetic with ether. The targeted skin was removed one day before the test. Cutting away a 300 mm2 complete thickness of skin from a preset shaved area resulted in an excision wound. The wounds were exposed to the outside air naked. To the control group, standard group, and treatment group, respectively, the base gel, standard medication ointment (povidone), and polyherbal gel were applied topically until the wound was fully healed. The period of wound contraction and epithelialization was tracked in this model. The amount of wound contraction was calculated as a percentage over the first two days following wound creation. Each rat had a sample of tissue removed from the healed wound for histological analysis[11].

Incision wound model: All of the animals in each group were put to sleep using light ether anaesthesia for the incision wound model. On either side of the rat's depilated back, two full thickness paravertebral long incisions were made through the skin at a distance of roughly 1 cm from midline. A curved needle (number 11) and black silk surgical thread (no. 000) were used to sew the skin's edges together after the incision was made. To ensure a complete closure of the wound, the continuous threads on both wound edges were tightened. After the stitches were removed, the wound was left uncovered and treated with ointment base, standard ointment, and extract ointment every day for 10 days. When the wounds had fully healed, the sutures were removed on day 10 and the tensile strength of the skin had been assessed using a tensiometer[12].

Dead space wounds: Light ether was used to anaesthetize the animals, and two polypropylene tubes (2.00.5), one on either side, were implanted to create wounds in the lumber region of the dorsal surface of each animal. The granuloma tissue that had developed on an implanted tube was carefully removed on the ninth post-wounding day. For the biochemical parameters and histological investigation, granuloma tissue from one tube was dried at 60°C and preserved in 10% formalin, while the other portion of the granuloma tissue was utilised to calculate tensile strength. A tensiometer was used to measure the tensile strength.

Wound healing evaluation parameters

Measurement of wound contraction: By tracking the incremental changes in the wound area plan metrically, excluding the day of wounding, an excision wound margin was identified. Throughout the monitoring period, the size of the wounds was recorded every two days on a transparent piece of paper. The wound surface area was assessed when the tracing was transferred to graph paper. After that, using the evaluated surface area and the following calculation, the original wound size of 300 mm2 was used to determine the percentage of wound contraction.

% wound contraction = initial wound size − specific day wound size initial wound size × 100

Epithelialization period: It was measured by keeping track of how long it took the Escher to remove itself from the wound surface without also leaving a raw wound.

Measurement of tensile strength: Tensile strength is the measure of the force needed to initiate the healing process. It is used to gauge how fully a person has recovered. Additionally, it shows how much the restored tissue can withstand tension without breaking, which may potentially hint at the quality of the mended tissue. On the ninth day following the wounding, the sutures were removed, and on the tenth day, the tensile strength was assessed. The newly produced tissue, including the scar, was removed for this purpose, and tensile strength was assessed using a tensiometer[13]. The weight of water per area of the specimen at the time the wound broke was used to calculate the wound-breaking strength in this method.

Hydroxyproline estimation: The collagen fibres of granulation tissues contain the unusual amino acid

hydroxyproline. Its estimation aids in the clinical understanding of the rate at which the wound's connective tissue is undergoing the healing process. The wound tissues were removed and dried in a hot air oven at 60 to 70°C to a consistent weight before being hydrolyzed in 6N HCl at 130°C for 4 h in sealed glass tubes to determine the amount of hydroxyproline. The hydrolysate underwent 20 minutes of Chloramine-T oxidation after being pH neutralised to 7.0. The reaction was stopped by adding 0.4 M perchloric acid, and colour was produced at 60 °C with the aid of the Ehrlich reagent. A UV spectrophotometer (Shimadzu, Japan) was used to measure the absorbance at 557 nm. A standard curve created with pure l-hydroxyproline served as the basis for calculating the amount of hydroxyproline present in the samples.

Stability studies: The rapid stability testing has received the most attention. For three very essential reasons patient protection, official concerns about the uniqueness, potency, transparency, and attributes of the drug, it is crucial to research the stability of pharmaceutical inventions. The medicine's shelf life was determined to prevent drug breakdown while being stored. Due to chemical changes or product instability, the degradation may cause the medication concentration in the patch formulation to decrease. The ICH guidelines are used to evaluate the patches' stability. In polythene bags, the created optimized gel was kept at three different temperatures and relative humidity levels. In-vitro characterization and in-vivo performance evaluation served as the foundation for the selection of formulation code PHF4 for stability tests. The chosen formulation was kept in twist-top amber painted bottles for 180 days in a humidity stability chamber at 22°C, 25°C & 60% RH, 40°C & 75% RH, 50°C & 75% RH, and 60°C & 75% RH. Samples were kept in stability chambers for 90 days at 25°C and 60% and 75% RH, respectively. Over the course of three months, these samples were checked for any changes in their physical characteristics at various intervals[13].

RESULTS AND DISCUSSION

Formulation *A. vera* and *A. indica* extracts were used in various concentrations to create PHFs 1 through 5. Evaluation criteria for the developed polyherbal gel formulation included physical appearance, pH measurement, extrudability measurement, viscosity measurement, spreadability, homogeneity, and grittiness (Table 3). Within 10 and 12 days, respectively, a superior healing pattern with full wound closure was seen in the standard and treated groups, whereas it took control rats roughly 24 days. Cutting away a 300 mm2 complete thickness of skin from a preset shaved area resulted in an excision wound. The wounds were exposed to the outside air naked. To the control group, standard group, and treatment group, respectively, the base gel, standard medication ointment (povidone), and polyherbal gel were applied topically until the wound was fully healed. The period of wound contraction and epithelialization was tracked in this model. The amount of wound contraction was calculated as a percentage over the first two days following wound creation. After 12 days, the wound in the Group VI animal that received the polyherbal gel PHF4 had contracted by %. In 21 days after receiving PHF1, Group III animal displayed a percent wound contraction. Table 4 and Figure 1 reveal that the animal group treated with Polyherbal Gel PHF4 had the best outcomes of any formulation. From the first day, the epithelialization time was calculated. Groups II and IV were shown to have considerably (P 0.01) shorter epithelialization times. The polyherbal gel PHF4-treated animal in Group VI displayed epithelialization after 12 days. The wound of the Group III animal that got PHF1 began to close in 19 days. Animals treated with polyherbal gel PHF4 displayed substantial recovery across all formulations. Figure 2 and Table 5. A significant portion of the ground substance of granulation tissue is hydroxyproline. Animals treated with polyherbal gel PHF4 had hydroxyproline concentration of 62.02 ±0.008 mg/g tissue, which is significant compared to the control group's 71.22 ±0.006 mg/g tissue. The hydroxyproline concentration of the Group III animal treated with PHF1 was 41.02± 0.009, in contrast. Animals treated with polyherbal gel PHF4 had the highest levels of hydroxyproline of all formulations. Table 6 and Figure 3. A wound's load capacity per unit area is measured by its tensile strength. Whatever its size, a wound's bursting strength is the amount of force needed to shatter it. Skin thickness affects the strength of the burst. The tensile strength of the polyherbal gel PHF4 treated group animal was 540.34± 3.01 g/mm2, which is significantly higher than the tensile strength of the control group (590.04± 4.01 g/mm2). The tensile strength of the Group III animal that got PHF1 was 475.31± 3.01 g/mm2, in contrast. Animals treated with polyherbal gel PHF4 displayed the highest tensile strength of any formulation. Figure 4 and Table 7. When compared to the control group, the treated group significantly increased the amount of hydroxyproline (P<0.01). Monitoring the level of hydroxyproline, a sign of collagen manufacturing, can be used to determine the amount of collagen present in the granulation during the healing period. Faster wound healing is associated with higher concentrations of hydroxyproline, which reflects enhanced cellular proliferation and thus higher collagen synthesis. Poor wound healing is indicated by lower hydroxyproline concentrations. The amount of hydroxyproline in the wound granulation tissue increased in Group VI following treatment with PHF4 polyherbal gel (Table 8 and Figure 5). On day 10, it was discovered that the treated group's tensile strength was significantly higher (P <0.01) than the control group's. Group VI animal treated with polyherbal gel PHF4 displayed tensile strength of 570.14 ±3.25 g/mm2, which is significantly equivalent to the untreated group's tensile strength of 591.04± 2.01 g/mm2. The tensile strength of the Group III animal that received PHF1 was 591.04 ±2.01 g/mm2, in contrast. Among all formulations, the polyherbal gel PHF4 treated animal group demonstrated the greatest tensile strength in rat dead space wound models. Figure 6 Table 9. Granuloma weight content on dead space anaemic wound models was calculated for Group VI and revealed a result of 435.1 ±9.7 (mg/100 g), which was the best result of any extract and significant with the treated group. The findings indicated that PHF4 had the best effect compared to other extracts and was significant for the treated group. Figure 7 and Table 10. According to Group VI results, the protein content on dead space anaemic wound models was 70.3 1.2 (mg/g wet tissue), the best result of any polyherbal group and significantly higher than the control group. The results were significant with the treatment group and demonstrated that PHF4 had the best effect of any polyherbal gel (Table 11 & Figure 8). The polyherbal gel PHF4 was a semi-solid gel made from polymeric materials with plant extract at temperatures of 22°C, 25°C and 60% relative humidity, 40°C and 75% RH, 50°C and 75% RH, and 60°C and 75% RH for 180 days. The ability of a formulation's ingredients to persist within predetermined boundaries for a predetermined period of time is known as the product's shelf life. The research showed that PHF4 did not change physically during storage at a temperature of 25°C and 60% RH. It might suggest that the formulas have a minimum 2-year shelf life. Table 12.

Table 3 Physical parameters of polyherbal gel formulation

Parameters	PHF1	PHF2	PHF3	PHF4	PHF5
Colours	Green colour	Green colour	Light green colour	Pale yellow colour	cream colour
Appearance	Translucent	Transparent	Translucent	Transparent	Translucent
Odour	Pleasant odour	Define odour	Pleasant odour	Define odour	Pleasant odour
Feel of application	Smooth	Smooth	Smooth	Smooth	Smooth
Spreadability (g.cm/sec)	8.9	10.7	11.9	10.0	9.2
Consistency	Poor	Fairly good	Good	Excellent uniform	Good
pH	6.88	6.88	6.43	7.54	6.22
Viscosity (cps)	0.91	0.99	0.91	1.06	0.91
Extrudability	Poor	Good	Good	Excellent	Good
Stability	Stable	Stable	Stable	Stable	Stable

Table 4: Effect of polyherbal gel formulation on % of wound contraction of excision wound models in rats

Group	0 Days	3 Days	6 Days	9 Days	12 Days	15 Days	18 Days	21 Days	24 Days
Group I	0	27.34	34.28	47.64	51.4	76.15	85.43	93.76	100
Group II	0	52.24	74.32	99.15	100				
Group III	0	20.12	39.14	58.21	65.11	71.02	91.02	100	
Group IV	0	21.14	41.24	61.02	69.24	78.17	96.24	100	
Group V	0	35.21	50.24	73.61	82.45	100			
Group VI	0	47.68	68.61	81.41	99.82	100			
Group VII	0	27.12	47.24	68.21	78.21	94.14	100		

Table 5: Effect of plant extract gel on epithelialization time of excision anaemic wound models in rats

Group	Epithelialization time (Days)
Group I	22
Group II	10
Group III	19
Group IV	16
Group V	14
Group VI	12
Group VII	18

Table 6: Effect of polyherbal gel formulation on hydroxyproline on incision wound models in rats

Group	Hydroxyproline (mg/g tissue)
Group I	25.11 ± 0.007
Group II	71.22 ± 0.006
Group III	41.02 ± 0.009
Group IV	48.13 ± 0.011
Group V	53.13 ± 0.002
Group VI	62.02 ± 0.008
Group VII	44.27 ± 0.001

Table 7: Effect of polyherbal gel formulation on tensile strength on incision wound models in rats

Group	Tensile strength (g/mm2)
Group I	394.21 ± 3.21
Group II	590.04 ± 4.01
Group III	475.31 ± 3.01
Group IV	502.21 ± 3.17
Group V	510.32 ± 2.31
Group VI	540.34 ± 3.01
Group VII	489.11 ± 2.14

Table 8: Effect of polyherbal gel formulation on hydroxyproline on dead space wound models in rats

Group	Hydroxyproline (mg/g tissue)
Group I	24.15 ± 0.005
Group II	70.26 ± 0.007
Group III	45.12 ± 0.002
Group IV	55.93 ± 0.001
Group V	59.13 ± 0.002
Group VI	63.02 ± 0.003
Group VII	50.27 ± 0.001

Table 9: Effect of polyherbal gel formulation on tensile strength on dead space wound models in rats

Group	Tensile strength (g/mm2)
Group I	389.14 ± 5.21
Group II	591.04 ± 2.01
Group III	480.31 ± 4.21
Group IV	529.11 ± 2.18
Group V	549.32 ± 2.31
Group VI	570.14 ± 3.25
Group VII	510.11 ± 3.65

Table 10: Effect of polyherbal gel formulation on granuloma weight on dead space wound models in rats

Group	Granuloma weight mg/100 g
Group I	210.8 ± 9.2
Group II	450.1 ± 9.4
Group III	380.5 ± 9.2
Group IV	405.2 ± 10.3
Group V	421.7 ± 10.1
Group VI	435.1 ± 9.7
Group VII	390.7 ± 9.5

Table 11: Effect of polyherbal gel formulation on protein content on dead space anaemic wound models in rats

Group	Protein content mg/g wet tissue
Group I	49.1 ± 1.4
Group II	78.5 ± 2.1
Group III	48.2± 1.2
Group IV	59.1± 2.3
Group V	62.1± 1.3
Group VI	70.3± 1.2
Group VII	54.8± 1.6

Table 12: Stability study of polyherbal gel PHF4

S. No.	Temperature (°C)	Room humidity (RH)	Physical nature				
			Time in days				
			0	30	60	90	180
1	2°C	-	No change in color	No change in color	No change in color	No change in color	No change in color
2	25°C	60% RH	No change in color	No change in color	No change in color	No change in color	No change in color
3	40°C	75% RH	No change in color	No change in color	No change in color	No change in color	No change in color
4	50°C	75% RH	No change in color	No change in color	No change in color	No change in color	No change in color
5	60°C	75% RH	No change in color	No change in color	No change in color	No change in color	No change in color

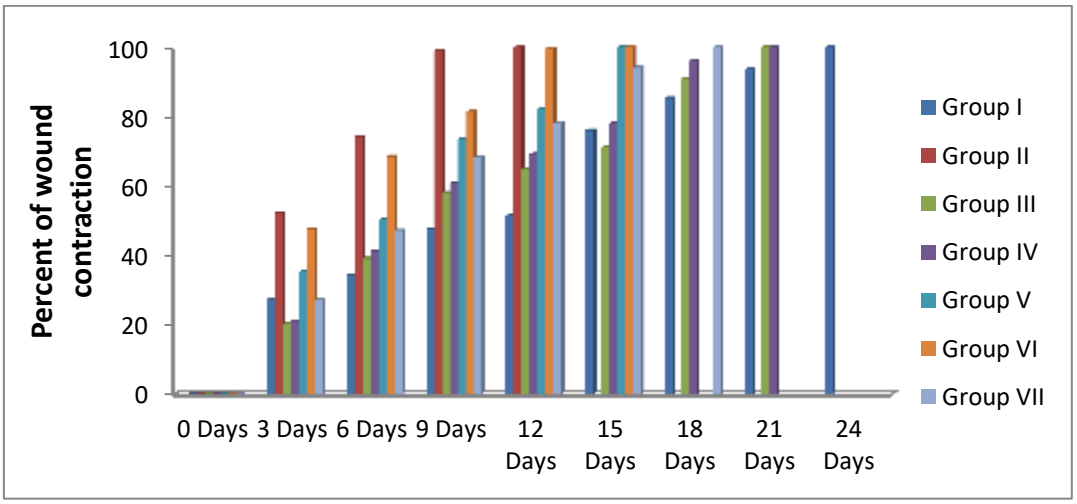

Figure 1: Effect of polyherbal gel formulation on % of wound contraction of excision wound models in rats

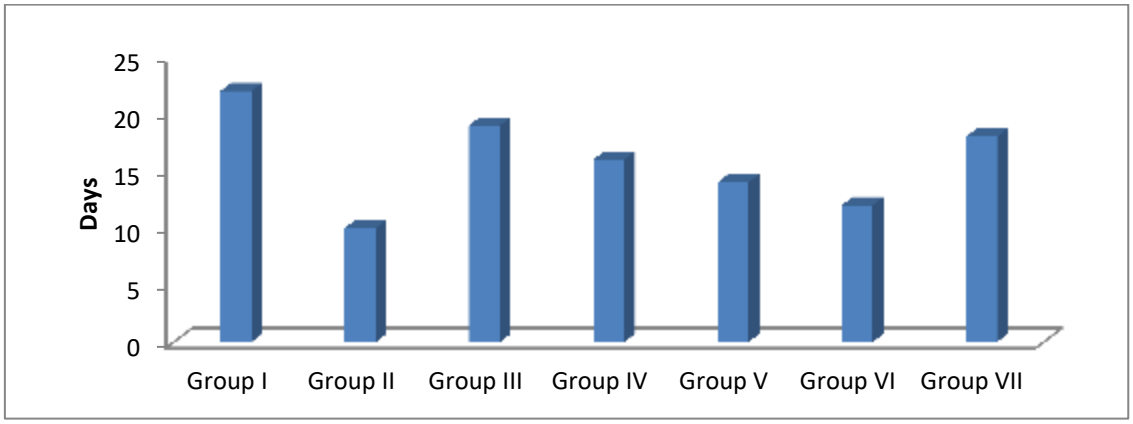

Figure 2: Effect of polyherbal gel formulation on epithelialization time of excision wound models in rats

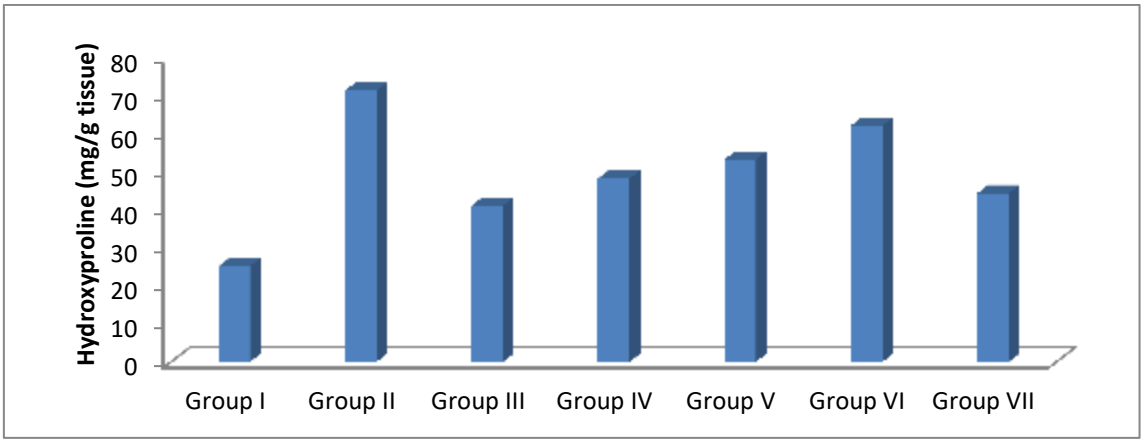

Figure 3: Effect of polyherbal gel formulation on hydroxyproline on incision wound models in rats

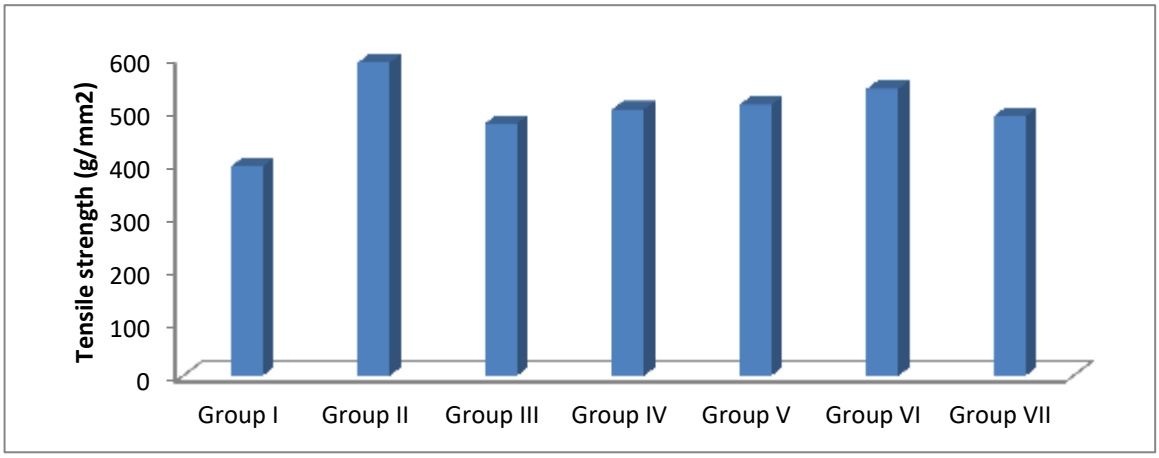

Figure 4: Effect of polyherbal gel formulation on tensile strength on incision wound models in rats

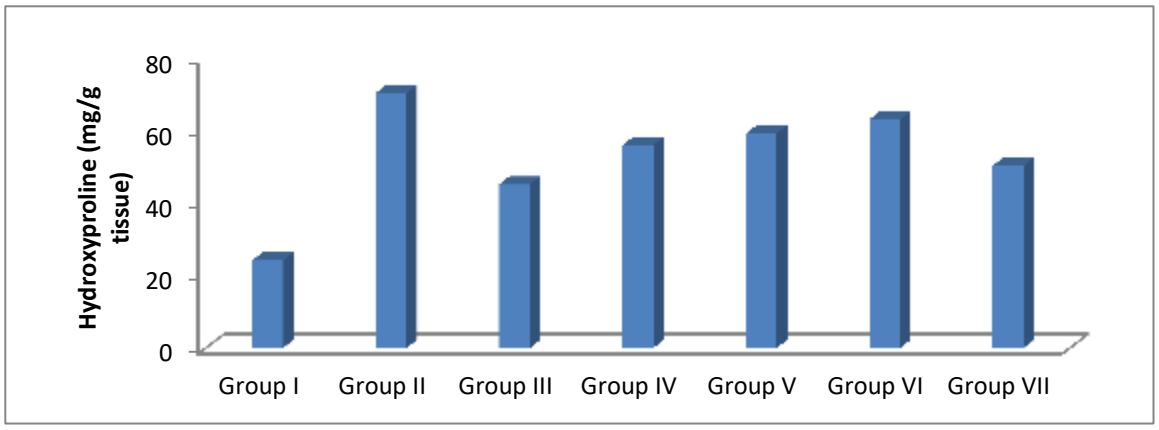

Figure 5: Effect of polyherbal gel formulation on hydroxyproline on dead space wound models in rats

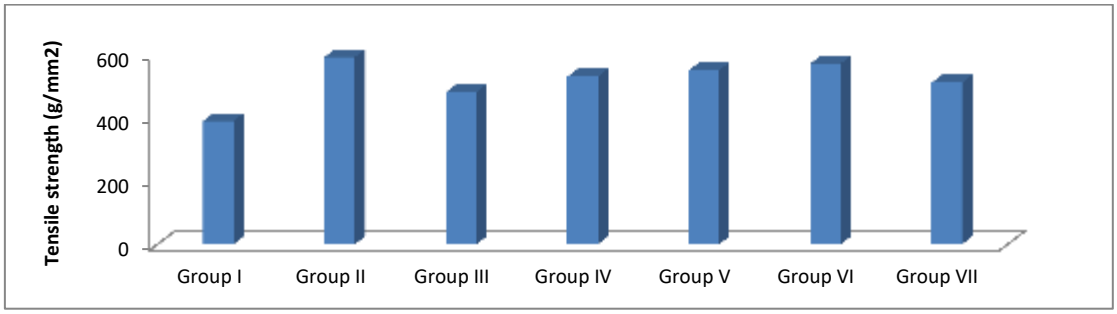

Figure 6: Effect of polyherbal gel formulation on tensile strength on dead space wound models in rats

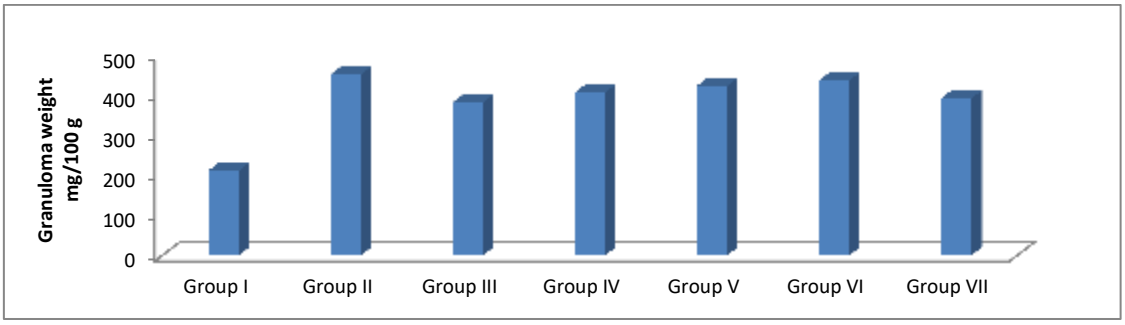

Figure 7: Effect of polyherbal gel formulation on granuloma weight on dead space wound models in rats

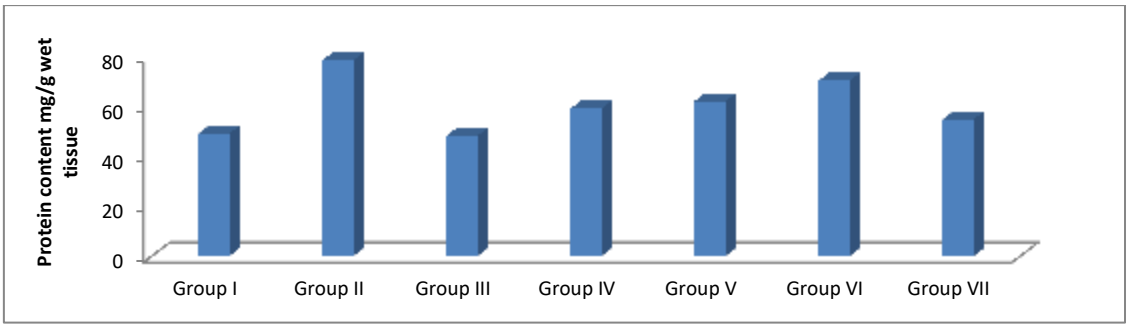

Figure 8: Effect of polyherbal gel formulation on protein content on dead space anaemic wound models in rats

CONCLUSION

In excision, incision, and dead space wound models, the ethanolic extract of the poly herbal formulation demonstrated considerable wound healing efficacy that is comparable to the commercial povidone formulation. This discovery sheds light on how the poly herbal mixture is applied in conventional wound or burn care for bacterial infections.

REFERENCES

1. Gonzalez ACDO, Costa TF, Andrade ZDA, Medrado ARAP. Wound healing- a literature review. An Bras Dermatol 2016; 91:614-620. https://doi.org/10.1590/abd1806-4841.20164741

2. Rittie L. Cellular mechanisms of skin repair in humans and other mammals. J Cell Commun Signal 2016; 10:103-120. https://doi.org/10.1007/s12079-016-0330-1

3. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Rep Regen 2008; 16: 585-601. https://doi.org/10.1111/j.1524-475X.2008.00410.x

4. Caley MP, Martins VL, O'Toole EA. Metalloproteinases and wound healing. Adv Wound Care 2015; 4:225-234. https://doi.org/10.1089/wound.2014.0581

5. Briquez PS, Hubbell JA, Martino MM. Extracellular matrix-inspired growth factor delivery systems for skin wound healing. Adv Wound Care 2015; 4:479-489. https://doi.org/10.1089/wound.2014.0603

6. Pereira RF, Bartolo PJ. Traditional therapies for skin wound healing. Adv Wound Care 2016; 5:208-229. https://doi.org/10.1089/wound.2013.0506

7. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phyto therapeutic agents). Braz J Med Biol Res 2000; 33:179-189. https://doi.org/10.1590/S0100-879X2000000200004

8. Maver T, Maver U, Stana Kleinschek K, Smrke DM, Kreft S. A review of herbal medicines in wound healing. Int J Dermatol 2015; 54:740-751. https://doi.org/10.1111/ijd.12766

9. Budovsky A, Yarmolinsky L, Ben-Shabat S. Effect of medicinal plants on wound healing. Wound Rep Reg 2015; 23:171-183. https://doi.org/10.1111/wrr.12274

10. Jain DK, Gupta S, Jain R, Jain N. Anti-inflammatory activity of 80% ethanolic extract of acorus calamus linn. leaves in albino rats. Research J Pharm Tech 2010; 3 (3):882-884.

11. Taranalli AD, Tipare SV, Kumar S, Torgal SS. Wound healing activity of Oxalis corniculata whole plant extract in rats. Indian J Pharm Sci 2004; 66:444-446

12. Hemalata S, Subramanian N, Ravichandran V, Chinnaswamy K. Wound healing activity of Indigofera ennaphylla Linn. Indian J Pharm Sci 2001; 63:331-333

13. Kuwano H, Yano K, Ohano S, Ikebe M, Kitampura K, Toh Y, Mori M, Sugimachi K. Dipyridamole inhibits early wound healing in rat skin incisions. J Surg Res 1994; 56:267-270. https://doi.org/10.1006/jsre.1994.1042