

Research Article

Costus Afer Stem Juice Extract Enhances Wound Healing Process Through the Modulation of the Expression of TGF- β 1 at Wound Site

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Abstract

Recent studies have explored the potential of Costus afer stem juice in supporting the process of tissue repair and regeneration. This study sought to expand on those discoveries by assessing the expression of transforming growth factor beta 1 (TGF- β 1) in wounds treated with C. afer stem juice extracts. Ninety-six adult Wistar rats, divided into four groups, were used to study the wound healing process over 24, 72 and 120 hours, as well as at complete healing. The rats were wounded on their dorsum and treated on the first day according to their group: Group A received no treatment, Group B was treated with honey gel, Group C with heat-dried C. afer stem juice extract and Group D with freeze-dried C. afer stem juice extract. The result of the study showed that the expression of TGF- β 1 was initially highest in group D which gradually declined to the lowest at 120 hours and peaked again in the healed tissues. Group C wounds showed the lowest level of expression of TGF- β 1 at 24 hours, which increased at 72 hours and gradually fell to be at the same level as group B and group A wounds in the healed tissues. The deposition of granulation tissues and extracellular matrix fibres in group D followed the same pattern as expression of TGF- β 1. In conclusion, the extract of C. afer stem juice enhanced the healing process through the modulation of TGF- β 1 expression.

Keywords: Costus Afer; Wound Healing; TGF- β 1

Introduction

The skin, being the outer covering of the body, is always at risk of injury. When injured, the skin can either fully regenerate or produce scar tissue at the site of injury [1,2]. The outcome of the healing process has been largely associated with the differential expression of TGF- β 1 [3-5].

Studies have shown that adult wounds, which heal with fibrosis, express higher levels of TGF- β 1, while embryonic wounds, which heal by complete regeneration, express lower levels of TGF- β 1. Notwithstanding, TGF- β 1 have been found to be important for the healing process as TGF- β 1 deficient mice showed impaired wound healing [6,7]. In addition, it has been shown that the level of TGF- β 1 expression in foetal wounds that heal without scar remained unchanged during the healing process 8-10. Thus, the relative level of TGF- β 1 and not the absolute expression, seems to be the determinant of the healing phenotype [4,5,11].

TGF- β 1 exerts both pro-inflammatory and anti-inflammatory effects at wound sites depending on the specific stage of the healing process [12,13]. The expression of TGF- β 1 at the early phase of the healing process prompts recruitment of inflammatory cells into the injury site, which are later involved in a negative feedback via release of superoxides from macrophages [14]. During this initial stage, TGF- β 1 prompts the expression of key components of extracellular matrix (ECM) proteins, such as fibronectin and collagen types III, thus promoting the formation of granulation tissues [15,16]. TGF- β 1 also inhibits various Matrix Metalloproteinases (MMPs), which further promote the accumulation of collagen fibers and perpetuating scar formation [17]. It is involved in the differentiation of fibroblast to myofibroblast, which are crucial to wound contraction [18,19]. When applied experimentally to wounds that have no repair deficiency, TGF- β 1 has been found to accelerate wound healing. However, the

increase in the healing rate is at the expense of increased fibrosis as excessive TGF- β 1 activity leads to the increased synthesis of collagen fibres [20,21].

Costus afer is a monocot plant mainly found in the wild within the forest region of tropical Africa [22]. The plant is a popular traditional medicine plant, used by the indigenous people for its therapeutic benefits. Recent scientific research has revealed several chemical compounds and therapeutic properties that may explain some of its traditional uses [23,24]. The stem of *C. afer* has been found to exhibit antimicrobial activity against some species of fungi and bacteria. This activity has been attributed to the presence of some phytochemicals in *C. afer*, such as flavonoids, alkaloids, triterpenes and phenolics compounds, which have been shown to exhibit antimicrobial activity [23,25]. In addition to its antimicrobial properties, *C. afer* stem has been reported to possess analgesic and anti-inflammatory activities. Researchers studying the analgesic effects of *C. afer* stem in animal models found that it was able to reduce pain in a dose-dependent manner [24]. Moreover, *C. afer* stem extract has been found to inhibit inflammatory cytokines and enzymes, such as interleukin-1 β , tumor necrosis factor-alpha and cyclooxygenase-2 [26-29]. Its antinociceptive and antioxidant properties have also been confirmed by several studies [26,30,31].

A study by Udoh, et al., showed that aqueous extract of *C. afer* stem juice significantly enhanced wound healing [32]. It was reported that application of the extract at wound site only on the first day was more potent at enhancing the healing process compared to continuous application for seven days. Continuous application for seven days was reported to stimulate the deposition of fibrous tissue below the scab, which resorbed when application was stopped. Notwithstanding, both modes of application were found to result in a better healing outcome compared with iodine ointment [32,33]. The healed skin tissues from treatment with aqueous extract of *C. afer* stem juice were found to have random and tight organization of dermal fibers with significantly higher tensile strength [32]. The researchers attributed the enhanced healing outcome to the biomolecules in *C. afer* stem juice including alkaloids, triterpenes and quinones, which have also been reported to modulate the activities of growth factors at wound site, including TGF- β 1.

Based on this improved healing outcome and the regulatory role of TGF- β 1 during the healing process, this study was carried out to assess the effect of *C. afer* stem juice on the expression of TGF- β 1 through the healing process using heat-dried and freeze-dried extracts of *C. afer* stem juice.

Materials and Methods

Ethical Approval

The ethical approval for this study was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee. The study was given the ethical number: UU_FBMSREC_2024_010 (Appendix III).

The health of the rats was regularly monitored through weight measurements and stress indicators. All experimental procedures followed the guidelines of the National Centre for the Replacement, Refinement and Reduction (3Rs) of Animals in Research, as well as the internationally accepted standards for laboratory animal care and use, as outlined by the National Institute of Health (NIH) publication number 85 (23), revised in 2011 [34,35]. The animals were treated humanely and all procedures were conducted with strict aseptic techniques.

Plant Collection

Fresh stems of *C. afer* were collected from bushes within Uyo Local Government Area, Uyo State, Nigeria with coordinate 5.0377° N, 7.9128°E. After removal of the foliage leaves, the stems were thoroughly washed and cut into pieces to be crushed in an electric blender. The juice obtained from the blender was filtered with a chess cloth. Equal volumes of the juice was concentrated by freeze-drying using a freeze-dryer and by evaporation in beaker placed in hot water bath at 40°C as modified from Zhang, et al.

Analyses of Phytoconstituents of Extracts

A portion of the extracts was collected and analyzed for their proximate, phytochemicals and mineral constituents. The qualitative and quantitative analyses of the phytochemicals in the extracts were carried out according to the procedures described by Ezeonu and Ejikeme, as well as Abubakar and colleagues [36,37].

Mineral Content

The analysis of the mineral content for the extracts was done using the wet digestion sample method and Atomic Absorption Spectrophotometer (AAS). For wet digestion of sample, 2 mL of the plant samples was taken in digesting glass tube. 12 mL of hydrochloric acid was added to the plant samples. The mixture was kept overnight at room temperature. 4.0 mL Perchloric Acid (PCA) was added to these mixtures and was kept in the fumes block for digestion. The temperature was increased gradually, starting from 50°C and increasing up to 150°C. The digestion was completed in about 70-85 minutes as indicated by the appearance of white fumes. The mixture was left to cool and the contents of the tubes were transferred to 100 mL volumetric flasks and the volumes of the contents were made to 100 mL with distilled water. The wet digested solution was transferred to plastic bottles and labelled accurately. The digest was stored and used for mineral determinations. Mineral contents of plant samples were determined by Atomic Absorption Spectrophotometer (AAS) while Na and K were determined using flame photometry according to the procedure specified in AOAC [38].

Experimental Animals

Ninety-six adult female and male Wistar rats, weighing between 240-320 g, obtained from the animal house of the Department of Pharmacology and Toxicology, University of Uyo were used for this study. The rats were housed individually in plastic cages and allowed to acclimatize for 14 days. They were fed with pelletized diet (Vital Feeds Growers, Green Cereals Nigeria Ltd.) and water ad libitum and exposed to 12 hour light/dark natural lighting.

The rats were equally divided into 4 treatment groups (24 rats each) of A, B, C and D for each treatment procedure as illustrated in Table 1. Each treatment group was subdivided into four observational groups of 6 rats each for the investigation of the healing process through time. For each treatment group, treatment was done only on the first day after which six rats were chosen at random after appropriate treatment for molecular and histological assessments on 24 hours, 72 hours, 120 hours and on complete healing (Table 1).

Group	Treatment	24 hrs	72 hrs	120 hrs	Healed	Total
A	No treatment	6 rats	6 rats	6 rats	6 rats	24 rats
B	Medihoney gel	6 rats	6 rats	6 rats	6 rats	24 rats
C	Heat-dried extract	6 rats	6 rats	6 rats	6 rats	24 rats
D	Freeze-dried extract	6 rats	6 rats	6 rats	6 rats	24 rats
Total		24 rats	24 rats	24 rats	24 rats	96 rats

Table 1: Experimental design.

Wound Creation

All surgical interventions were carried out under local anaesthesia with lidocaine. A designated area of fur at the centre of the rat's dorsum was carefully shaved using scissors and a razor blade. The shaved area was cleaned using savlon and methylated spirit. Within the shaved area, a circular marking of 1.5 cm in diameter was made using methylene blue to guide the excision process. The target area was excised with a surgical blade, scissors and forceps to create a full thickness circular wound (Appendix 1). Immediately after wounding, sterile gauze was used to apply pressure on the wound until haemostasis was attained. Thereafter, the wounded rats were treated according to their treatment groups.

Treatment of Wounds

All groups were treated only on the first day of injury. Group A served as control without treatment; group B was treated with honey gel (Medihoney); group C was treated with heat-dried extract of C. afer stem juice; and group D was treated with freeze-dried extract of C. afer stem juice. Medihoney gel was obtained commercially from pharmaceutical stores, while other treatment substances were developed during the study. A spatula was used to apply 2.5 mL of each treatment agent on the wound. Then the rats were left for observation according to the experimental protocol as illustrated in Table 3.

Microbiological Assessment Procedure

The microbial profile of the breeding environment was carried out to determine the presence or absence of certain microbes that could affect the healing process in case of future study replication. About 1 g of the bedding was placed in a sterile universal

container and 10 mL of tryptone soya broth was added. It was incubated at 37°C for 24 hrs. The overnight broth was sub-cultured on nutrient agar, Macconkey agar and Sabouraud dextrose agar. The plates were incubated at 37°C for 24 hrs. The isolate bacteria were identified based on colony morphology, gram staining, reaction and biochemical characteristics using established standardized methods according to Bergey's Manual of Determinative Bacteriology [39].

Laboratory Procedure for Histological Processing

Samples of 0.2 cm around the wound site was collected from each rat for histological processing and assessment. The tissue samples, which included healing tissues from the observational groups and the healed skin from the wound healed tissue were appropriately labeled and fixed in 10% neutral buffered formalin for 72 hours before dehydration using automatic tissue processor (Shandon-ElliotR). After dehydration, the tissues were embedded in paraffin wax and sectioned at 5 microns using a rotary microtome (AM-202A Manual Rotary Microtome) and mounted on glass slides. The stepwise protocol for the automatic tissue processor for histological examination of slide was as described by Winsor and Hopwood [40,41]. The tissues were cleared using xylene and mounted using DPX. The tissues were stained using haematoxylin and eosin staining procedures as also described by Winsor and Hopwood [42,43].

Microscopy

Normal tissues were identified and the pathological changes observed in the microscopic examination were described accordingly based on histological structure of the tissues. Photomicrographs were taken with the aid of computerized digital camera (Amscope MU900, United States).

Immunohistochemical Staining of Skin Tissues

Paraffin embedded tissues were micro-sections (4 μ), floated and mounted on charged glass slides. The slides were labeled, arranged in racks and placed in oven at 50-60°C for 20-30 minutes to melt excess paraffin. The slide-mounted tissue were further deparaffinized and prepared for heat induced antigen retrieval (in citrate buffer solution (10 mM citric acid, pH 6.0). The staining was performed using the Thermo Scientific Pierce Peroxidase IHC Detection Kit (36000, Thermo Scientific, USA) with slight modification of the procedure. Endogenous peroxidase activity was quenched by incubating tissue for 30 minutes in Peroxidase Suppressor, washed three times in Wash Buffer. Blocking buffer was added to the slides and incubated for 30 minutes. Excess buffer was blotted from the tissue sections, before addition of primary antibodies at a dilution of 1:100 and left overnight in a humidified chamber at 4°C. Afterward, slides were washed two times for 3 minutes with Wash Buffer. The tissue sections were treated with Biotinylated Secondary Antibody and incubated for 30 minutes. The slides were washed three times for 3 minutes each with Wash Buffer, treated and incubated with the Avidin/Streptavidin-HRP for another 30 minutes and again washed three times for 3 minutes each with Wash Buffer.

The tissues were incubated with Metal Enhanced DAB (3,3 diaminobenzidine) Substrate Working Solution for 5 minutes for desired staining to be achieved. The slides were rinsed with distilled water and drained. Adequate amount of Mayer's hematoxylin stain was dropped on the slide to cover the entire tissue surface and incubated for 1-2 minutes at room temperature. Drained off the hematoxylin and the slides were washed several times with distilled water. The slides were mounted with cover slips using DPX mountant. Photomicrographs were taken with digital camera (Amscope MU900) attached to the microscope. The images were quantified for staining intensity using the open source Fiji (ImageJ) software.

Results

Phytoconstituent Analyses of C. afer Stem Juice Extract

Both extracts of *C. afer* stem juice were found to contain alkaloids, terpenoids, tannins, steroids, saponins, glycosides, phenols and flavonoids (Table 2). The freeze-dried extract contained more concentrations of the biomolecules than the heat-dried extract, with terpenoids having the highest concentration in both extracts, while steroids was the lowest (Table 2).

Biomolecules	Freeze-dried Extract (mg/100g)	Heat-dried Extract (mg/100g)
Flavonoids	372.95	363.84
Tannins	134.42	122.16
Saponins	240.10	238.54

Alkaloids	259.86	248.11
Terpenoids	628.33	618.52
Phenols	482.55	478.51
Glycosides	407.62	402.13
Steroids	39.86	25.96

Table 2: Phytochemicals in *C. afer* stem juice extract.

Both extracts were found to contain copper, magnesium, calcium, zinc, iron, manganese, chloride, potassium and sodium (Table 3). The freeze-dried extract contained slightly higher concentrations of the minerals than the heat-dried extract.

Minerals	Freeze-dried Extract (mg/100 g)	Heat-dried Extract (mg/100 g)
Magnesium	6.60	6.54
Potassium	2.40	2.12
Calcium	8.45	8.20
Sodium	3.12	3.04
Iron	4.69	4.62
Copper	0.46	0.44
Manganese	0.79	0.71
Zinc	2.85	2.62

Table 3: Mineral content of *C. afer* stem juice extracts.

Microbial Profile

The classes of microbes identified and isolated from the beddings that the rats were bred on were *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Candida spp* (Table 4). While these microbes were present in the environment of the rats, no clinical signs of infection such as purulent discharge and foul odour were observed in the wounds through the healing process.

S/N	Microbe	Identification
1	<i>Pseudomonas aeruginosa</i>	Present
2	<i>Klebsiella pneumonia</i>	Present
3	<i>Escherichia coli</i>	Present
4	<i>Staphylococcus aureus</i>	Present
5	<i>Proteus mirabilis</i>	Present
6	<i>Candida spp</i>	Present

Table 4: Microbes identified in experimental environment.

Histology Micrograph showed that at 24 hours, there was no observable changes at wound site in groups A and B, while groups C and D showed (Fig. 1). At 72 hours, there was increased deposition of granulation tissues in group C wounds with no observable changes at the wound site (Fig. 2). At 120 hours, groups A and B showed deposition of granulation tissues with inflammation at the wound site, while the granulation tissue in group D was gradually resolving (Fig. 3). On complete healing, the deposition of dermal fibres were dense and random in groups C and D in group B, the deposition of dermal fibres were also random but loosely organized (Fig. 4).

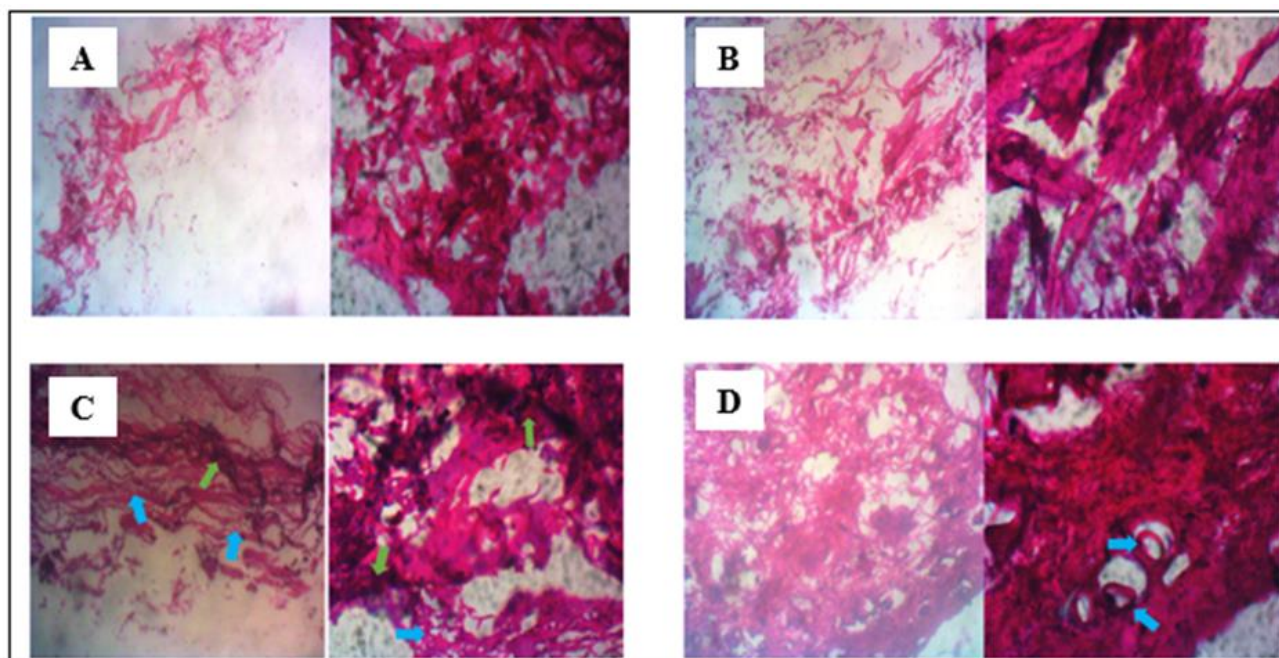


Figure 1: Wound site at 24 hours after treatment. A) Wound site from group A without treatment. There is no observable changes; B) Wound site from group B treated with honey gel. There is no observable lesion; C) Wound site from group C treated with *C. afer* stem juice heat-dried extract at 24 hours after treatment. There is moderate deposition of connective tissue (blue arrows) and keratinization (green arrows); D) Wound site from group D treated with *C. afer* stem juice freeze-dried extract. There is deposition of collagen and growth of adnexal structures (blue arrows). HE $\times 100$, 400.

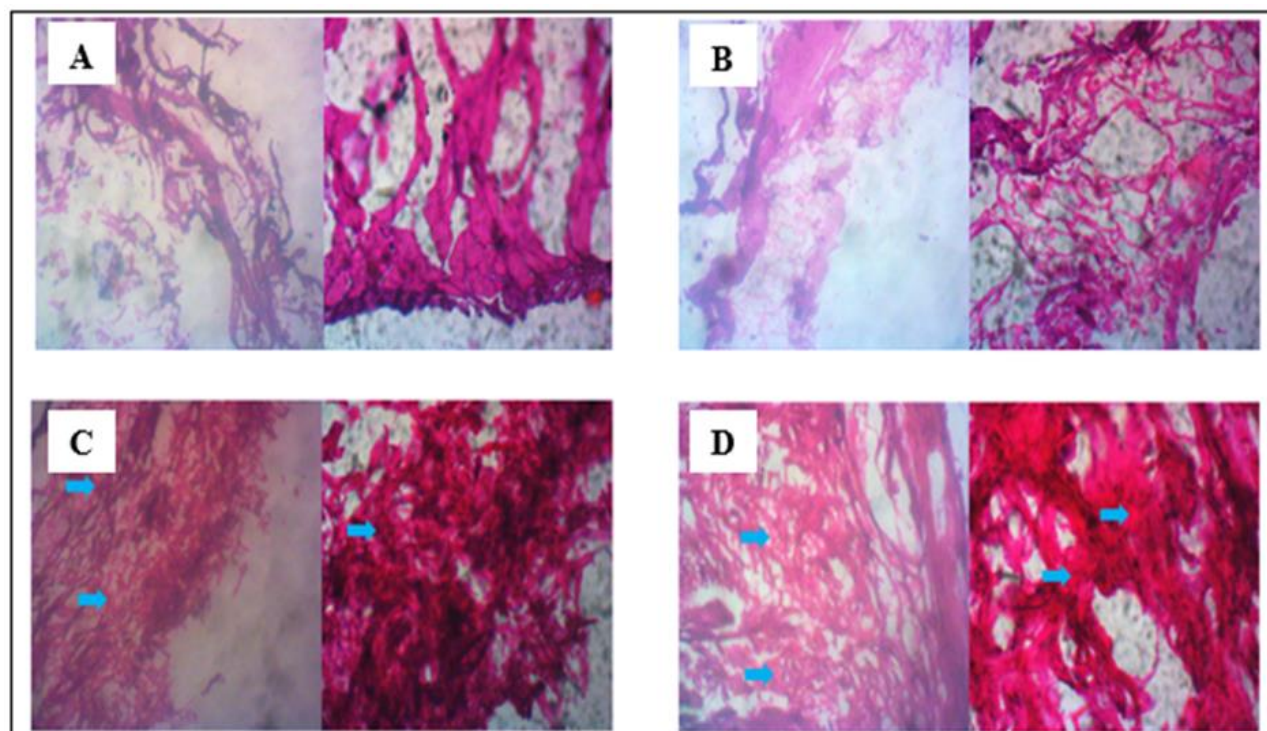


Figure 2: Wound site at 72 hours after treatment. A) Wound site from group A without treatment. There is no observable lesion. B) Wound site from group B treated with honey gel. There is no observable lesion. C) Wound site from group C treated with *C. afer* stem juice heat-dried extract. There is increased deposition of granulation tissue (blue arrows). D) Wound site from group D treated with *C. afer* stem juice freeze-dried extract. There is increased deposition of granulation tissue (blue arrows). HE $\times 100$, 400.

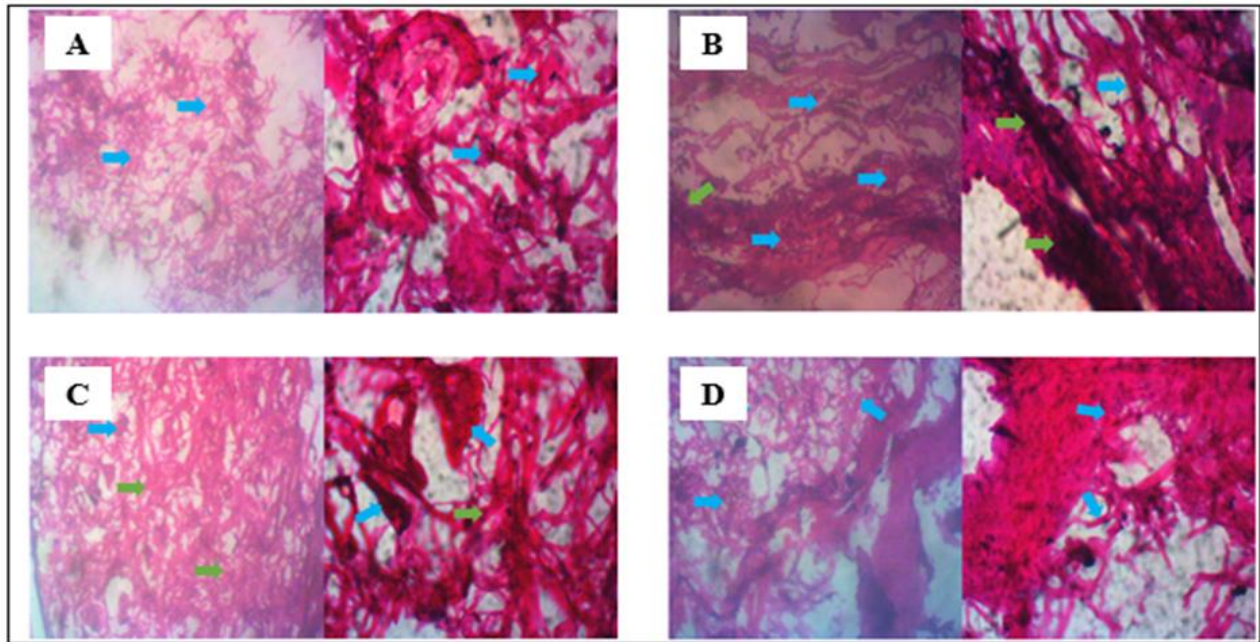


Figure 3: Wound site at 120 hours after treatment. A) Wound site from group A without treatment, showing deposition of granulation tissue (blue arrows). B) Wound site from group B treated with honey gel, showing acute inflammation (green arrows) and granulation tissue (blue arrows). C) Wound site from group C treated with *C. afer* stem juice heat-dried extract. There is deposition of granulation tissue (green arrows) and growth of adnexal structures (blue arrows). D) Wound site from group D treated with *C. afer* stem juice freeze-dried extract. There is gradual resolution of granulation tissue (blue arrows). HE x100, 400.

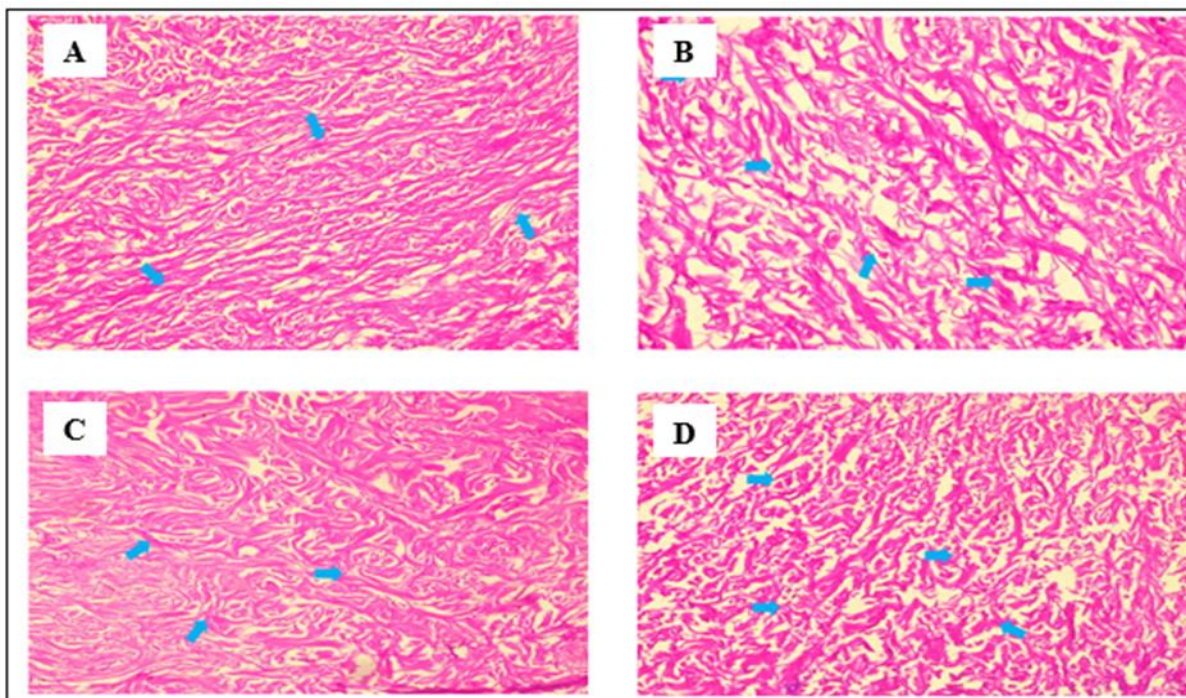


Figure 4: Healed dermal tissues. A) Healed dermal tissue from group A without treatment showing parallel organization of dermal fibres (blue arrows). B) Healed tissue from group B treated with honey gel showing loose and haphazard organization of dermal fibres (blue arrows). C) Healed tissue from group C treated with *C. afer* stem juice heat-dried extract showing tight and haphazard organization of dermal fibres (blue arrows). D) Healed tissue from group D treated with *C. afer* stem juice freeze-dried extract showing tight and haphazard organization of dermal fibres. HE x100.

Expression of TGF- β 1 at Wound Sites

At 24 hours, 72 hours and complete healing, group D showed the highest expression of TGF- β 1 (Fig. 5,6). However, at 120 hours the expression was lowest in group D (Fig. 7). While group A showed highest expression of TGF- β 1 at 120 hours (Fig. 7), it had the lowest expression at 72 hours (Fig. 6). Groups C and B showed the lowest expression of TGF- β 1 at 24 hours and on complete healing respectively (Fig. 5, 8).

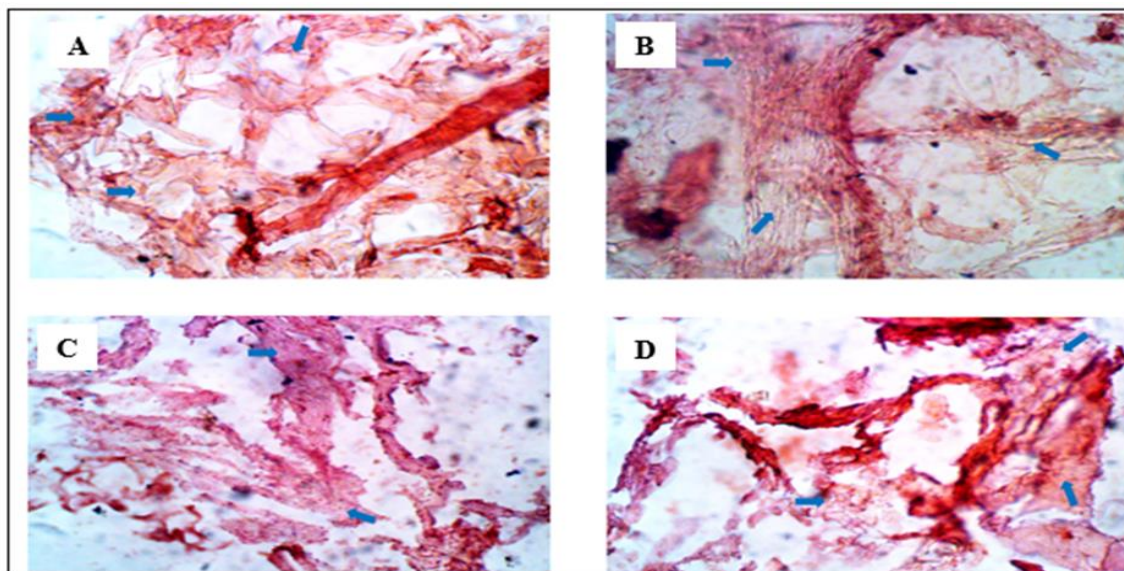


Figure 5: TGF- β 1 expression at 24 hours after treatment. A) Wound site from group A without treatment, showing high expression of TGF- β 1 (brown pigment, blue arrows). B) Wound site from group B treated with honey gel, showing high expression of TGF- β 1 (brown pigment, blue arrows). C) Wound site from group C treated with C. afer stem juice heat-dried extract, showing medium expression of TGF- β 1 (brown pigment, blue arrows). D) Wound site from group D treated with C. afer stem juice freeze-dried extract, showing high expression of TGF- β 1 (brown pigment, blue arrows) (x100).

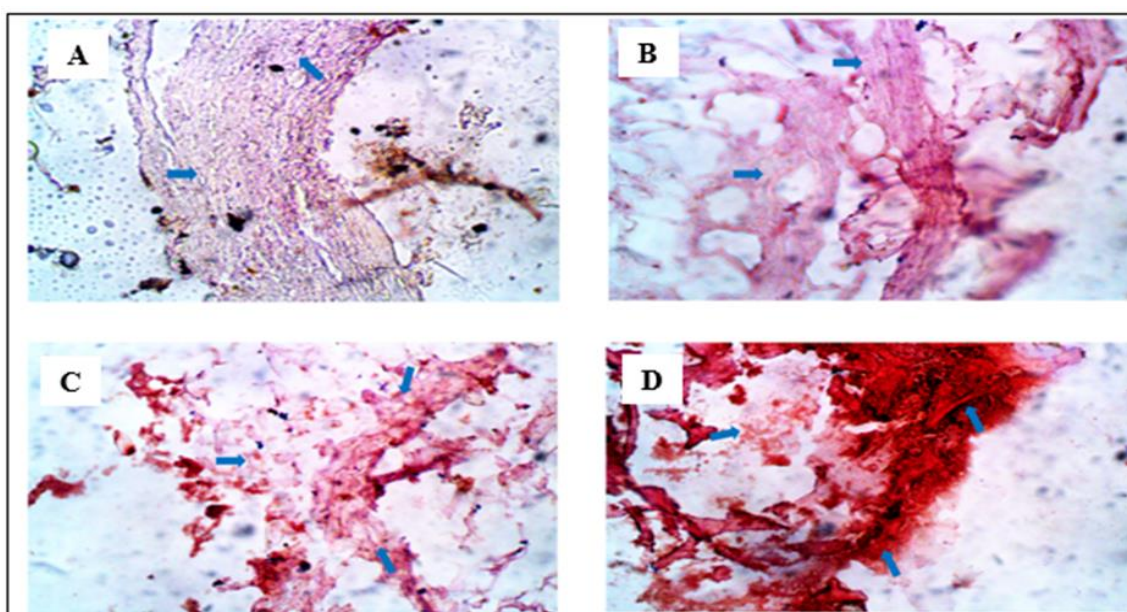


Figure 6: TGF- β 1 expression at 72 hours after treatment. A) Wound site from group A without treatment, showing low expression of TGF- β 1 (brown pigment, blue arrows). B) Wound site from group B treated with honey gel, showing medium expression of TGF- β 1 (brown pigment, blue arrows). C) Wound site from group C treated with C. afer stem juice heat-dried extract, showing medium expression of TGF- β 1 (brown pigment, blue arrows). D) Wound site from group D treated with C. afer stem juice freeze-dried extract, showing high expression of TGF- β 1 (brown pigment, blue arrows) (x100).

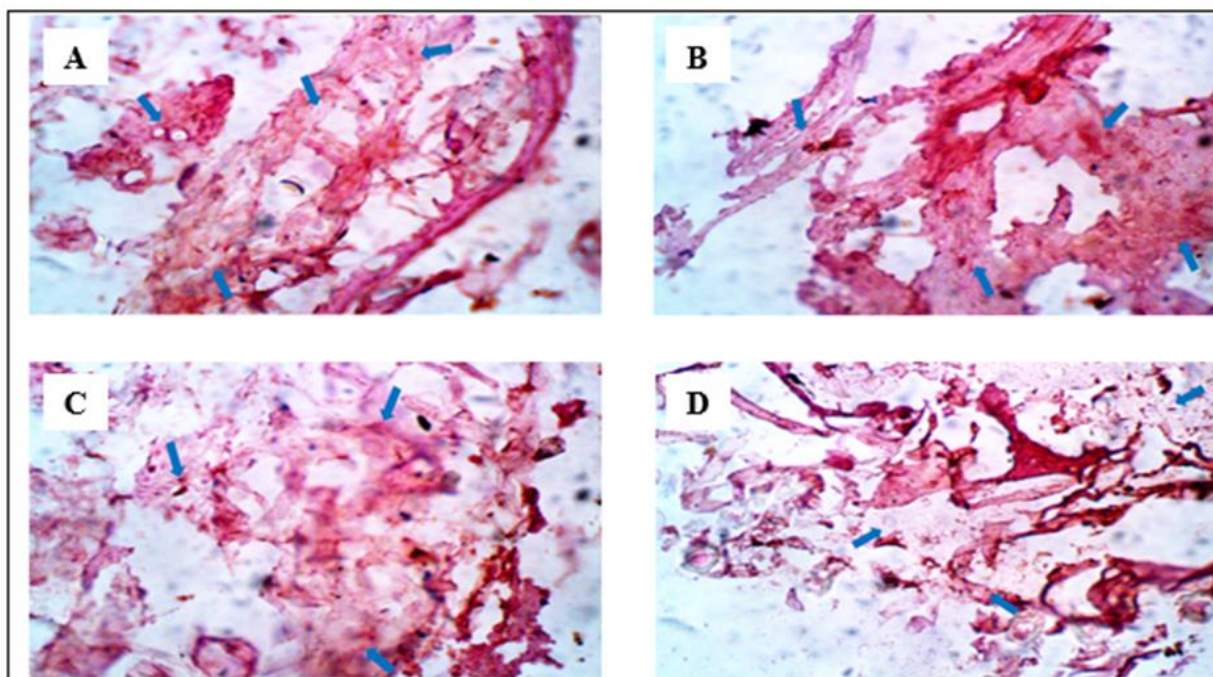


Figure 7: TGF- β 1 expression at 120 hours after treatment. A) Wound site from group A without treatment, showing medium expression of TGF- β 1 (brown pigment, blue arrows). B) Wound site from group B treated with honey gel, showing medium expression of TGF- β 1 (brown pigment, blue arrows). C) Wound site from group C treated with C. afer stem juice heat-dried extract, showing medium expression of TGF- β 1 (brown pigment, blue arrows). D) Wound site from group D treated with C. afer stem juice freeze-dried extract, showing medium expression of TGF- β 1 (brown pigment, blue arrows) (x100).

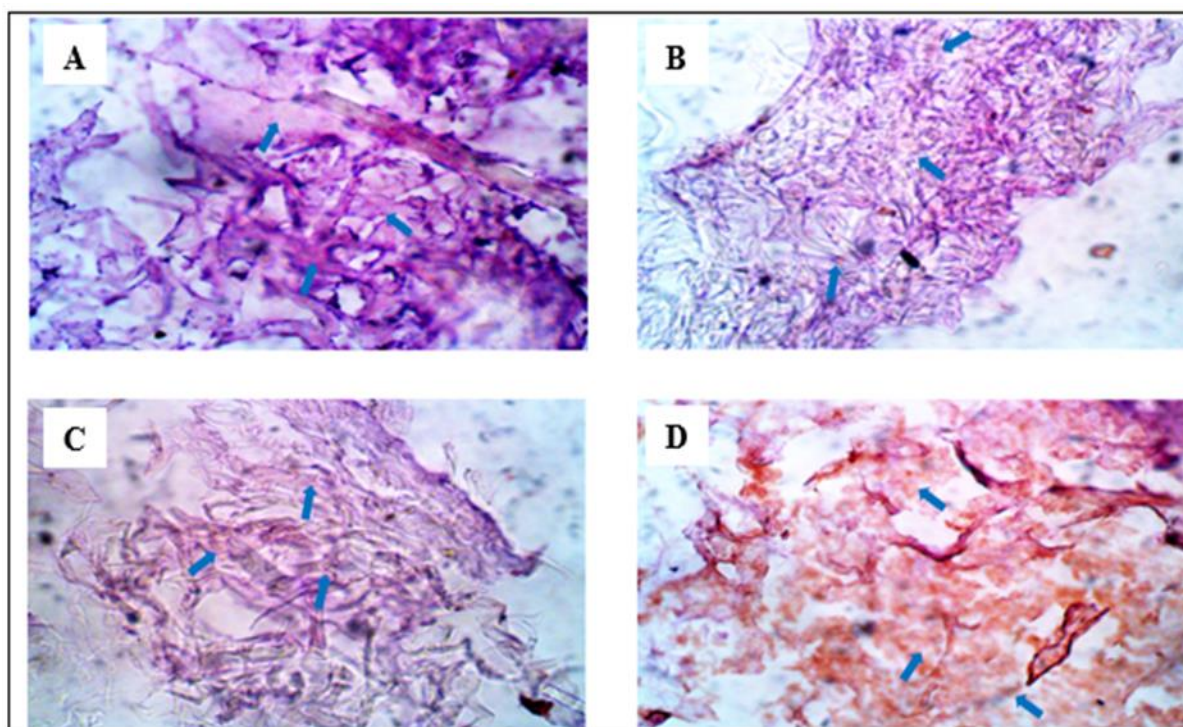


Figure 8: TGF- β 1 expression in healed skin tissues. A) Healed skin tissue from group A without treatment, showing low expression of TGF- β 1 (brown pigment, blue arrows). B) Healed tissue from group B treated with honey gel, showing low expression of TGF- β 1 (brown pigment, blue arrows). C) Healed tissue from group C treated with C. afer stem juice heat-dried extract, showing low expression of TGF- β 1 (brown pigment, blue arrows). D) Healed tissue from group D treated with C. afer stem juice freeze-dried extract, showing medium expression of TGF- β 1 (brown pigment, blue arrows) (x100).

Intensity Quantification of TGF- β 1 Expression

Group D wounds treated with *C. afer* freeze-dried extract showed the highest expression of TGF- β 1 with a gradual decrease through 72 hours and 120 hours, which slightly increased again after healing (Fig. 9). On the other hand, group C wounds treated with *C. afer* heat extract showed the lowest expression of TGF- β 1 at 24 hours, which significantly increased at 72 hours and gradually decreased at 120 hours such that the level of expression when the wounds healed was below the observation for 24 hours (Fig. 9). Group A (no treatment) and group B (treated with honey gel) wounds showed similar pattern of TGF- β 1 expression with little deviation from their values.



Figure 9: Intensity quantification showing pattern of expression of TGF- β 1 across treatment groups.

Discussion

From the result of the study, the *C. afer* stem juice treatment groups showed similar pattern of TGF- β 1 expression, although at varying levels. For instance, while the group treated with *C. afer* freeze-dried extract had the highest expression of TGF- β 1 at 24 hours, the group treated with heat-dried extract of *C. afer* stem juice showed the lowest expression of TGF- β 1. Similarly, while TGF- β 1 expression in the group treated with freeze-dried extract of *C. afer* stem juice was higher in the healed tissue compared to the expression at 120 hours, in the group treated with heat-dried extract of *C. afer* stem juice, TGF- β 1 expression was lower in the healed tissue compared to the expression at 120 hours. The variations in the level of expression of TGF- β 1 among the *C. afer* treatment groups may be attributed to the differences in the concentration of active biomolecules in the extracts as observed in the phytochemical analysis of the extracts, in which freeze-dried extract contained more concentrations of the prohealing biomolecules than the heat-dried extract. According to Tsala and colleagues 44, triterpenes and glycosides can modulate the expression and activity of TGF- β 1 as well as other growth factors. Phenols and lignans have also been reported to influence the activities and expression of growth factors including TGF- β 1 [45].

The low expression of TGF- β 1 at 24 hours in the group treated with heat-dried extract suggests that the extract suppresses the expression of TGF- β 1 in the early phase wound healing. Despite this inhibitory effect, the group treated with heat-dried extract of *C. afer* stem juice showed enhanced healing outcome, as observed in the deposition and organization of collagen fibres and tensile strength. This is in agreement with the finding that one-time application of neutralizing antibodies to TGF- β 1 in the initial phase of wound healing significantly enhanced the healing process with markedly reduced scarring [4,9]. By 72 hours, the group treated with heat-dried extract exhibited an elevated expression of TGF- β 1 compared to 24-hour levels, ensuring the availability of TGF- β 1 for cellular proliferation and migration, which are essential as the wound enters the proliferative phase. The subsequent increase in the expression of TGF- β 1 at 72 hours in wounds treated with heat-dried extract despite being applied

only on the first day, suggests that the extract influences the activities of other critical biomolecules involved in TGF- β 1 regulation during wound healing.

TGF- β 1 is also well known for its promotion of collagen synthesis and suppression of ECM degradation during the wound healing process [17,46]. Accordingly, in the group treated with freeze-dried extract of *C. afer* stem juice, the expression of TGF- β 1 and deposition of ECM fibres throughout the observation periods followed similar pattern. At 24 hours and 72 hours, there was increased expression of TGF- β 1 along with progressive deposition of connective tissues and granulation tissue formation at the wound site. Similarly, at 120 hours, low levels of TGF- β 1 expression were associated with resolution of granulation tissue. These findings correspond with that of Noverina, et al., as well as Pakyari and colleagues in which TGF- β 1 was reported to stimulate the expression of key ECM components such as fibronectin and collagen types I and III [15,16]. In addition, this pattern of TGF- β 1 expression allows efficient transition of the healing process through its various phases. Several studies have confirmed the role of TGF- β 1 in enhancing inflammation and ECM deposition. Thus, the high expression of TGF- β 1 in the group treated with *C. afer* stem juice extract, allowed for inflammation and formation of granulation tissue, which provides a framework for wound healing cells, such as stem cells and keratinocytes, to migrate into the wound site. In the same manner, low expression levels at 120 hours allowed for the resolution of inflammation and gradual transition of ECM from granulation tissue fibres to mature ECM fibres. Subsequent increase in TGF- β 1 expression in the dermis of healed skin, thus, promoted the deposition of thick bundles of dermal fibres with dense organization. This finding is in agreement with those of Sorg, et al., in which TGF- β 1 was found to promote the expression and organization of collagen fibres in the dermis [21]. This pattern of TGF- β 1 thus, promote proper wound healing with improved healing outcome as observed in the group treated with the freeze-dried extract of *C. afer* stem juice with random organization of dermal fibres.

The group treated with heat-dried extract of *C. afer* stem juice showed similar random organization of dermal fibres in the healed skin to the group treated with freeze-dried extract of *C. afer* stem juice. This is in agreement with the findings of Udoh and colleagues [33], in which treatment of wounds with the aqueous extract of *C. afer* stem juice resulted in random organization of dermal fibres in the healed skin. Although the expression of TGF- β 1 in the group treated with heat-dried extract of *C. afer* stem juice followed a different pattern with lower expression of TGF- β 1, it is possible that the heat-processed extract could promote dermal fibre deposition and organization through other molecular pathways not involving TGF- β 1. For instance, copper, zinc and calcium, which had similar levels of concentration in both extracts, have been found to play important roles in modulating the activities of inflammatory cells, as well as the deposition and organization of collagen fibres in the ECM [47-51].

Conclusion

This study found that while both freeze-dried and heat-dried extracts of *C. afer* stem juice had positive healing outcomes, they affected TGF- β 1 expression differently during the healing process. These differences indicate that *C. afer* may influence various molecular pathways in wound healing, suggesting a need for further research into its broader effects in tissue repair and regeneration.

Conflict of Interests

The authors declare no conflict of interest in this publication.

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