Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2023: 85.868
Volume 14, Issue 4; 2025, 48-64

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Research Article

In Vivo Healing Potential of Prunus Africana in Excision and Incision Wound Models

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Article Info: Received: 07-05-2025 / Revised: 25-05-2025 / Accepted: 28-06-2025
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DOI: https://doi.org/10.32553/jbpr.v14i4.1329
Conflict of interest statement: No conflict of interest

Abstract:

Introduction: Prunus africana (PA) bark has traditionally been used for treatment of wounds. Its antioxidant and anti-inflammatory activities are well described in scientific literatures. Proceeding with these findings, it is the purpose of the proposed study to assess the wound healing its potential in Wistar rats.

Methods: chloroform (PACE) and hydroethanolic (PAHE) extract were prepared as 5 and % w/w gel. These formulations were subjected to acute dermal toxicity test based on OECD guideline 402 and wound healing activity were also determined using excision and incision wound models. The following parameters were measured: wound contraction rate, epithelialization period, tensile strength, , the total content of protein, hexosamine and hydroxyproline content. The histopathological changes in the granulation tissue were also measured.

Results: The current study on excision wound model showed that treatment demonstrated a significant improvement in wound contraction and the protein, hexosamine and hydroxyproline levels in both 5 percent and 10 % w/w gels of PAHE and PACE than control group ($p < 0.01$; $p < 0.001$). A major increase in the tensile strength was recorded with the incision model ($p < 0.01$; $p < 0.001$). Histology revealed that there was larger collagen deposition and decreased macrophage infiltration, less edema, and more fibroblasts in the treatment groups.

Conclusion: The results establish the fact that PACE and PAHE extracts share good wound healing activity.

Keywords: Prunus africana, Excision wound model, Incision wound model, Wound healing.

Introduction

The Prunus africana, otherwise known as the African cherry or Pygeum is an important fruit of Rosaceae and it is part of the genus Prunus that has more than 400 species across Africa. This is an evergreen tree that is common in the forest and this tree grows very high over its 40 meters and the dimensions of the stem can extend to 1 meter and the crown is very wide and that is why it can reach to a height of 10-20 meters. It is characterized by dark brown, glossy-leaves and small greenish-white-coloured flowers. It is mainly located in mountainous areas and islands in 22 countries of eastern Africa, including parts of central (e.g. Katanga and Congo), western Africa, Comoros and Madagascar. It is native however in montane

tropical forests at levels of about 1500 meters above the sea level. Traditionally, P. africana has had wide use in different parts of Africa, such as the southern, eastern, and central countries, in the treatment of disorders of the prostate, especially prostate cancer. The bark of the herb is used commonly by herbalists to treat diseases like prostate, or diseases of the stomach and urinary system, chest pains, malaria, viral infections and those related to the kidney. In Ethiopia it is used as a treatment by traditional healers who use infusions made using its leaves and decoctions made using its stem and root bark to treat urinary problems, diarrhea, abdominal pains, wounds as well as bacterial infections. It is hypothesized that the plant pharmacological activity is due to the synergistic effect of multiple bioactive constituents (known and unidentified). Its major contents are pentacyclic triterpenoids (including Ursolic and oleanolic acids), which have anti-edematous effect of inhibiting glucosyl-transferase and phytosterols (β-sitosterol and β-sitostenone), which have anti-inflammatory effects through inhibition of prostaglandin synthesis.

Wound can be taken as an abnormality in the makeup and operation of living structure. The process of wound healing is a complicated biological procedure and it commences with damage to the tissue and typically ends with the formation of scars. This wound healing process takes different stages, which entail coagulation, epithelialization, granulation, collagen, and tissue reconstruction. It mostly touches on the connective tissue whereby acute inflammation occurs first after which collagen components and extracellular matrix structures are formed and remodelled in the end thus re-establishing tissue integrity.

Trees and shrubs are used in the treatment of wounds through cleaning, disinfection, and establishment of a good environment favouring the type of natural healing. Herbal medicines tend to possess few side effects and low toxicity compared with conventional ones. Hence, there is need to scientifically substantiate the therapeutic allegations of the traditional medicine.

In this regard, the research will focus on trying to determine the wound healing effect of Prunus africana in Wistar rats. Two important notions underpin the research:

1. The PA stem bark has proven to be scientifically antioxidant and anti-inflammatory.
2. As per Traditional and ethnomedical claim, PA bark has been used in the treatment of wounds

Materials and methods

Plant Collection and Extracts Preparation

Prunus africana (Rosaceae) stem bark was obtained in Mabira Forest, Near Lugazi Town, Uganda situated in East Africa, The plant was authenticated in Veer Narmad South Gujarat University, Surat vide: specimen no: VNSGU/BVBRC/2021/05/TC-06. Powdered Bark were shade dried and successive extractions were carried out using Chloroform and Hydroethanolic solvent in Soxhlet apparatus to produce Chloroform (PACE) and Hydroethanolic extract (PAHE) which were concentrated by using vacuum rotary evaporator respectively.

Phytochemical analysis

Qualitative analysis of different phytoconstituents was done on both the chloroform extract (PACE) and hydroethanolic extract (PAHE) through a series of standard chemical tests (21, 22, 23).

Preparation of Topical Gel Formulation

Topical gel containing the chloroform extract (PACE)and hydroethanolic extract (PAHE) were developed. To make the gel, Carbopol 940 was put in a different container and then dissolved in distilled water by constantly stirring it up energetically. The other constituents were added in turn (methyl paraben, propyl paraben and glycerine) and were allowed to stand overnight after adding in the order in increasing amounts. Powdered PACE and PAHE were

dissolved in different proportions (5 % w/w & 10% w/w) to make their extracts and added them into propylene glycol. These mixtures were later mixed with the polymer dispersion. The proper amount of distilled water was added to each of the formulations and the pH adjusted to 7.1 with triethanolamine keeping the mixture stirred continuously within a time period of about 10 minutes. The same procedure was followed in preparing a control gel, in another case not involving the extracts. The parameters based on which the resulting formulations were assessed included visual appearance, pH, Spreadibility, among others.

Animals

Either of the sexes, Wistar albino rats that were healthy and weighed 150-200 g were kept in controlled environmental conditions, with the temperatures (25±0.5) and the relative humidity (60±5%) and the 12-hour light/dark cycle were maintained. The standard pellet diet was offered to the animals and they had free water intake. The conduct of all the animal experiments was at the institute and it was first approved by the Institutional Ethics Committee of the institute (SETCPD/IAEC/June/2024/01).

Acute Dermal Toxicity Study

Suitable therapeutic dose was determined by testing the standardized extract (PACE and PAHE) gel in accordance to OECD guideline No. 402. A total of six, healthy adult female rats selected randomly and marked individually for identification. Five days before testing, the animals were acclimatized to laboratory condition. To train the rats before the test, intraperitoneal injections of ketamine (50 mg/kg) and diazepam (5 mg/kg) were used to anesthetize these rats. A day prior to the test, dorsal/ flank region of about 10 % body surface area was shaved.

The initial limit-test dose of 200 mg/kg of extract gel was spread on the skin uniformly on the shaved part of two rats. Animals were under closer observations immediately after application and at fixed intervals within the initial 24 hours of the application, especially during the initial 2 to 6 hours. Twenty-four hours after the first search, a remnant extract was eliminated, and the rats were tested once again.

The animals those previously not exposed to the extract gel were then administered with the rising dosage-1000 mg/kg, and 2000 mg/kg in the same manner. During 14 days, all animals were examined on any clinical signs which could touch skin, fur, eyes, mucous membranes and respiratory, circulatory, autonomic, central nervous systems and motor activity and behaviour. Symptoms of tremors, convulsions, salivation, diarrhea, lethargy, sleep disturbances, and coma were given the special attention (26).

Study design

The PACE (chloroform extract) and PAHE (hydroethanolic extract) were experimented with both excision and incision wound models. In each model, the experimental animals were randomly subdivided into 7 groups with each group containing 6 rats. Group 1 acted as normal control. In Group 2, the plain gel base was applied, which acted as the negative control and In Group 3, 5% povidone-iodine ointment was applied, which acted as the positive control. Group 4 and 5 were treated with 5 and 10 %w/w PACE gel respectively and those Group 6 and 7 were treated with 5 and 10 %w/w PAHE gel respectively.

Determination of Wound Healing Activity

Excision wound

The sample of the wound was created by the surgical wound model on anesthetized rats. Before wound creation, the intra peritoneal application of ketamine (80 mg/kg) and diazepam (5 mg/kg) was used as the form of anesthesia. On each of the shaved rat dorsal regions, a full-thickness circular excision wound measuring about 500 mm^2 area and 2 mm deep was inflicted. Any bleed was stopped by patting the injury using a piece of cotton swab smeared with normal saline. These wounds were not covered, and the wounds were not dressed.

These wounds were open and exposed them to the environment.

Daily application of the different gel formulations was topically done on each wound and the outcomes observed until the wound healed completely. Day 0 was chosen as the day during which wound was created. The wound healing process was followed by marking the wound edges on transparent papers using a permanent marker each other day during a 21-day period. Such tracings were then put on graph papers to compute the area of the wound. Documentations including the wound pictures were also made using cameras during the treatment period. To compute the percentage of contraction in wound, the following formula was used [27,30]:

$$\% \text{ of wound closure} = \frac{\text{Wound area on day0-wound area on day n}}{\text{Wound area on day0}} X100$$

When n = n days: 1st, 3 rd, 5 th, 7 th, etc., till the 21 st day.

Estimation of Biochemical Markers

Hydroxyproline Content

On the 20th day of the experiment, the hydroxyproline of the newly formed tissues excised in a wound was determined. The tissue samples were initially dried under hot air oven at 60° C. They were afterwards hydrolysed at 130 ° C, 4h with 6N hydrochloric acid. The samples underwent hydrolysis after which they were neutralized to a pH of 7.0 and oxidized with Chloramine-T to last a time of 20 minutes. After 5 minutes of reaction, 0.4 M perchloric acid was added to end the reaction and some steps of colour development done with the help of Ehrlich reagent, at 60 ° C. The samples were mixed properly and measured using UV spectrophotometer at 557nms. Levels of hydroxyproline were quantified based on a standard calibration curve that was composed of the pure L-hydroxyproline (30).

Hexosamine Content

The samples of granulation tissue were weighed into the precise weights to determine the content of hexosamine contents, hydrolysed in 6 N hydrochloric acid in an incubator at 98 ° C, 8 hours. The neutralization of the hydrolysate was done to pH 7 by 4 N sodium hydroxide and the same was diluted with distilled water. The diluted solution in acetyl acetone solution was warmed at 96 ° C. in duration of 40 minutes. It was cooled after and 96 percent ethanol and p-dimethylaminobenzaldehyde solution (Ehrlich reagent) added. This was thoroughly stirred and left to stand on the table and at room temperature of 1 hour. The absorbance was next determined at 530 nm by double-beam UV-vis spectrophotometer. The hexosamine was measured based on absorbance values with a straight-line figure and expressed as milligrams per gram of dry tissue weight (32).

Total Protein Content

On the 21st day of the experiment protein content was estimated. One millilitre of 0.1 N NaOH was added to tissue samples and then the samples were homogenized overnight. 2 ml was removed and the homogenate and then centrifuged at 5000 rpm during 15 minutes. This was followed by the addition of 1 ml of the resultant supernatant into a reagent mixture consisting of 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 2 ml of 0.5 percent CuSO4 in 1 percent sodium potassium tartrate. This was incubated at room temperature during 15 minutes. The samples were incubated and 100 ml of Folin-Ciocalteu reagent was added to the sample and left to stand at room temperature for 30 minutes. The spectrophotometer that measured UV-visible light was then used to assess absorbance at 670 nm every 30 minutes (30).

Pro-inflammatory Cytokine (IL-6, TNF-α) and Anti-inflammatory Cytokine (IL-10):

All reagents, standards as well as serum samples were prepared in line with the manufacturer recommendations and left them at room temperature prior to analysis. The standard curve was produced by reconstituting the cytokine standards and serial diluting the range of 1,000 pg/ml to 15.6 pg/ml Each of the

standards was pipetted in the appropriate amount: 50–100 µL to each of the corresponding wells. Serum samples were suitably diluted (e.g. 1:2 or 1:10 using sample diluent), 50-100 ul of each of the diluted samples in respective well. In the case of non-pre-coated plates, they were exposed to capture antibodies and incubated for 1-2 hours. with 37º C .

The wells were washed with wash buffer, 3-5 times after incubation to remove unbound substances. After that biotinylated detection antibodies (50 -100 µL per well) specific to TNF- / IL6 / IL10 were added and incubated at room temperature at 30- 60min. This was then proceeded with addition of 50-100 µL streptavidin-conjugated horseradish peroxidase (HRP) and another 30-minute incubation. The colour reaction was started by adding 100 µL of tetramethylbenzidine (TMB) substrate to each well, after which the plate was incubated in dark length 15 to 30 minutes until a blue colour appeared. A mixture of 50- 100 µL of 2N sulphuric acid (H_2SO4) was added which abolished the reaction resulting in a yellow solution. The ELISA reader was utilized in measuring the absorbance at 450 nm, with a background correction undertaken by either taking the difference at 570 or 630 nm (33).

Histopathology

At the last day of wound healing experiment, the animals were killed by first anesthetizing them with ketamine HCl (50 mg/kg, intraperitoneally) and then humane killing. The wound area along with the healthy nearby tissue was taken as the sample to obtain the tissue. The above samples were fixed in 10 percent formalin and examined under standard histopathological examination. Hematoxylin and eosin staining was performed on the wound tissues, and the samples were studied at a light microscope. Moreover, in order to evaluate the amount of collagen the Van Gieson stain that stains only the collagen fibers was used to stain the tissue sections and viewed microscopically (30).

Incision wound

The dorsal surface of the rat was shaved 30 minutes after administering anesthesia by use of incision wounds that had a length of about 6 cm and depth of 2 mm. The excised skin was positioned and sewed with the black silk threads at an interval of 0.5 cm. These stitches were done with the surgical threads (No. 000) through the curved needles (No. 9) making sure that the edges of the wound were carefully closed by tightening the continuous sutures (Figure 2). Treatment of Groups Topical application of formulations was done in each group, once a day/10 days. Day 0 was the day of wounding. The sutures were laid off after 8 days of wounding when appropriate healing was noted. The tensiometer was used to determine the tensile strength of healed skin (weight in grams at which the wound is ruptured) on Day 10 and the rupture force was measured on Day 15 [27,31].

Statistical Analysis

The data of the experiment were given in mean + SEM. One-way ANOVA test was applied to identify statistical values and was followed by Tukey post hoc test with the help of GraphPad Prism 5 software (30).

Results and Discussion

Phytochemical Analysis

The results of the phytochemical screening of the chloroform extract (PACE) showed presence of flavonoids, tannins, phytosterols and triterpenoids. On the contrary, flavonoids, glycosides, tannins, phytosterols, saponins and triterpenoids were identified in the hydroethanolic extract (PAHE).

Acute Dermal Toxicity Study

In the acute dermal toxicity test both chloroform extract (PACE) and hydroethanolic extract (PAHE) gels were safe at the maximum dose of 2,000 mg/kg. During the 14 days of observational period, there were no symptoms of skin reactions, including inflammation, erythema, irritation, redness, or any other

negative outcome, shown by the treatment animals.

Determination of Wound Healing Activity

Excision wound

Wound Contraction

Tables 1-3[*p < 0.05 (a), ** p < 0.01 (b). *** p < 0.001 (c)] (Figures 1 and 3) show the percentage of wound contraction in PACE and PAHE gels as well as Povidone-Iodine. Periodical records of wound contraction were noted to determine the rate of healing. The most complete healing and rapid healing were observed on standard group (Povidone-Iodine) on the 17th day with complete wound closure. A comparable healing pattern was also observed in the treatments group where PAHE 10% w/w gel achieved complete closure on day 19 after which PACE 10% w/w and PAHE 5% w/w gels followed. Even though PACE 5% w/w and the base gel groups also showed an on-going wound contraction, the healing process was a bit slower. The slowest recovery was recorded in the control group of which only 99.88 ± 0.08 % is closed on day 21. These findings suggest that either PACE or PAHE gels, especially with 10% w/w concentration has a dose-dependent wound healing mechanism

Period of Epithelialization

The use of PACE and PAHE gels at the level of 5% w/w and 10% w/w greatly minimized the period of the epithelialization compared to the control group and the group in which the base gel has been used. The healing periods of the epithelialization of PACE gel and PAHE gel treated groups were almost comparable to the Povidone-Iodine ointment. When the reduction in epithelialization time expressed as a percentage is compared then it might be possible that higher concentrations of PACE and PAHE gels could be used more effectively in promoting wound healing compared to Povidone-Iodine. The epithelialization occurred earlier in all the treated groups when compared with the control. The control group took the shortest time (less than/equal to 17 days) to recover and PACE 10% w/w and PAHE 10% w/w gels also healed with re-epithelialization at about day 19. Control group on the other hand required 21 days which means that healing is delayed with respect to the resurfacing of the wound (Table 4 & Figure 2) [** p < 0.01 (b). *** p < 0.001 (c)]. These observations indicate the increased epithelial regeneration capacity of both the extracts, particularly in higher concentrations).

Biochemical Markers Analysis

Content of hydroxyproline

Table 5 and Figure 4 show the effect of PACE and PAHE gel on the extent of hydroxyproline in the healed tissue. Hydroxyproline, the key indicator of collagen synthesis was found to be significantly higher when compared between the treated groups and control. Strong wound healing activity was confirmed in the standard treatment of the highest quantity of the hydroxyproline component (80.19 0.6938 mg/g). The PACE 10% (76.41 ± 0.7958 mg/g) and PAHE 10% (71.73 ± 0.2884 mg/g), however, among the experimental groups were more or less accordant with the original and were notable higher than the control group (32.87 ± 0.5382 mg/g) with p < 0.001. Initiating with lower concentration, PACE 5% and PAHE 5%, the moderate increase was observed (35.87 ± 0.2129 mg/g and 35.72 ± 0.2015 mg/g, respectively, p < 0.01). Based on these findings, both gels showed considerable promotion of collagen production and their wound healing given that at 10% w/w, these gels promoted collagen than at 5% w/w.

Content of Hexosamines

The impact of PACE and PAHE gels on the level of hexosamine as an indicator of extracellular matrix (ECM) formation was also revealed in Table 5 and Figure 4. There was a strong elevation of the content of hexosamine in treated groups as compared to the control in all of them. Belonging to the standard group, the highest levels (28.82± 0.1674 mg/g) were demonstrated, which indicates increased ECM development. The PACE 10% (26.80 ± 0.2220 mg/g) and

PAHE 10% (21.86 ± 0.1272 mg/g) also exhibited significant results (($p < 0.001$) and were even more elevated than PACE and PAHE at enhanced percentages of the treatments by comparison to the control (10.05 ± 0.3172 mg/g) and was highly significant ($p < 0.001$). According to the findings, both extracts enhance tissue repair by stimulating the synthesis of ECM in the dose-dependent fashion.

Total Protein Content

Total protein levels, which indicate tissue repair and cell activity at the wound site, were significantly higher in the treatment groups. The standard-treated group had the highest protein concentration (81.69 ± 0.2441 mg/g), showing strong tissue repair. There were also significant increases with PAHE 10% w/w gel (77.06 ± 0.2270 mg/g) and PACE 10% w/w gel (68.06 ± 0.2997 mg/g) compared to the control group (43.28 ± 0.3344 mg/g), with a statistical significance of $p < 0.001$. Moderate but still significant increases were found in groups treated with PACE 5% w/w (44.85 ± 0.2224 mg/g) and PAHE 5% w/w (44.92 ± 0.2094 mg/g) gels ($p < 0.01$). These results suggest that both PACE and PAHE gels, especially at higher concentrations, improve protein production, which supports the wound healing process.

Pro-Inflammatory Cytokines (IL-6, TNF-α) and Anti-Inflammatory Cytokine (IL-10)

To assess the inflammation during wound healing, levels of TNF-α, IL-6 (pro-inflammatory), and IL-10 (anti-inflammatory) were measured. The control group showed high levels of TNF-α (106.9 ± 2.963 pg/mL) and IL-6 (72.40 ± 1.762 pg/mL), indicating ongoing inflammation, with lower IL-10 (17.47 ± 1.910 pg/mL). Both PACE and PAHE gels resulted in a significant, dose-dependent decrease in TNF-α and IL-6. The 10% w/w gels of PACE (76.40 ± 2.597 pg/mL for TNF-α; 55.97 ± 1.386 pg/mL for IL-6) and PAHE (77.70 ± 2.871 pg/mL; 57.87 ± 1.073 pg/mL) showed the largest reductions ($p < 0.001$), closely aligning with the standard treatment (67.67 ± 2.659 pg/mL; 47.30 ± 1.308 pg/mL). At the same time, IL-10 levels rose significantly in these groups, reaching 33.10 ± 1.882 pg/mL (PACE 10%) and 32.87 ± 2.526 pg/mL (PAHE 10%), nearly matching the standard (36.60 ± 1.484 pg/mL) (Table No. 6 & Figure No. 5). These results suggest that both PACE and PAHE gels effectively manage inflammation by lowering pro-inflammatory markers and boosting anti-inflammatory responses, creating a better environment for healing.

Histopathological Evaluation

Histological analysis of wound tissues (Figure No. 6 A & B), stained with Hematoxylin-Eosin (H&E) and Van Gieson (VG), highlighted significant differences in inflammation, epithelial regeneration, collagen formation, and granulation tissue among the groups. In the control group, H&E staining showed incomplete epithelial coverage, limited fibroblast activity, and a high number of inflammatory cells. VG staining confirmed poor collagen deposition, indicating slow tissue remodelling. In contrast, the standard treatment group showed complete epithelialization, organized granulation tissue, and dense collagen fibers, which indicated effective healing. Tissue samples from animals treated with PACE and PAHE extracts demonstrated a dose-dependent improvement in tissue structure. The 10% gel groups for both extracts showed nearly full epithelial regeneration, fewer inflammatory cells, and greater fibroblast activity. VG staining in these groups revealed increased and more structured collagen deposition, like the standard treatment. Meanwhile, the gel base group showed only partial healing, with noticeable inflammation and loosely arranged collagen. These histological results support the biochemical and biomechanical findings, showing that PACE and PAHE gels significantly improve wound healing by promoting tissue regeneration and collagen maturation.

Incision Wound Model

Tensile strength assessment was conducted to evaluate the mechanical strength and collagen quality in the healed skin. The control group

showed the weakest tensile strength at 363.47 ± 6.291 g, reflecting poor tissue repair. The standard group (treated with Povidone iodine) achieved the highest tensile strength at 625.51 ± 8.627 g ($p < 0.001$), indicating effective collagen cross-linking and tissue regeneration. Among the experimental treatments, PACE 10% w/w gel (610.46 ± 8.741 g) and PAHE 10% w/w gel (595.74 ± 9.639 g) showed a notable improvement in tensile strength, nearly matching the standard group ($p < 0.001$). Moderate yet significant increases were seen with PACE 5% (525.91 ± 7.421 g) and PAHE 5% (511.74 ± 8.432 g) gels ($p < 0.01$). The base gel group showed limited improvement at 375.62 ± 9.251 g, close to the control. These results suggest that PACE and PAHE gels, especially at 10% strength, significantly enhance the tensile strength of the healed tissue, indicating better collagen deposition and structural remodelling (Table No. 7 & Figure No. 7 & 8).

Table No.1 Effect of extract on wound diameter across different stages of the study

Groups	D1	D3	D5	D7	D9	D11	D13	D15	D17	D19	D21
Control	25±0	23.5±0.057	21.5±0.024	19.25±0.095	17.75±0.05	15.5±0.057	14±0.081	9.25±0.125	7±0.05	3.75±0.05	0.75±0.05
Base	25±0	22.5±0.057	20±0.070	17.75±0.095	15±0.081	13±0.081	11±0.081	8.5±0.057	5.5±0.05	1.75±0.05	0±0
STD	25±0	21.5±0.057	17.25±0.082	14.5±0.057	12.75±0.05	11.25±0.095	8±0.095	2.5±0.057	0±0	0±0	0±0
PACE 5%	25±0	22.5±0.057	19±0.024	15.25±0.095	13.75±0.05	10.25±0.095	8±0.0817	5.5±0.0577	1.75±0.05	0±0	0±0
PACE 10%	25±0	22.25±0.05	19±0.070	16.25±0.125	14.25±0.125	12±0.081	8.25±0.05	1±0.0817	0±0	0±0	0±0
PAHE 5%	25±0	22.75±0.05	20.75±0.05	17.25±0.05	13.25±0.095	10.25±0.095	5.75±0.095	3.75±0.095	1.75±0.05	0±0	0±0
PAHE 10%	25±0	21.25±0.05	17.25±0.043	14.5±0.1	12.5±0.057	9±0.081	5±0.081	1.25±0.05	0±0	0±0	0±0

Table No 2: Effect of Extract on Wound Area

Groups	**Area mm2**										
	D1	D3	D5	D7	D9	D11	D13	D15	D17	D19	D21
Control	490.6 ± 0	433.7 ± 0.213	363.0 ± 0.194	291.4 ± 0.286	247.4 ± 0.137	188.7 ± 0.140	154.2 ± 0.179	68.0 ± 0.189	38.8 ± 0.089	11.1 ± 0.027	0.5 ± 0.003
Base	490.6 ±0	397.6 ± 0.203	314.3 ± 0.256	247.8 ± 0.269	177.0 ± 0.192	133.0 ± 0.166	95.3 ± 0.141	56.9 ± 0.077	23.94 ± 0.049854	2.55 ± 0.011775	0±0
STD	490.6 ±0	363.0 ± 0.194	234.1 ± 2.290	165.2 ± 0.131	127.7 ± 0.098	99.8 ± 0.166	41.8 ± 0.105	5.1 ± 0.022	0±0	0±0	0±0
PACE 5%	490.6 ±0	397.6 ± 0.203	283.7 ± 0.243	183.1 ± 0.226	148.5 ± 0.105	83.0 ± 0.151	50.6 ± 0.102	23.9 ± 0.0498	2.5 ± 0.011	0±0	0±0
PACE 10%	490.6 ±0	388.7 ± 0.176	283.7 ± 0.243	208.2 ± 0.328	160.3 ± 0.288	113.4 ± 0.153	53.5 ± 0.066	1.1 ± 0.013	0±0	0±0	0±0
PAHE 5%	490.6 ±0	406.4 ± 0.176	338.1 ± 0.160	233.7 ± 0.137	138.3 ± 0.196	83.0 ± 0.151	26.4 ± 0.089	11.5 ± 0.059	2.5 ± 0.011	0±0	0±0
PAHE 10%	490.6 ±0	354.6 ± 0.168	233.7 ± 0.137	165.6 ± 0.219	122.8 ± 0.113	63.9 ± 0.115	20.0 ± 0.064	1.3 ± 0.011	0±0	0±0	0±0

Table No 3: Percentage of Wound Closure of Different Test Samples

Groups	Percentage wound closure										
	D1	D3	D5	D7	D9	D11	D13	D15	D17	D19	D21
Control	0±0	11.6 ± 4.341	26 ± 3.972	40.6 ± 5.835	49.56 ± 2.8	61.52 ± 2.863	68.56 ± 3.659	86.12 ± 3.871	92.08 ± 1.831	97.72 ± 0.56	99.88 ± 0.08
Base	0±0	18.96 ± 4.156	35.92 ± 5.226	49.48 ± 5.501	63.92 ± 3.920	72.88 ± 3.397	80.56 ± 2.875	88.4 ± 1.570	95.12 ± 1.016	99.48 ± 0.24	100 ± 0±
STD	0±0	26 ± 3.972 a	52.28 ± 5.222 a	66.32 ± 2.678 a	73.96 ± 2 a	79.64 ± 3.384 a	91.48 ± 2.16 a	98.96 ± 0.461 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
PACE 5%	0±0	18.96 ± 4.156	42.16 ± 4.965 a	62.68 ± 4.610 a	69.72 ± 2.16 a	83.08 ± 3.078 a	89.68 ± 2.092 a	95.12 ± 1.016 a	99.48 ± 0.24 a	100 ± 0 a	100 ± 0 a
PACE 10%	0±0	20.76 ± 3.6 c	42.16 ± 4.965 a	57.56 ± 6.688 b	67.32 ± 5.883 a	76.88 ± 3.136 a	89.08 ± 1.36 a	99.76 ± 0.277 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
PAHE 5%	0±0	17.16 ± 3.6	31.08 ± 3.28	52.36 ± 2.8 c	71.8 ± 3.997 a	83.08 ± 3.078 a	94.6 ± 1.826 a	97.64 ± 1.214 a	99.48 ± 0.24 a	100 ± 0 a	100 ± 0 a
PAHE 10%	0±0	27.72 ± 3.44 a	52.36 ± 2.8 a	66.24 ± 4.48 a	74.96 ± 2.309 a	86.96 ± 2.353 a	95.92 ± 1.309 a	99.72 ± 0.24 a	100 ± 0 a	100 ± 0 a	100 ± 0 a

* Indicates

$p < 0.05$ (a). ** indicates $p < 0.01$ (b). *** indicates $p < 0.001$ (c).

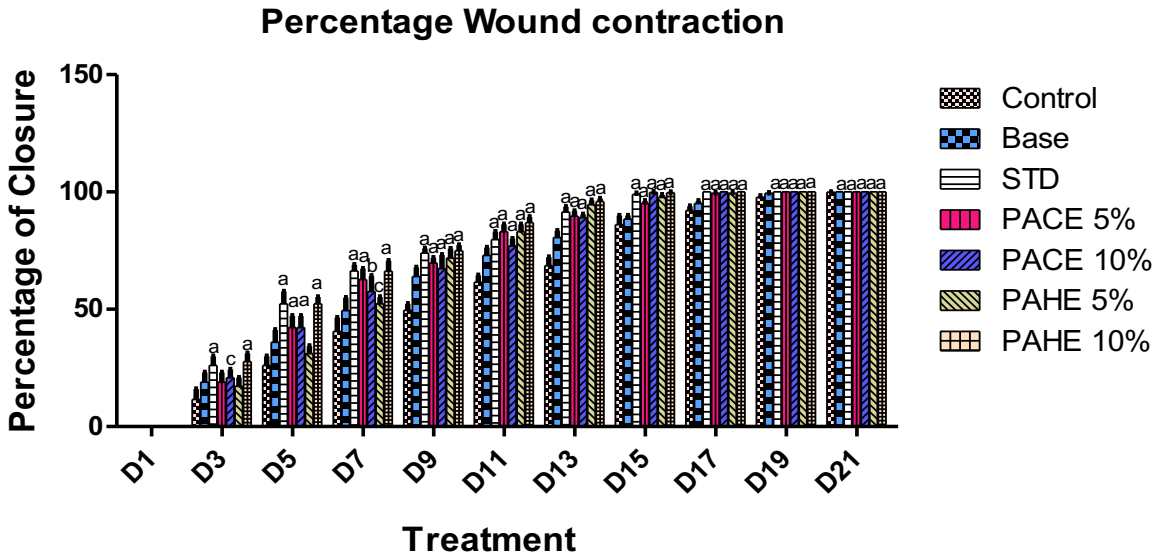


Figure No.1 Wound area contraction percentage was used as a key factor to measure in vivo wound healing efficiency in the Excision Wound Model. Data are shown as Mean ± SEM based on six animals per group. Statistical significance was noted as *p < 0.01 and **p < 0.001 compared to the control group.

Table no.4: Period of Epithelialization

Groups /Animals	Mean±SEM
Control	20.666±0.5163
BASE	19.666±0.8164
STD	16.833±0.4082**
PACE 5%	17.5±1.0488*
PACE 10%	17±0.6324**
PAHE 5%	17.666±0.5163*
PAHE 10%	17.166±0.7527**

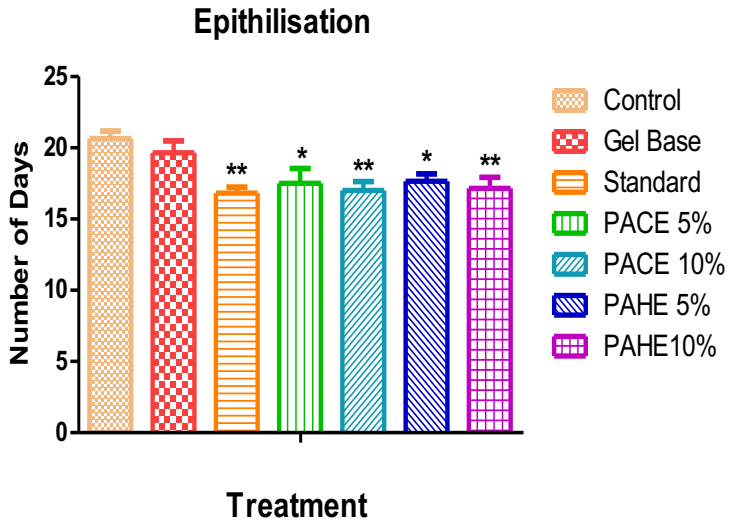


Figure No. 2 shows the period of epithelialization. This was used to measure in vivo wound healing in the excision wound model. The data are represented as Mean ± SEM for six animals in each group. Statistical significance is indicated by *p < 0.01 and **p < 0.001 compared to the control group.

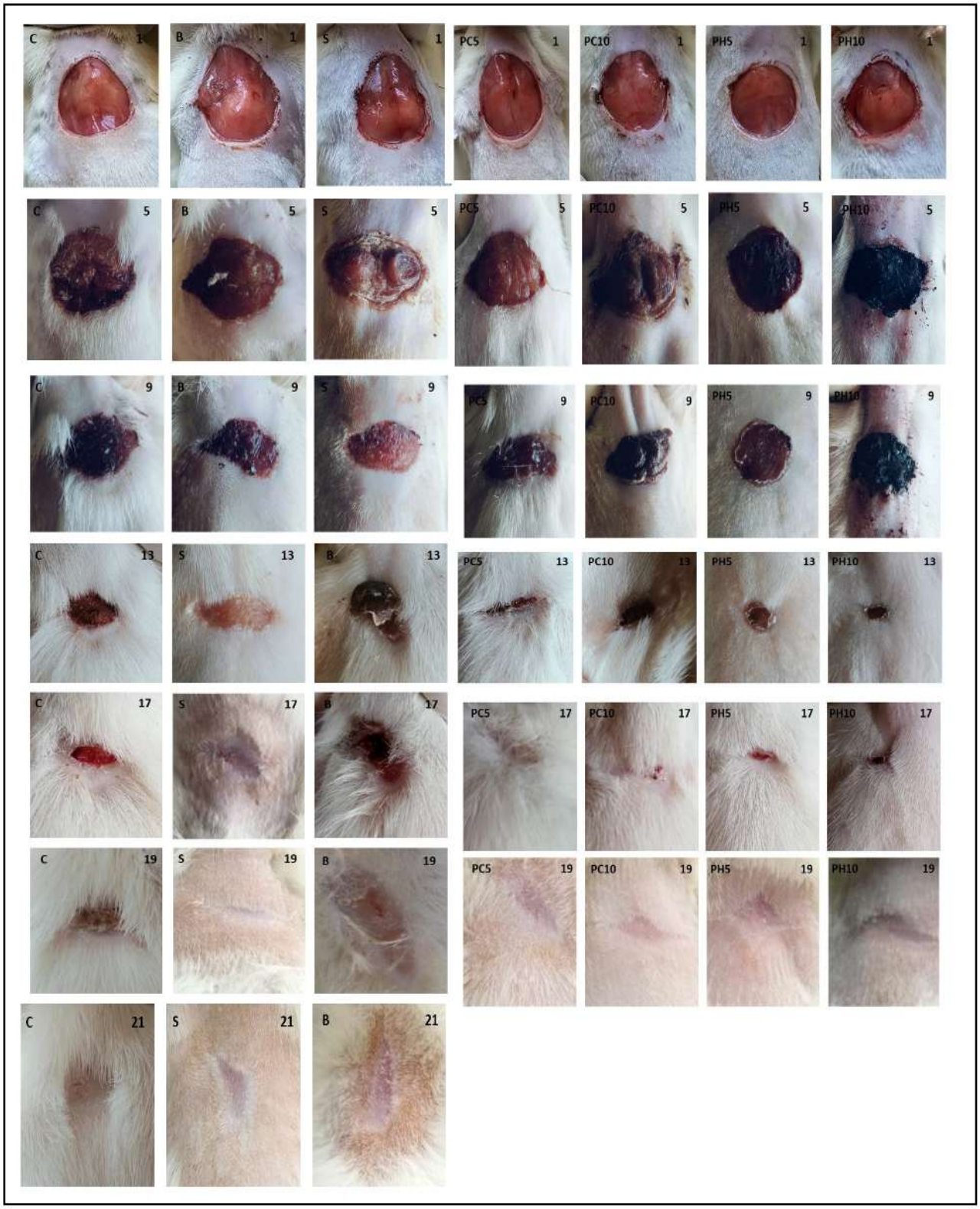


Figure No. 3: Photographic documentation of wound contraction on different post-excision days in various animal groups (Control, Base, Standard, PACE 5% w/w, PACE 10% w/w, PAHE 5% w/w, and PAHE 10% w/w).

Table no 5: Estimation of Hydroxyproline, Total Protein and Hexosamine

Treatment	Hydroxyproline (mg/g tissue)	Total Protein (mg/g tissue)	Hexosamine (mg/g tissue)
Control	32.87± 0.5382	43.28±0.3344	10.05±0.3172
Base	28.51±0.5431	31.88±0.3013	5.215±0.2233
Standard	80.19±0.6938***	81.69±0.2441***	28.82±0.1674***
PACE 5%	35.87±0.2129**	44.85±0.2224**	11.41±0.3881**
PACE 10%	76.41±0.7958***	68.06±0.2997***	26.80±0.2220***
PAHE 5%	35.72±0.2015**	44.92±0.2094**	11.60±0.1567**
PAHE10	71.73±0.2884***	77.06±0.2270***	21.86±0.1272***

* indicates $p < 0.05$ (significant).** indicates $p < 0.01$ (highly significant).*** indicates $p < 0.001$ (very highly significant).

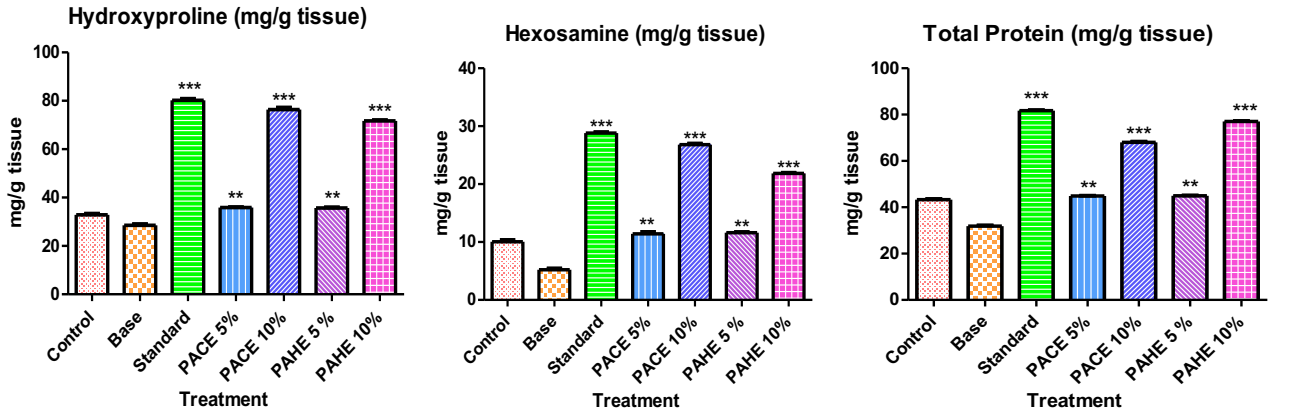


Fig No 4: Effect of PACE &PAHE gel on Hydroxyproline content, Hexosamine content and Total Protein content

Table No 6: Estimation of Proinflammatory (TNF-α & IL-6) & Anti-inflammatory Cytokines (IL-10)

Groups	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control	106.9±2.963	72.40±1.762	17.47± 1.910
Base	101.6±3.728	70.27±1.099	21.37± 2.085
Standard	67.67±2.659***	47.30±1.308***	36.60± 1.484***
PACE 5% w/w	85.10±3.593**	59.93±1.885**	29.43± 1.849**
PACE 10% w/w	76.40±2.597***	55.97±1.386***	33.10± 1.882***
PAHE 5% w/w	87.17±2.791**	61.30±1.587**	29.30±1.701**
PAHE10 w/w	77.70±2.871***	57.87± 1.073***	32.87± 2.526***

* Indicates $p < 0.05$ (significant). ** indicates $p < 0.01$ (highly significant). *** indicates $p < 0.001$ (very highly significant).

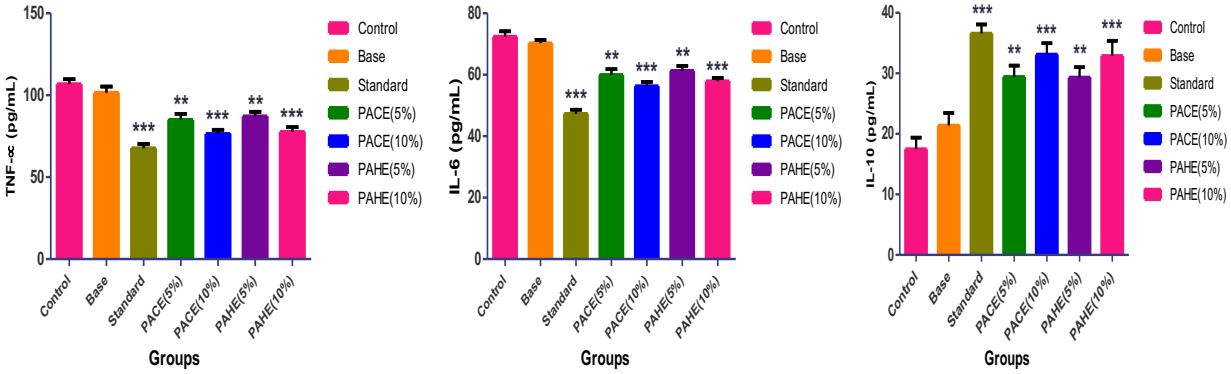
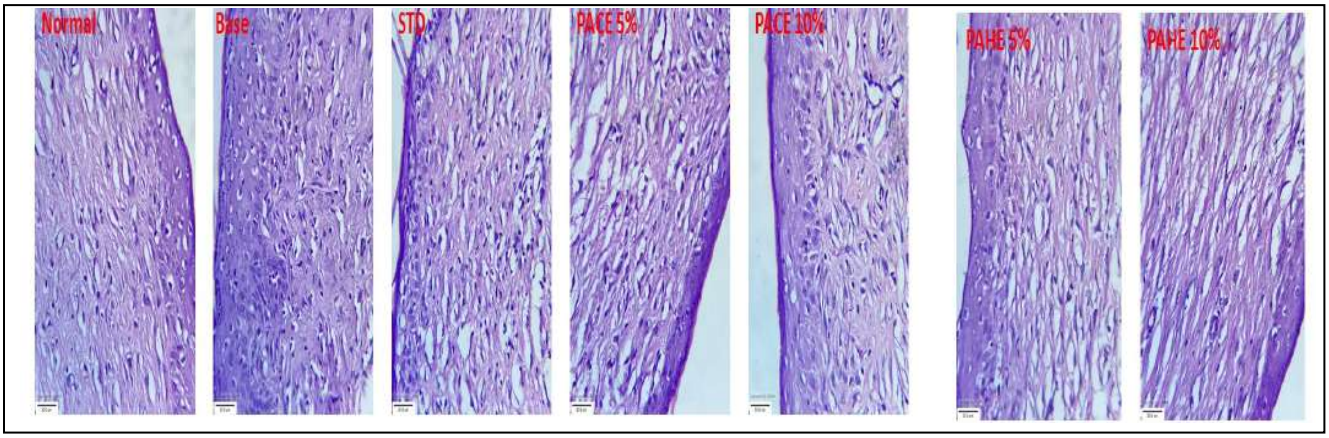
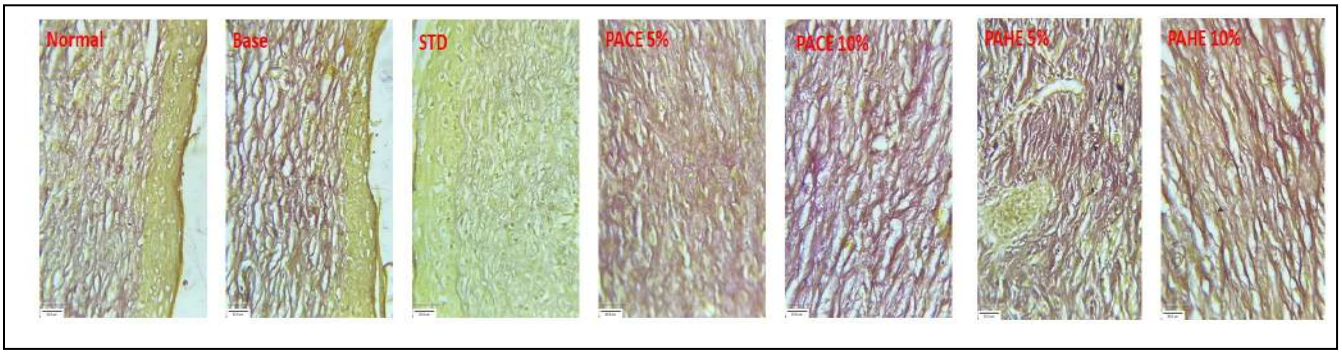


Fig No 5: Effect of PACE &PAHE gel on Proinflammatory (TNF-α & IL-6) & Anti-inflammatory Cytokines (IL-10)



6.A. Hematoxylin-Eosin Stain



6.B Van Gieson Stain

Figure No. 6: Histological analysis of newly formed tissue on day 21
A. Hematoxylin-Eosin Staining B. Van Gieson's Staining (magnification 10X)

Figure No. 6 (A & B) shows histopathological images of wound tissue stained with Hematoxylin-Eosin (H&E) and Van Gieson (VG). It highlights important differences among experimental groups in inflammation, epithelial regeneration, collagen formation, and granulation tissue development.

In the control group, H&E staining showed incomplete epithelial coverage, low fibroblast activity, and a significant number of inflammatory cells. VG staining revealed poor

collagen deposition, indicating delayed wound healing. The standard-treated group had fully restored epithelium, well-structured granulation tissue, and a dense presence of collagen fibers, indicating improved healing. Animals treated with PACE and PAHE extracts showed concentration-dependent improvements in tissue structure. In the 10% gel groups, there was nearly complete epithelialization, reduced inflammation, and increased fibroblast activity. VG staining confirmed better-organized collagen deposition, similar to the standard group. In contrast, the base-treated group had only partial tissue regeneration, with moderate inflammation and loosely arranged collagen fibers.

Table No 7: Determination of Tensile Strength:

Sl.No	Tensile Strength (g)
Control	363.47±6.291
Gel Base	375.62±9.251
Standard (Povidone iodine ointment)	625.51±8.627***
PACE 5% w/w gel	525.91±7.421**
PACE 10% w/w gel	610.46±8.741***
PAHE 5% w/w gel	511.74±8.432**
PAHE10% w/w gel	595.74±9.639***

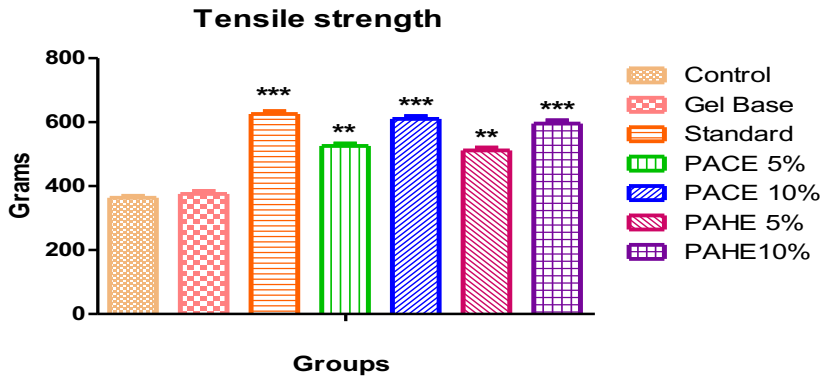


Fig No 7: Effect of PACE &PAHE gel on Tensile Strength

* Indicates $p < 0.05$ (significant). ** indicates $p < 0.01$ (highly significant).

*** indicates $p < 0.001$ (very highly significant

8.A **8.B**

Figure 8. (A) Wound suturing on day 0; (B) Measurement of Tensile strength of test drug-treated animal

Conclusions

In this study, both the chloroform extract (PACE) and the hydroethanolic extract (PAHE) of Prunus africana bark showed improved wound healing. They increased levels of hydroxyproline, hexosamine, total protein, and the anti-inflammatory cytokine IL-10. Additionally, these extracts significantly lowered pro-inflammatory cytokines IL-6 and TNF-α, sped up the epithelialization process, and increased tissue strength. These results clearly show that Prunus africana bark has strong potential for healing wounds in living organisms.

Acknowledgement

I am thankful to all those who directly help and support during this research

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