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Assessment of Wound Healing Potential of Pachai Pakku Thailam Using Excision and Incision Wound Models in Rats

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ABSTRACT

Introduction: Wound healing involves complex biological processes such as inflammation, tissue proliferation and extracellular matrix (ECM) remodeling. Although conventional treatments like Povidone Iodine and Silver Nitrate are effective, traditional herbal formulations offer potential alternatives. This study investigates the wound healing efficacy of Pachai Pakku Thailam (PPT)-a Siddha formulation in rat models of excision and incision wounds.

Methodology: In excision model rats were divided into four groups: Group I (Control), Group II (Povidone Iodine), Group III (Silver Nitrate) and Group IV (PPT). In incision model rats were divided into three groups: Group I (Control), Group II (Povidone Iodine) and Group III (PPT). Excision and incision wounds were created and treated topically for 21 and 11 days respectively. Wound contraction, tensile strength and biochemical markers—hydroxyproline, hexosamine and hexuronic acid—were measured. Histological examination was performed to assess tissue regeneration.

Results: The wound contraction in the PPT group reached 95.8 ± 0.636% by day 21, higher than the control (88.5 ± 1.6%) and comparable to Povidone Iodine (90.2 ± 1.41%) and Silver Nitrate (91.4 ± 1.08%). Hydroxyproline content was significantly elevated in the PPT group (77.4 ± 0.339 µg/g tissue) compared to control (41.9 ± 0.586 µg/g, $p < 0.001$). Increases were also seen in hexosamine (91 ± 37.1 µg/mg protein) and hexuronic acid (51.7 ± 2.41 µg/mg protein) in the PPT group. Tensile strength of incision wounds improved with PPT but was slightly higher than standard group value. Histopathology studies showed that the PPT group had a good tissue regeneration, indicating successful healing at the cellular level.

Conclusion: PPT significantly enhances wound healing by promoting collagen deposition and extracellular matrix (ECM) remodeling. However, further formulation refinement, comprehensive safety evaluation, and additional preclinical and clinical studies are necessary before it can be considered for further clinical application.

Keywords: Pachai Pakku Thailam, Wistar rats, Incision and Excision, Histopathology

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INTRODUCTION

Wounds are defined as physical injuries that result in a disruption of the skin's continuity. The healing of such injuries involves a complex biological process aimed at restoring the skin barrier and functional integrity. This process progresses through a well-orchestrated sequence of healing phase- inflammation, cell proliferation, cell migration and tissue remodeling which collectively contribute to tissue repair and regeneration [1]. Wounds may arise from a variety of causes including mechanical trauma, chemical exposure, infections and are considered a universal health concern due to their inevitability across the human lifespan. However, the wound healing process does not always proceed in a uniform or predictable manner. It can be disrupted, leading to outcomes such as under-healing (chronic wounds), over-healing (hypertrophic scars or keloids) or non-healing wounds, all of which pose significant medical and economic challenges. In particular, chronic wounds—those that fail to progress through the normal healing stages—are difficult to treat and place a substantial burden on healthcare systems [2]. In patients with chronic conditions such as diabetes, vascular diseases or immune disorders, prolonged wound healing is a common complication. These conditions impair the body's natural repair mechanisms making the development of more effective wound therapies a medical priority. In this context, there has been growing interest in the therapeutic potential of herbal and traditional remedies drawn from systems like Siddha, Ayurveda and Traditional Chinese Medicine (TCM). These systems often utilize plant-based formulations believed to promote healing through anti-inflammatory, antimicrobial and regenerative actions [3,4].

One such traditional remedy, rooted in the Siddha medical system of South India especially in Tamil Nadu, involves the use of a polyherbal preparation made from green leafy herbs infused in sesame oil or coconut oil. This formulation has been historically applied to treat cuts, burns and ulcers. It is claimed to possess antioxidant and antibacterial properties that may aid in wound healing [5]. Despite its extensive use in ethnomedicine and anecdotal reports of effectiveness, scientific validation of this preparation remains limited. There is a clear need for rigorous pharmacological and clinical studies to assess its safety, efficacy and mechanisms of action in wound care.

Recent research has demonstrated that plant extracts such as *Centella asiatica*, *Curcuma longa* and *Azadirachta indica* significantly promote wound healing in rat models. These effects are attributed to enhanced wound contraction, accelerated epithelialization, increased collagen synthesis and improved angiogenesis [6,7]. Additionally, Thai herbal mixtures containing *Garcinia mangostana, Areca catechu* and *Oryza sativa* have shown antibacterial, anti-inflammatory and antioxidant properties, which further support the wound healing process [4]. To evaluate wound healing efficacy, excision and incision wound models are commonly employed in laboratory animals. These models assess parameters such as granulation tissue formation, wound contraction and tensile strength of the healing tissue. Biochemical markers—including hydroxyproline, hexosamine and hexuronic acid—are also analyzed to gauge healing progress [8]. Hydroxyproline is a critical component of collagen and serves as an indicator of collagen deposition and matrix remodeling. Elevated levels of hexosamine and hexuronic acid are associated with the active synthesis of glycosaminoglycans which are essential for maintaining the hydration and structural stability of the extracellular matrix (ECM) [9].

Herbal formulations contribute to wound healing by modulating inflammatory cytokines and reducing reactive oxygen species (ROS), both of which play key roles in the inflammatory phase of healing [10]. Antioxidant compounds found in herbs like *Glycyrrhiza glabra, Nigella sativa* and Aloe vera have been shown to stimulate fibroblast proliferation, promote angiogenesis and decrease the risk of wound infection. These actions collectively improve both the rate and quality of wound healing, particularly in chronic or infected wounds [11, 12]. In this study, excision and incision wound models are utilized to investigate the in vivo effects of Pachai Pakku Thailam on wound healing in Wistar rats. The efficacy of this traditional Siddha formulation is compared with standard treatments such as Povidone Iodine and Silver Nitrate, focusing on the rate of wound closure, collagen deposition and restoration of tissue integrity.

MATERIALS AND METHODS

Preparation and formulation of Pachai Pakku Thailam

Pachai Pakku Thailam, a Siddha herbal oil from ancient text is empirically used by traditional healers in Tamil Nadu for treating non-healing ulcers and burns. Its preparation begins with carefully sourced raw ingredients authenticated (certificate. Num- 854.15052408-15) by the Siddha Central Research Institute in Chennai, Ministry of AYUSH, Government of India. The formulation includes equal parts of Varattu pakku (*Areca catechu nut*), Vellai kungliyam (*Shorea robusta resin*), Manjal (*Curcuma longa*), Puliyankottai thodu (*Tamarindus indica* seed coat), Masikkai (*Quercus infectoria*), Kadukkai thol (*Terminalia chebula*) and Kaichchukkatti (*Acacia catechu*), along with unripen *Areca catechu* juice and virgin coconut oil. The juice is extracted from coarsely ground tender betel nuts sourced from local farms. Herbal ingredients are powdered, mixed with juice and oil, heated in a clay vessel without overheating, stirred till required consistency and preserved in amber bottles after being filtered through muslin cloth.

Animals

The study used healthy albino rats (Wistar strain) either sex weighed between 160 and 180 g and were 6 to 8 weeks old. The animals were kept in a typical laboratory setting with a 12-hour light/dark cycle and a temperature of 22 ± 2°C. They were also given unrestricted access to water and a commercial pellet meal. One week before to the experiment, they underwent adaptation. All procedures were authorized by the Institutional Animal Ethics Committee (IAEC) with approval number KMCRET/ReRc/Ph.D./91/2024 and according to CPCSEA rules.

Wound Healing Activity

Grouping Animals

Animals in the excision wound models were divided into four groups at random each consisting of six rats (n = 6). Group I acted as the control and underwent no treatment other than the induction of the wound. Group II received topical treatment with 5% Povidone Iodine, a widely utilized standard antiseptic. Group III was administered Silver Nitrate topically, a typical therapy for wound management. Group IV received treatment with the test formulation, Pachai Pakku Thailam, administered topically to the wound area. All animals were anesthetized by intra-peritoneal injection of ketamine (50mg/kg). The rats were depilated on the back and a predetermined area of 500 mm^2 full thickness skins was excised in the dorsal interscapular region. Rats were left undressed to the open environment. All procedures were conducted under sterile conditions and the animals were consistently observed for wound healing indicators during the study duration.

In the incision wound models, three groups of six animals each were randomly selected from the total population of animals (n = 6). In Group I acted as the control group underwent no treatment. In Group II, the animals received topical treatment with povidone iodine ointment. In Group III, the animals were administered Pachai Pakku Thailam, the experimental medication, applied externally once each day for ten consecutive days. The animals were anesthetized by intra-peritoneal injection of ketamine (50mg/kg). The animals were kept on the operating table in the common position. One paravertebral straight incision of six centimeters was made on either side of the vertebral segment with the help of a scalpel blade without using antibiotics. The edges held together and stitched using black silk surgical thread and a curved needle. Threads were pulled tight to ensure proper wound closure [13].

During the study, all animals were observed for wound healing indicators like visible wound closure, tissue regeneration and other specific metrics related to each wound model. This facilitated the evaluation of the impacts of various topical therapies on wound recovery.

Wound Contraction Measurement

Healing rate was assessed by wound contraction in excision wound model. To measure the wound area, transparent sheets were used to trace the wound margins on the day of wound induction (Day 0) and at 3-day intervals until Day 21. These tracings were then transferred onto 1 mm² graph paper to calculate the wound area in square millimeters (mm²) [14]. The percentage of wound contraction was computed and changes in wound size were noted using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Wound area on day 0} - \text{Wound area on day n}}{\text{Wound area on day 0}} \text{x}100$$

Where, n= the days when measurement was taken.

Estimation of Total protein

Protein content was determined via the Lowry method (1951) [15]. 0.1 ml homogenate was made up to 1ml with distilled water and to this; 5ml of alkaline solution was added, mixed well and allowed to stand for 10 min. Then a volume of 0.5 ml Folin's reagent was added, mixed well and incubated at room temperature for another 10 min. The blue colour developed was measured at 660 nm against blank. Bovine serum albumin (1mg/ml) served as the standard and form the standard graph obtained; the amount of protein in the sample was calculated and expressed as mg/100mg tissue.

Estimation of Hydroxyproline

Hydroxyproline content was determined using Newman and Logan's technique (1950) [16]. 0.3 ml of hydrolysate, 2.5 N NaOH, 0.01 M $CuSO_4$ and 6% H_2O_2 were added to each tube stirred and incubated for 15 minutes at 80°C. After cooling, 0.6 ml of 5% p-dimethylaminobenzaldehyde in n-propanol and 1.2 ml of 3 N H_2SO_4 were added, followed by incubation at 75°C for 15 minutes. Color was measured at 540 nm with a spectrophotometer and reported in mg/100 mg tissue.

Estimation of hexosamine

Hexosamine was quantified using Dische and Borenfreund's method (1950) [17]. Tissue specimens (50 mg) were hydrolyzed with 5 ml of 2 N HCl at 100°C for 6 hours. Post-hydrolysis HCl was removed by evaporation and the residue was dissolved in distilled water to a final volume of 10 ml. Aliquots (10–50 µg) were combined with 1 ml of 2% acetyl acetone in 0.5 M sodium carbonate, heated in boiling water for 15 minutes then allowed to cool. After adding 5 ml of 95% ethanol and 1 ml of Ehrlich's reagent, the absorbance was measured at 530 nm with results expressed as µg hexosamine per 100 mg of tissue.

Estimation of Hexuronic acid

Hexuronic acid content was determined using Bitter and Muir's method (1962) [18]. A buffered copper solution was prepared with NaCl, sodium acetate, glacial acetic acid and $CuSO_4$, adjusted to pH 4.8. Equal volumes of solution and sample were heated, Folin–Ciocalteu reagent was added and absorbance was measured at 750 nm with a Bicinchoninic acid reagent variant at 560 nm.

Determination of tensile strength in incision model

Tensile strength is essential for tissue repair during the healing of wounds. The mechanical characteristics of the skin are associated with networks of collagen and elastic fibers in the dermis. The degree of healing, the strength of the wound tissue and the efficacy of healing are all indicated by the minimal force needed to separate the incision [19]. Sutures were removed on the ninth day following surgery and on the tenth day one side of the wound was examined while bearing additional weight. The load that completely divided the wound represented the breaking strength obtained from para-vertebral incision in animals [20, 21].

Histopathology of Incision Wound Model

Rats in the incision model were sacrificed and a portion of the skin was taken for histopathological studies. On Day 5, granulation tissue was removed. Healed skin samples were collected on Day 11, preserved in 10% formalin and treated histologically. H&E staining was applied to the sections for microscopic examination. Histopathological evaluation assessed re-epithelialization, collagen deposition, fibroblast proliferation and inflammatory cell infiltration using light microscopy at 10× and 40× magnifications.

Statistical Analysis

Treated groups were analyzed via one-way ANOVA against the control and reporting results as mean ± SD, significance at $P < 0.01$.

RESULTS AND DISCUSSION

The efficacy of Pachai Pakku Thailam (PPT) in promoting wound healing was evaluated using excision wound model in rats with its performance compared to both untreated wounds and those treated with conventional antiseptics such as Povidone Iodine and Silver Nitrate. Wound contraction was assessed over a 21-day period and presented as percentage reduction in wound area (Table 1). On Day 3, all treatment groups showed a similar level of wound contraction compared to the control group, with no statistically significant differences ($p > 0.05$). This indicates that during the initial inflammatory phase of wound healing, none of the interventions including PPT had a distinct early advantage in promoting contraction. By Day 7, however, a marked increase in wound contraction was observed in the PPT treated group (24.5 ± 2.6%), which was statistically significant ($p < 0.01$) compared to all other groups including those treated with Povidone Iodine (13.1 ± 2.68%) and Silver Nitrate (12.4 ± 1.05%). This suggests that PPT may enhance the proliferative phase of healing, potentially by promoting fibroblast activity and collagen deposition, as supported by findings from previous studies on polyhedral formulations [22,23]. On Day 11, although the differences were not statistically significant, the PPT group maintained a slightly higher wound contraction (32.7 ± 7.14%) compared to the other treatments. By Day 14, the wound contraction in the PPT group (49 ± 2.86%) was significantly greater than in the control (39.4 ± 5.87%) and both conventional treatment groups (Povidone Iodine: 40.3 ± 2.88%, $p < 0.05$; Silver Nitrate: 41.4 ± 3.01%, $p < 0.01$), indicating sustained enhancement of tissue regeneration. During the remodeling phase (Day 17 onward), wound contraction progressed steadily in all groups. On Day 17, the PPT group (63.4 ± 0.857%) showed contraction rates comparable to the other treated groups, with no statistically significant difference. However, by Day 21, the PPT treated wounds demonstrated a significantly higher wound contraction (95.8 ± 0.636%) than all other groups, including the Silver Nitrate group (91.4 ± 1.08%) and Povidone Iodine group (90.2 ± 1.41%,), with a significance level of $p < 0.01$.

Table 1: Impact of conventional ointment and Pachai Pakku Thailam on the contraction of rats' excision wound models.

Day	Groups			
	Group I-Only wound (Control)	**Group II-Wound + Povidone Iodine**	**Group III-Wound + Silver Nitrate**	**Group IV-Wound + Pachai Pakku Thailam (PPT)**
3	12.2 ± 3	13 ± 2.53	14.7 ± 1.22	13.3 ± 3.19
7	13.3 ± 1.7	13.1 ± 2.68	12.4 ± 1.05	24.5 ± 2.6 (P < 0.01)
11	27.4 ± 2.7	27 ± 5.69	31.5 ± 2.26	32.7 ± 7.14
14	39.4 ± 5.87	40.3 ± 2.88 (*P* < 0.05)	41.4 ± 3.01 (P < 0.01)	49 ± 2.86 (*P* < 0.001)
17	60.4 ± 1.88	64.4 ± 1.02	64 ± 1.4	63.4 ± 0.857
21	88.5 ± 1.6	90.2 ± 1.41	91.4 ± 1.08	95.8 ± 0.636 (P < 0.01)

Values expressed as mean ± SD; statistical significance (p) assessed using one-way ANOVA and Dunnett's test, comparing control and treated group.

The superior performance of PPT in later stages may be attributed to its potential antioxidant, anti-inflammatory and antimicrobial properties which likely contribute to reduced oxidative stress and enhanced tissue remodeling. Similar trends have been observed with other traditional herbal formulations rich in phytochemicals such as flavonoids, tannins and terpenoids which are known to facilitate wound healing by stimulating angiogenesis, fibroblast proliferation and extracellular matrix synthesis [24,25].The data suggest that while conventional antiseptics provide moderate support for wound healing, Pachai Pakku Thailam demonstrates a significantly greater effect on wound contraction, especially during the proliferative and remodeling phases. This reinforces the therapeutic potential of traditional Siddha formulation in modern wound care and warranting further more scientific validation.

Table 2: Total Protein Content in Granulation Tissue of Different Treatment Groups

Group	Treatment	Total Protein (mg/g dry tissue)
Group I	Only Wound (Control)	49.54 ± 0.2951
Group II	Wound + Povidone Iodine	58.32 ± 0.5797 (P < 0.001)
Group III	Wound + Silver Nitrate	58.58 ± 0.3736 (P < 0.001)
Group IV	Wound + Pachai Pakku Thailam (PPT)	59.26 ± 0.336 (P < 0.001)

Values expressed as mean ± SD; statistical significance (p) assessed using one way ANOVA and Dunnett's test, comparing control and treated group.

The total protein content in granulation tissue is critical in wound healing indicating the synthesis of necessary proteins and enzymes. This study found that all treatment groups had a significant rise in total protein compared to the control group (Table 2). The control (Group I) had the lowest protein content (49.54 ± 0.2951 mg/g), reflecting a slower healing rate without topical interventions. All treated groups showed significantly higher protein levels ($p < 0.001$), demonstrating the efficacy of the agents in boosting protein synthesis during repair. Group II (Wound + Povidone Iodine) and Group III (Wound + Silver Nitrate) showed protein contents of 58.32 ± 0.5797 mg/g and 58.58 ± 0.3736 mg/g, respectively indicating better outcomes than the control. These findings corroborate earlier studies emphasizing the role of antiseptics in fostering the proliferative healing phase [26,27]. Group IV (Wound + Pachai Pakku Thailam, PPT) had the highest protein content at 59.26 ± 0.336 mg/g, significantly exceeding the control ($p < 0.001$). PPT's role in enhancing protein synthesis appears linked to its phytochemicals, which promote fibroblast activity and collagen formation. The increased protein levels in the PPT group align with hydroxyproline and hexosamine data, affirming its beneficial effects in promoting granulation tissue. Overall, the results indicate that PPT can be a valuable natural adjunct in wound healing, particularly where synthetic treatments may pose risks.

Table 3: Effect of treatments on biochemical parameters in excision wound tissues

Group	Hydroxyproline (µg/g tissue)	Hexosamine (µg/mg protein)	Hexuronic Acid(µg/mg protein)
Group I – Only Wound (Control)	41.9 ± 0.586	82.4 ± 0.299	17.8 ± 0.427
Group II – Wound + Povidone Iodine	81.2 ± 0.534*	129 ± 3.65	56.5 ± 0.444
Group III – Wound + Silver Nitrate	71.5 ± 0.426*	127 ± 2.62	48.8 ± 0.437
Group IV – Wound + PPT (Pachai Pakku Thailam)	77.4 ± 0.339*	91 ± 37.1	51.7 ± 2.41

*Values are expressed as mean ± S.D. Statistical significance was assessed using one-way ANOVA followed by Dunnett's test. $P < 0.05$ compared with the wound control group.

The biochemical evaluation of wound tissue showed significant enhancement of hydroxyproline, hexosamine and hexuronic acid levels (Table 3) in all treatment groups compared to the untreated control, indicating improved collagen deposition and extracellular matrix (ECM) formation. Hydroxyproline, a major component of collagen, was lowest in the control group (41.9 ± 0.586 µg/g tissue), while all treated groups showed significant increases, with the Povidone Iodine group reaching 81.2 ± 0.534 µg/g and Pachai Pakku Thailam (PPT) showing 77.4 ± 0.339 µg/g, both statistically significant ($p < 0.001$). This suggests that both standard and herbal treatments stimulate collagen synthesis during tissue repair. Recent studies have confirmed that enhanced hydroxyproline content reflects better collagen fiber cross-linking, contributing to tensile strength and structural stability of the healed [28,29]. Similarly, hexosamine levels were elevated in all treated groups with the highest values observed in the Povidone Iodine (129 ± 3.65 µg/mg) and Silver Nitrate (127 ± 2.62 µg/mg) groups, while the PPT-treated group demonstrated a moderate but consistent increase (91 ± 37.1 µg/mg). Hexosamine plays a key role in glycoprotein synthesis, essential for ECM remodeling and re-epithelialization, supported by recent research highlighting the role of plant-based polysaccharides in promoting ECM stabilization [30,31]. The pattern of hexuronic acid levels followed a similar trend, with the control group at 17.8 ± 0.427 µg/mg and all treated groups showing marked increases, especially in the PPT group (51.7 ± 2.41 µg/mg), comparable to standard drugs. Hexuronic acid enhances tissue hydration and elasticity, facilitating fibroblast migration and wound closure, consistent with findings from herbal hydrogel-based therapies that improve hydration and granulation quality [32].
These results demonstrate that Pachai Pakku Thailam promotes both the quantitative and qualitative aspects of wound healing by stimulating collagen synthesis, ECM formation and tissue hydration. While standard antiseptics achieved slightly higher or faster responses in certain parameters, the efficacy of PPT was comparable and supports its traditional use in Siddha medicine. These observations align with the growing body of evidence that polyherbal formulations enhance wound healing via multi-targeted actions, including antioxidant, anti-inflammatory and proliferative effects [27,28,33].

Tensile strength in incision wound model in rats

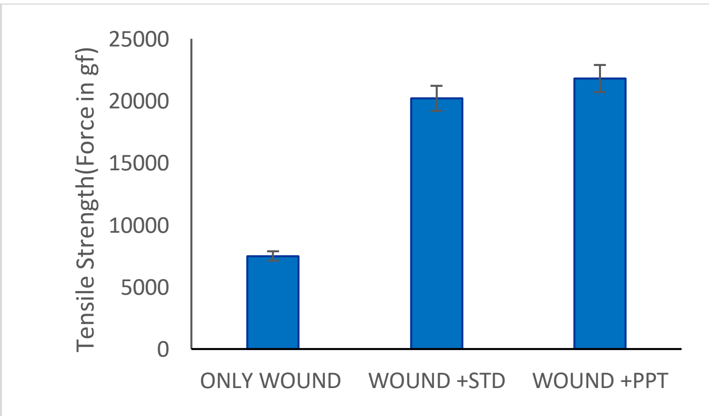

FIGURE 1: Effect of Pachai Pakku Thailam on wound parameters of tensile strength in the incision wound model in rats.

Tensile strength is crucial for assessing wound healing quality, reflecting skin's mechanical integrity post-injury. Increased tensile strength signals effective collagen synthesis, maturation, and cross-linking during the remodeling phase [34]. As illustrated in Figure 1, the tensile strength in the untreated control group (Only Wound), tensile strength was significantly lower at approximately 7500 gf, indicating poor structural integrity and suboptimal collagen organization. Treatment with the standard drug (Wound + STD) improved tensile strength to about 20500 gf, showing enhanced collagen synthesis. Earlier studies also indicated that topical agents like Povidone Iodine and Silver Nitrate facilitate collagen maturation and fibroblast proliferation. Notably, Pachai Pakku Thailam (PPT) treatment yielded the highest tensile strength at around 22000 gf, outpacing standard treatment, suggesting accelerated healing and enhanced biomechanical tissue strength [26, 33]. This efficacy is likely due to PPT's bioactive compounds such as flavonoids, terpenoids and polyphenols that promote angiogenesis, fibroblast proliferation and collagen deposition. The tensile strength findings correlate with biochemical results, including elevated hydroxyproline and total protein content in PPT treated wounds, underscoring PPT's role in enhancing wound healing. Compared to untreated and standard-treated groups, PPT's significant increase in tensile strength highlights its therapeutic potential as a natural alternative for managing non healing wounds. Compared to conventionally used antiseptics for wound management, the efficacy of PPT is more.

Histopathology of Incision Wound Model

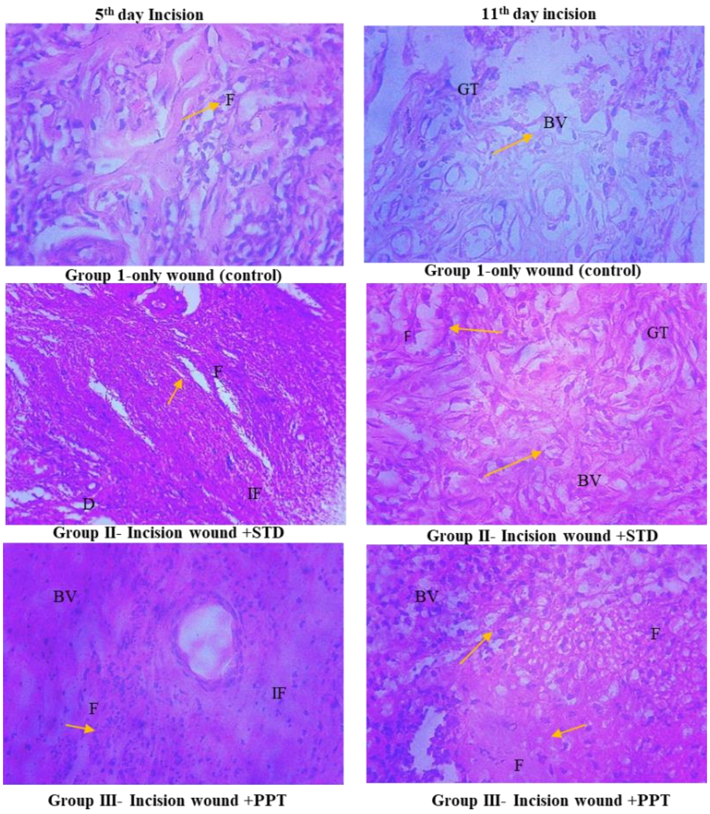

FIGURE 2: Histopathological studies of rat's skin on 5th day and 11th day of Group I, Group II and Group III in Incision wound model

F- Fibroblasts; IF- Inflammatory cells; BV- Blood vessels; GT-Granulation tissue.

Group-I: 5th day-only incision wound-40x shows granulation tissue composed of inflammatory infiltrates; Group – II : Incision Wound + STD 5th day- 40x shows fibro collagenous stroma; Group – III : Incision Wound + Pachai Pakku Thailam - 5th day -40x shows dermis with dense fibro collagenous stroma

Group-I. 11th day of incision model-40x shows fibro collagenous stroma; Group -II 11th day of incision model 40x shows fibro collagenous stroma.; Group -III 11th day of incision model- 40x shows thin-walled congested vessels.

Histopathological analysis of tissue in the incision wound showed notable differences in healing across three groups (Figure 2). In Group I (Only Incision Wound), the 5th-day examination showed early healing characterized by inflammatory cells, transitioning to a fibro collagenous stroma by the 11th day, indicating progression. In Group II (Incision Wound + Standard Treatment), a well-defined fibro collagenous stroma was evident on day five, reflecting an advanced healing stage, with continued dense stroma on the eleventh day. Group III (Incision Wound + Pachai Pakku Thailam) demonstrated rapid collagen deposition on day five compared to day eleventh indicating its roll in early stage of wound healing. Histopathological analysis revealed further insights into the tissue-level changes. On the 5th day, PPT-treated wounds showed dense fibro collagenous stroma formation, indicating early collagen deposition and on 11th day showed formation of thin-walled congested vessels indicating angiogenesis which accelerates wound healing activity.

The findings suggest that Pachai Pakku Thailam significantly accelerates early phase of wound healing, wound contraction and enhances biochemical parameters associated with tissue regeneration. These effects are more pronounced after the first week post-injury, indicating PPT's potential as an early phase healing promoter. However, its impact on tensile strength warrants further investigation into its formulation and application duration, in line with modern approaches to herbal wound therapy development [35].

Excision and Incision Animal Model Images

Figures 3 through 6 illustrate how wounds healed over 21 days in an excision model, comparing four groups: an untreated control, wounds treated with Povidone Iodine, Silver Nitrate and PPT. Group I, the control group showed a steady decrease in wound size, representing normal healing and slow wound contraction with incomplete closure observed by day 21. Group II, treated with Povidone Iodine, exhibited accelerated excision wound contraction with significant size reduction and near-complete closure by day 21. Group III, in excision model using Silver Nitrate, also achieved notable wound contraction rates similar to Group II. Both Povidone Iodine and Silver Nitrate treatments sped up the wound closure process relative to the control, suggesting their effectiveness in enhancing the healing phases. In excision model Group IV, treated with Pachai Pakku Thailam (PPT), demonstrated substantial wound area reduction and complete healing by day 21, indicating that PPT may influence the tissue repair process. These findings highlight how different treatments can affect the speed and quality of wound healing.

Figures 7 to 9 show the healing process in an incision wound model from day 1 to day 11 across three groups: untreated wounds (Group I), wounds treated with Povidone Iodine (Group II) and wounds treated with PPT (Group III). The untreated group exhibited gradual healing typical of natural tissue repair. In comparison, the group treated with Povidone Iodine demonstrated faster wound closure and improved tissue recovery, reflecting the antiseptic's supportive role in the healing process. The PPT treated group also showed improved tensile strength in the incision model, indicating that PPT may contribute to enhancing wound repair. Overall, these figures highlight differences in healing rates and outcomes depending on the treatment applied. The biochemical data indicated enhanced collagen synthesis and ECM formation, supporting PPT's efficacy. The phytochemicals in PPT likely provide antioxidant, anti-inflammatory and proliferative effects, promoting granulation tissue formation and fibroblast proliferation. Overall, findings suggest that PPT is a promising herbal treatment, showing comparable effectiveness to conventional antiseptics such as Povidone Iodine and Silver Nitrate reinforcing previous research on herbal formulations in wound healing [28,30].

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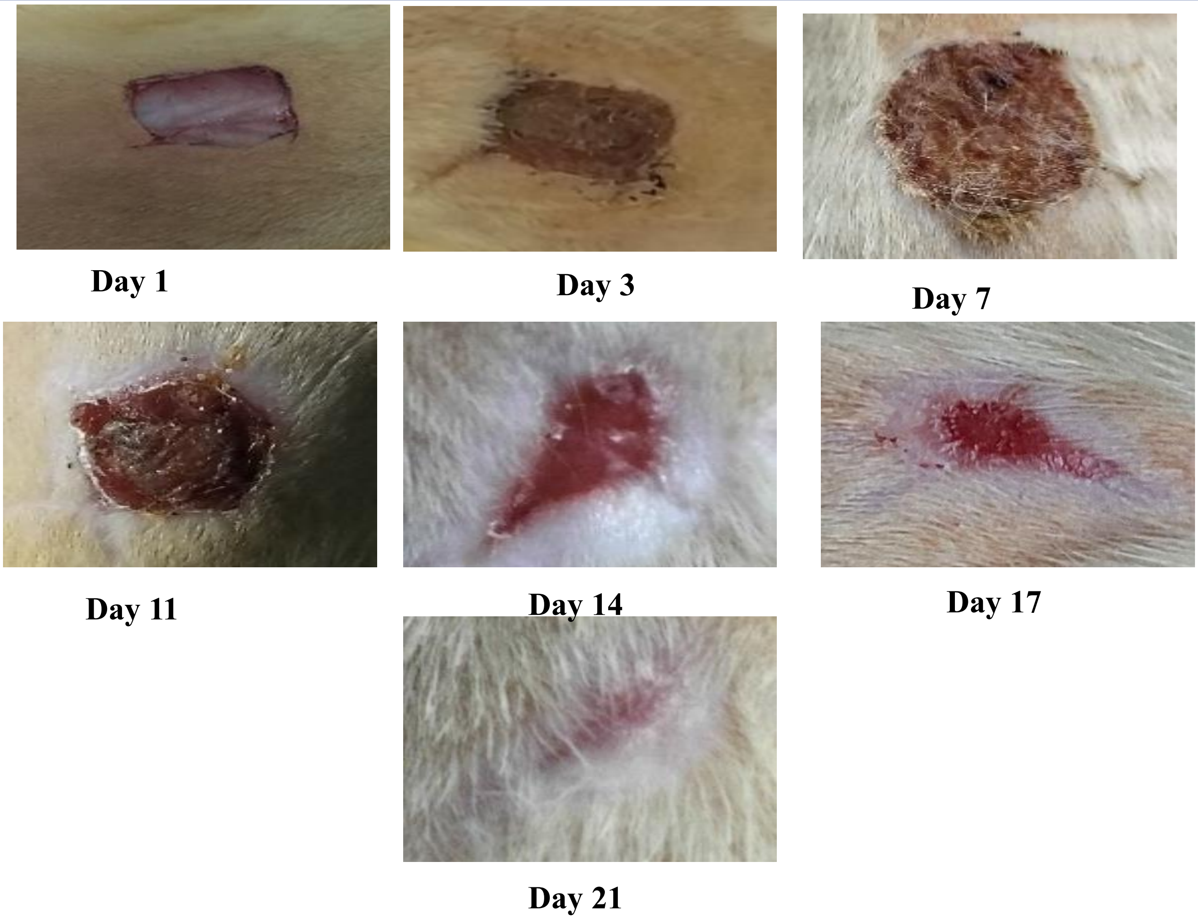

FIGURE 3: Group I. Wound only (Control). Excision wound model on day 1–day 21 with different test groups

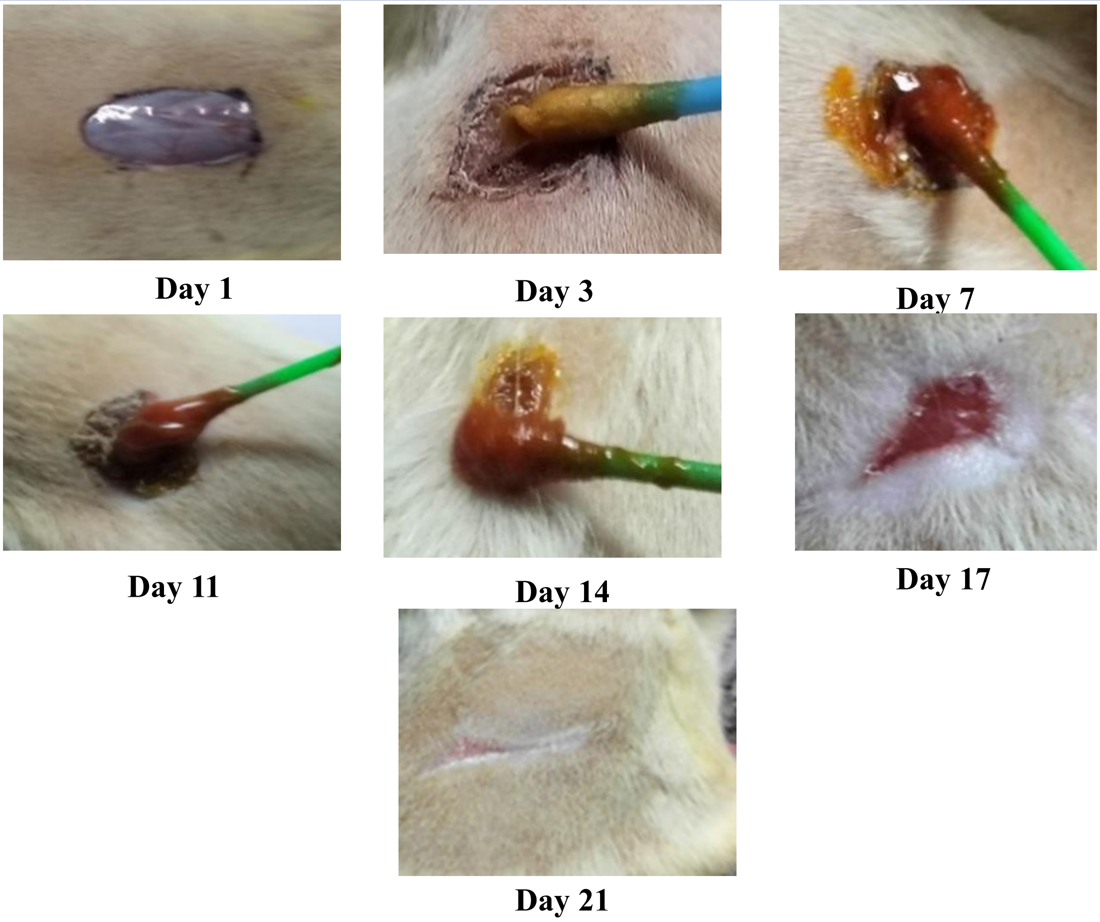

FIGURE 4: Group II Wound + Povidone Iodine. Excision wound model on day 1–day 21 with different test groups

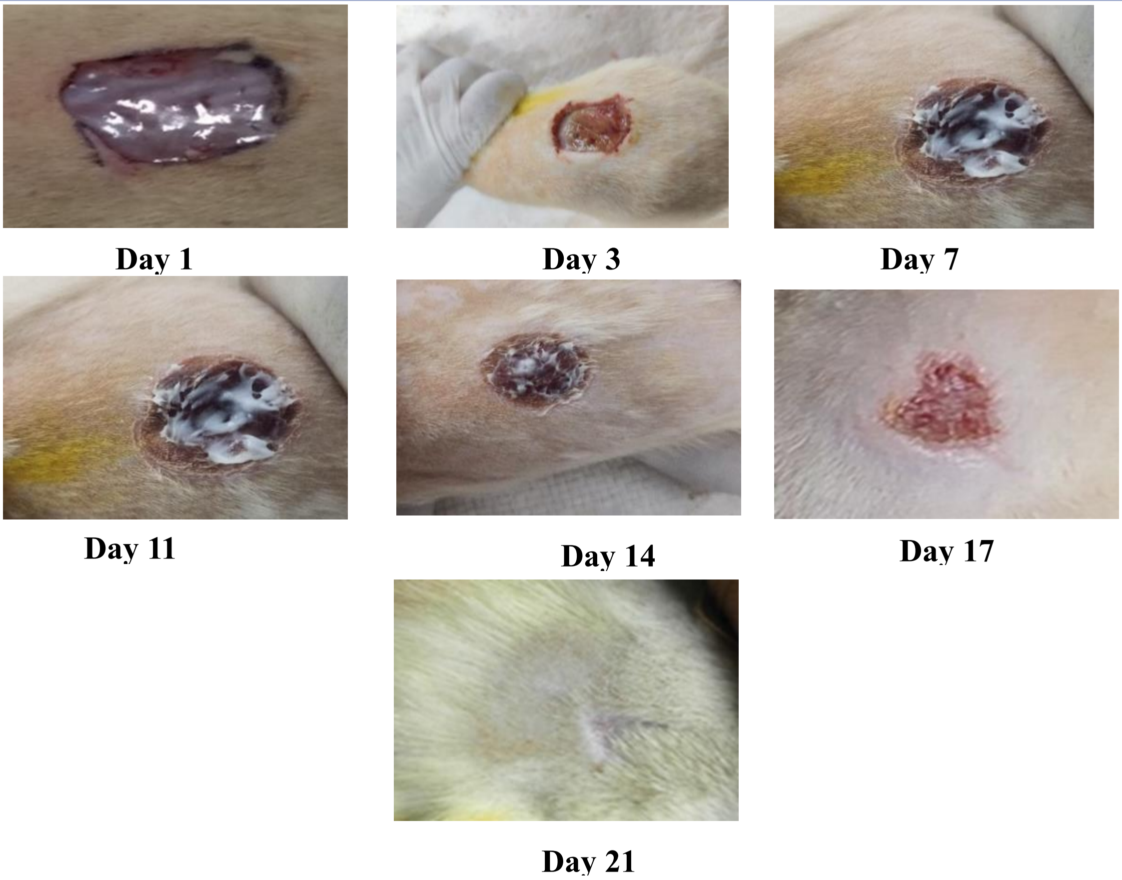

FIGURE 5: Group III Wound + Silver Nitrate. Excision wound model on day 1–day 21 with different test groups

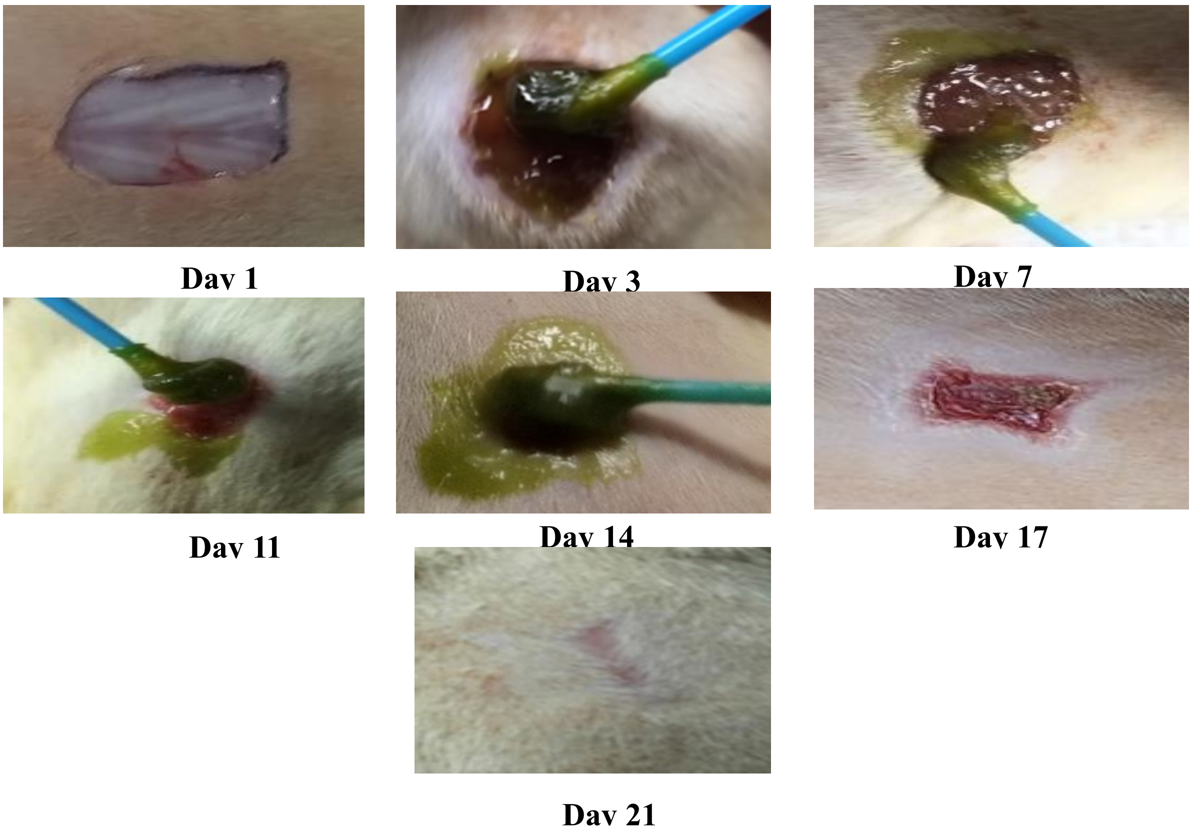

FIGURE 6: Group IV Wound + PPT. Excision wound model on day 1–day 21 with different test groups

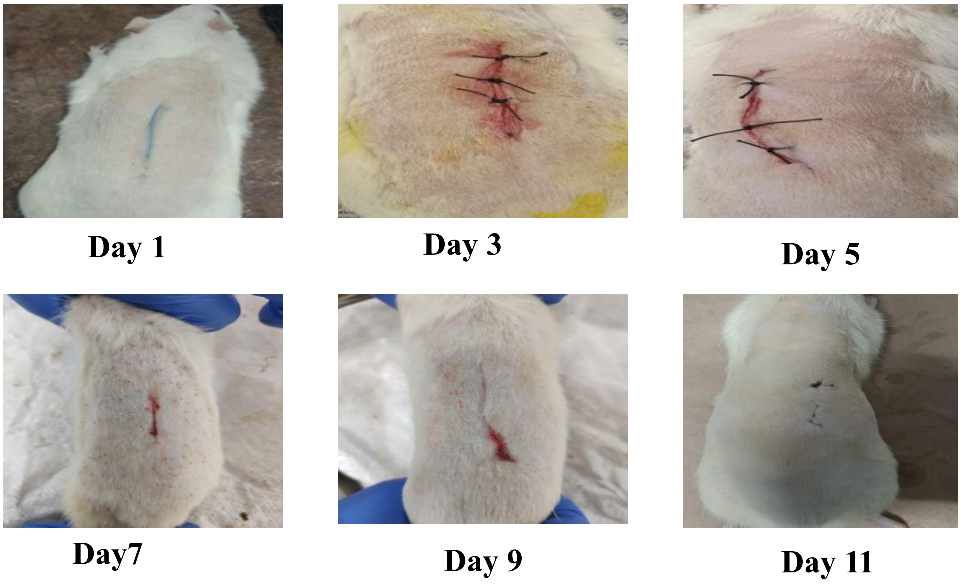

FIGURE 7: Group I. Wound only (Control). Incision wound model on day 1 – day 11 with different test groups

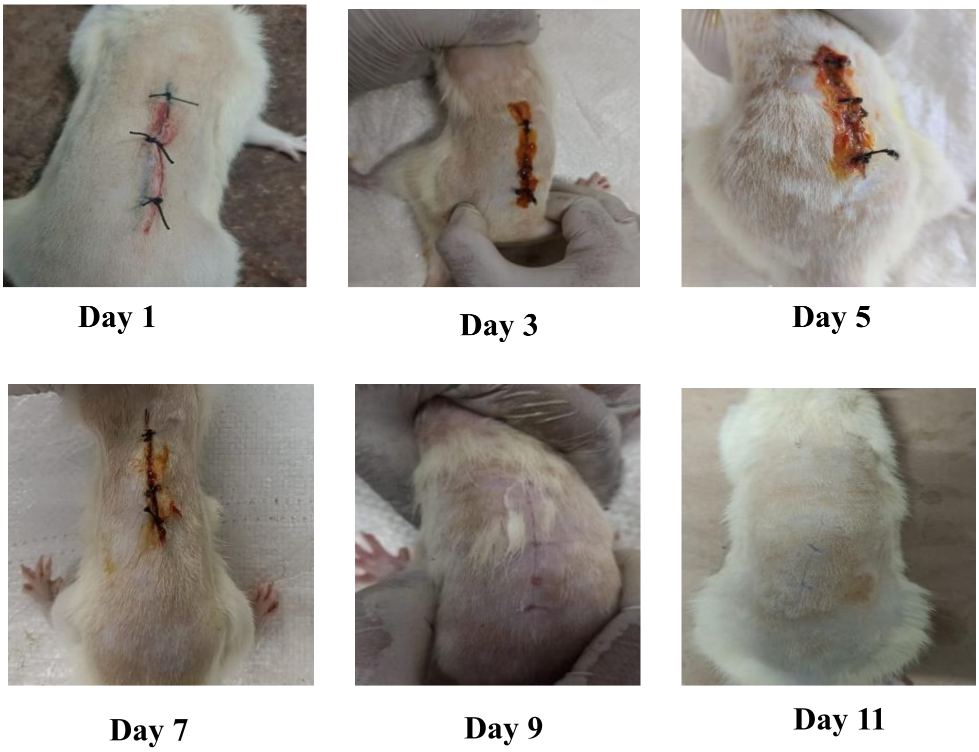

FIGURE 8: Group II Wound+ Povidone Iodine. Incision wound model on day 1- day 11 with different test groups

FIGURE 9: Group III Wound+ PPT. Incision wound model on day 1-day 11 with different test groups

CONCLUSION

This study demonstrates that Pachai Pakku Thailam (PPT) possesses significant wound healing potential in both excision and incision wound models in rats. The formulation effectively accelerated wound contraction, enhanced tensile strength and elevated key biochemical markers such as hydroxyproline, hexosamine and hexuronic acid, all of which play critical roles in collagen formation, extracellular matrix development and overall tissue repair. Visual assessments of the wounds further supported these findings, with the PPT-treated group showing quicker closure and more organized granulation tissue compared to the untreated control. When compared to standard wound care agents like Povidone Iodine and Silver Nitrate, PPT exhibited comparable efficacy, particularly in promoting collagen deposition and tissue regeneration. However, further formulation refinement, comprehensive safety evaluation and additional preclinical and clinical studies are necessary before it can be considered for further clinical application. Clinical trials will also be necessary to determine efficacy, safety and standardization, ultimately guiding the integration of such herbal therapies into mainstream biomedical practice.

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