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Anti-hypertrophic Scar Properties of *Musa cavendishii* Lamb. Peel Extracts in New-Zealand Rabbit Ear Wound: The Involvement of Hydroxyproline and Histamine

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ABSTRACT

Introduction: Hypertrophic scars, a frequent outcome of abnormal wound healing, can lead to significant disfigurement and functional challenges, with no universally accepted treatment currently available. Research indicates that *Musa cavendishii* peel extract may play a role in controlling hypertrophic scar formation, though its mechanisms remain unclear. This study investigates the effects of different extracts from *M. cavendishii* peel on hydroxyproline and histamine deposition in a rabbit model of hypertrophic scars.

Methods: The study employed a randomized design, including negative and positive controls (distilled water, 1 mL/kg and triamcinolone acetonide, 40 mg/kg respectively) and three dose levels (375, 750, and 1500 mg/kg) of hexane, ethyl acetate, methanol, and aqueous extracts, with three rabbits per group. Dermal excisions were created on the ventral surface of the rabbits' ears using an X-ray film template to allow consistent application of the treatments. Preventive and curative effects were assessed through hydroxyproline and histamine measurements using enzyme-linked immunosorbent assay (ELISA).

Results: In the preventive study, the aqueous extract at 1500 mg/kg significantly ($p<0.05$) reduced hydroxyproline levels compared to the control groups, while the other extracts showed no significant impact. In the curative study, all extracts, except the hexane extract at lower doses (375 and 750 mg/kg), significantly ($p<0.001$) reduced hydroxyproline levels. Moreover, histamine concentrations were significantly ($p<0.05$) reduced by all extracts at doses of 750 and 1500 mg/kg in both preventive and curative settings, with the highest dose showing superior effects compared to the standard treatment.

Conclusion: These results suggest that *M. cavendishii* peel extracts could offer potential benefits in managing hypertrophic scars, though further studies are required to confirm their efficacy and elucidate the underlying mechanisms.

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Introduction

The healing process in wound is a complex phenomenon involving four main sequences namely hemostasis, inflammation, proliferation, and tissue remodeling (Schultz et al. 2011). Collagen and histamine remain the two main important components in wound healing as collagen is mainly involved in the final two phases when the scar tissue begins to form over the wound. The process also brings about vasodilatory changes enhancing the volume of blood proceeding into the wound. Invariably, histamine results in the regulation of collagen content in myofibroblast via H_2, H_3 and H_4 histamine receptors (Piera et al., 2021).

Collagen, a structural protein in the extracellular matrix of the body, provide supports to many tissues and gives structural building block which can be found in bones, cartilage, tendons, ligaments, skin, as well as many other tissues and organs. Collagen in scar formation consist primarily of type 1 and 3, and is one of the reasons for scars to appear differently from each other in which small amount of collagen will form a sunken atrophic scar and higher amount will form hypertrophic scar and sometime keloids (Gozali and Zhou, 2015).

Histamine showed potency in the regulation of healing *in-vivo*, with mast cell believed to maintain angiogenesis in order to supply vital nutrients in healing of open skin wounds (Wolak et al., 2021). Mast cells are resident inflammatory membrane bound unit having increased number in exposed organs to the external environment such as the skin but also play other functions in a number of physiological and pathologic processes by maintaining normal homeostasis, defense against parasitic, viral, and bacterial infections etc (Dong et al., 2020 and Galli et al., 2020).

Researches especially those in animal models and human wound have revealed that mast cells pass through degranulation as a result of skin injury thereby increasing in numbers during repair of wound and it has also been suggested that their role in wound repair is enormous (Lateef et al., 2019; Ud-Din et al., 2019). They also enhance the production of hypertrophic scar tissue through the release of mediators that increase inflammatory reaction, producing pro-fibrotic growth factors that stimulate fibroblasts and directly interacting with fibroblast in the gap junctions (Wilgus and Wulff, 2014). Hence increase in collagen and mast cell production during wound healing can easily lead to the formation of hypertrophic scar.

Hypertrophic scar, an abnormal pattern of wound healing process (Rabello et al., 2014) is a thick raised scar arising from an insult to the deep dermis which may be from burns, lacerations, abrasions, surgery and vaccination etc (Manna et al., 2022). The components of extracellular matrix (ECM) re-organization are all involved in the formation of hypertrophic scar which are combination of proteins (collagens and elastin) and other smaller quantities of structural proteins like histamine (Diller and Tabor, 2022). This abnormality remain the most irritating outcome of wound healings and are highly common to human population characterized by disorganized platelet degranulation and excessive deposition of extracellular matrix components (Seo et al., 2013; Tracy et al., 2016). The array of potent cytokines from platelet degranulation are also involved in the formation of hypertrophic scar of which its' components such as insulin-like growth factor-1, platelet derived growth factor, epidermal growth factor and transforming growth factor β serve as a chemotactic agents for the recruitment of macrophages, neutrophils, endothelial cells, epithelial cells, mast cells and fibroblasts in wound healing processes (Daian et al., 2003; Tredget et al., 2006; Moreno-Sanchenz et al., 2012).

One hundred (between 32 to 72 %) million patients in the developed world alone are said to be affected with hypertrophic scar (Lawrence et al., 2012; Marshall et al., 2018). It was specifically reported that the prevalence rate among the young adult darker patients, female sex with burns is greater than 20 % of total body surface area and those with burns on the neck and upper limbs are with highest risk occurrence of developing hypertrophic scar (Thompson et al., 2013).

Prevention is key to every wound healing process and to date, no single therapeutic modality is best for hypertrophic scar as one of the aftermath of wound healing, although orthodox therapy and a large number of scientific research using medicinal plant as a natural source in the management of hypertrophic scar have been reported (Ye et al., 2015). The existing treatment for hypertrophic scar includes but not limited to surgery (both excision and cryosurgery), radiotherapy, verapamil, silicone gel, corticosteroid (including hydrocortisone acetate, methylprednisolone, and dexamethasone), statins, 5-Fluorouracil (5-FU), bleomycin, mitomycin C, paclitaxel etc and non-prescription drugs which can be assessed easily from outpatient outlets (e.g. chemists) like onion extract; combination of hydrocortisone, silicon, and vitamin E (Kumar and Ghosh, 2017, Ouyang et al., 2018 and Kim, 2021). Hypertrophic scar have been reduced over the years with the above measured therapies post-surgery or either after superficial radiation therapy and other promising potential therapies that can be used in its management which include bevacizumab, a vascular endothelial growth factor inhibitors, phototherapy, ingenol mebutate gel, transforming growth factor–beta inhibitors, tumor necrosis factor–alpha inhibitors (etanercept), recombinant human epidermal growth factor, recombinant human interleukin (rhIL)–10, small interfering RNA and oligonucleotide anti–connective-tissue growth factor etc.

Musa cavendishii Lamb. (Family: Musaceae) is an eatable fruit obtained from several herbaceous flowering plants. The peel serves as a traditional medicine all over the world and has been confirmed by orthodox medicine to have antibacterial activity against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and may other microbes (Kapadia et al., 2015). These properties exhibited by the peel are said to be attributed to their secondary metabolites; where tannins and terpenoids have been proven to be active detoxifying agents, inhibitors of bacterial growth and antimicrobial agents in wound healing process (Bondenstein and Du, 2012, Sheidu et al., 2020). Despite its popular use in ethno-medicine for treatment of wound and hypertrophic scar, no published data is available to back it up. This study is aimed at providing scientific

justification for the use of *M. cavendishii* peel in the management of hypertrophic scar in the local communities of Nigerian states.

Materials and Methods

Plant Material

Musa cavendishii peel with intact fruit was collected by way of cutting it from the whole sample plant in the month of September in Obehira, Okene Local Government of Kogi State, Nigeria (7° 33' 4.39" N, 6° 14' 9.20" E). The whole plant was taken to the Herbarium Section of Botany Department, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria which was then identified, authenticated by Mallam Namadi Sunusi and a voucher specimen number (28003) was assigned which was subsequently deposited in the department for future reference. The peels were removed from the fruit, air dried to a constant weight and reduced into coarse powder using pestle and mortar. The method described by Abubakar et al. (2019) was employed for the successive extraction process by maceration where dried plant material, 600 g was extracted by first mixing it with 2.5 L of hexane which was divided into two (2) bottles for convenience. The procedure was allowed for three days thereafter the hexane layer was filtered using a muslin cloth for about 15 minutes for complete drainage. The marc collected after the drainage was spread on a card board paper to allow drying and the hexane filtrate was poured into an evaporating dish which was allowed to dry for another 3 days. The resulting dried marc was put into a jar and the same procedure was repeated to obtain other extracts using ethyl acetate, methanol and water. The extracts were labeled and stored in a desiccator until required for further studies.

Laboratory Animals

Healthy forty-two (42) New-Zealand male and female rabbits (1.2 to 2.2 kg) were obtained from National Animal Production Research Institution (NAPRI), Shika-Zaria, Kaduna State. The rabbits were housed in the Rabbit Section, Animal House of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were maintained under ambient environmental conditions and fed with standard diet of vegetable and water *ad libitum*. The experimental protocols were approved by the Ahmadu Bello University Committee on Animal Use and Care with approval number of ABUCAUC/2018/075. The experiments were also done in accordance with the criteria outlined in the Guide for the Care and use of Laboratory Animals by the National Institutes of Health (Publication No 80-23, revised, 1996).

Drugs, Chemicals, Consumables and Equipment

Atropine sulphate (American Remedies Healthcare, India), adhesive silicone gel (Cica-Care; Smith & Nephew, Largo, FL), germ-x hand sanitizer (Deedat Medical Investment Ltd, Nigeria), Ketamine hydrochloride USP (Jawa International limited, Nigeria), xylazine (Akorn, Inc.), methylated spirit B.P (Ugolab, Nigeria), Purit antiseptic (Saro Life Care Ltd, Nigeria). Enzyme-Linked Immunosorbent Assay (ELISA) kits for histamine (EU0382, Finetest®, China) and hydroxyproline (CK-bio-14800, Shanghai Coon Koon Biotech Co., Ltd, China), microplate pipette, microplate reader (Rayto, RT 2100C, China), Rabbit cages, weighing balance (Xiamen Jadever Scale Co. Ltd, China), pestle and mortar, stop watch, syringes, thread, ruler, surgical gloves, conical flask (Lab Glassware TVET Lab Equipment, Japan), Whatman filter paper (Merck, Darmstadt, Germany), spatula, water bath (Malvern Panalytical Ltd, Beijing), X-ray film (Shanghai Allecard Image Material Co., Ltd, Shanghai, China).

Experimental Design

A total of forty-two (42) healthy rabbits were used for the study. The rabbits were divided into eleven groups of three rabbits each (n=3) and treated dermally as described below:

Group I: Received distilled water 1 mL/kg (Negative control)
Group II: Received triamcinolone acetonide 40 mg/kg (Positive control)
Group III: Received hexane extract of *M. cavendishii* peel 375 mg/kg
Group IV: Received hexane extract of *M. cavendishii* peel 750 mg/kg
Group V: Received hexane extract of *M. cavendishii* peel 1500 mg/kg
Group VI: Received ethylacetate extract of *M. cavendishii* peel 375 mg/kg
Group VII: Received ethylacetate extract of *M. cavendishii* peel 750 mg/kg
Group VIII: Received ethylacetate extract of *M. cavendishii* peel 1500 mg/kg
Group IX: Received methanol extract of *M. cavendishii* peel 375 mg/kg
Group X: Received methanol extract of *M. cavendishii* peel 750 mg/kg
Group XI: Received methanol extract of *M. cavendishii* peel 1500 mg/kg
Group XII: Received aqueous extract of *M. cavendishii* peel 375 mg/kg
Group XIII: Received aqueous extract of *M. cavendishii* peel 750 mg/kg
Group XIV: Received aqueous extract of *M. cavendishii* peel 1500 mg/kg

These doses of the extract were selected based on the acute toxicity profile and previous doses reported (Sheidu et al., 2023).

Skin preparation

The procedure described by Morris et al. (1997) and Nabai and Ghahary (2017) was adopted. Briefly, 6 mm diameter was created on X-ray used film for scar creation in the ear of rabbits. The dermal excision was created using surgical scissor and sterile razor blade on the region where the marked X-ray film was positioned on the frontal part of the rabbit ear and marked for dermal excision. Meanwhile before the excision, each rabbit was safeguarded carefully and anesthetized with ketamine (50 mg/kg) and xylazine (3 mg/kg). Full thickness excisional wound was made down to the cartilaginous surface over the marked area on the rabbit ear. Gentle application by way of digital pressure on the exact region where the wound was created was done so as to achieve hemostasis before the treatment procedures (Figure 1).

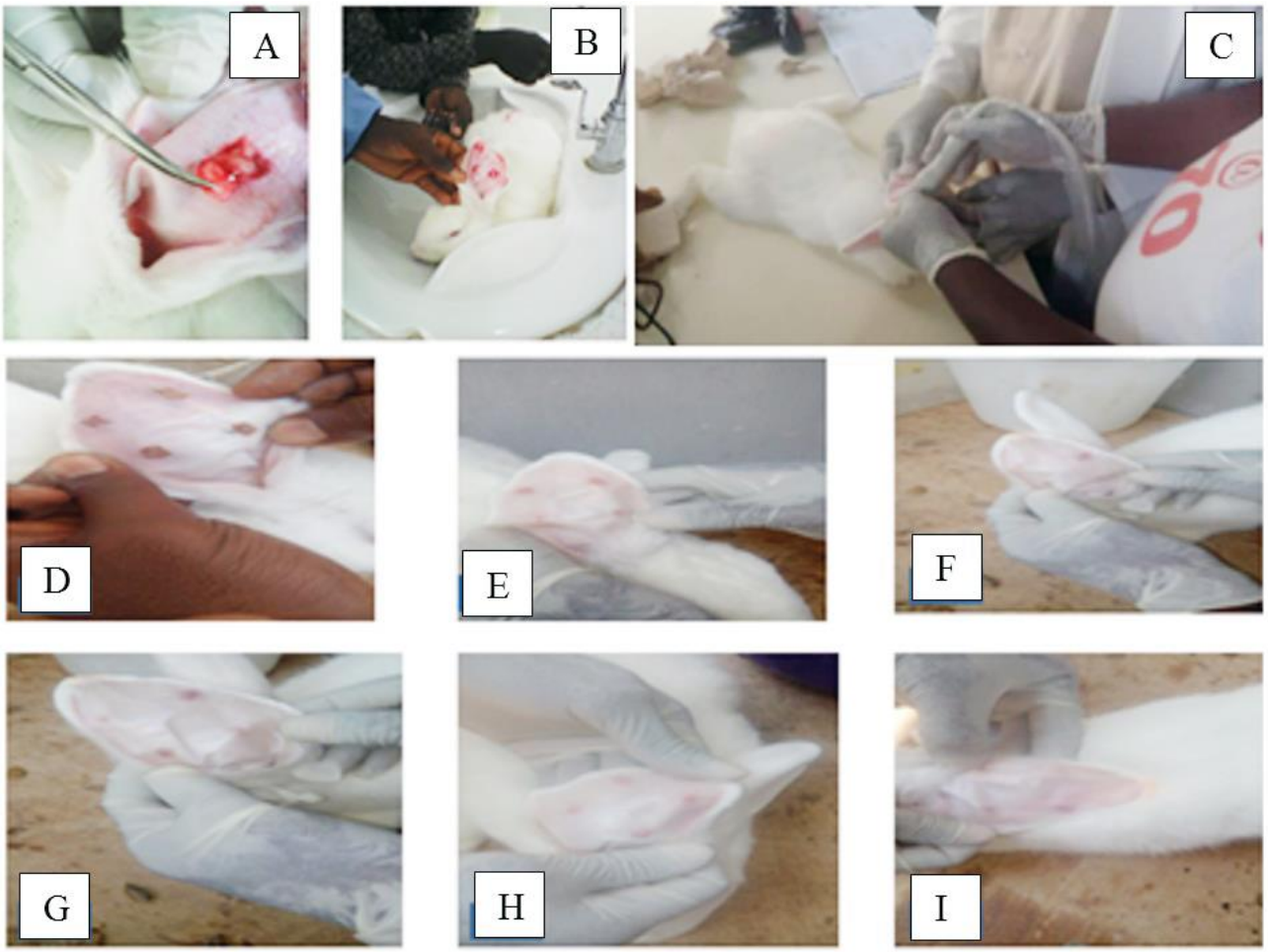

Figure 1: Graphical representation of scar creation
A: Immediately after excision of the rabbit ear; B: day 1 after excision; C: day 8 after excision with ultrasound monitoring; D, E and F: Prevention period; G, H and I: Curation period.

Anti-Hypertrophic Scar Evaluation

Innate hypertrophic scar were created using excision rabbit model on the ventral area of the ears, where the left ear was used for preventive study and the right ear for curative study (Seo et al., 2013). In the preventive study (which lasted for 35 days), digital pressure was applied to the excised area immediately after the wound excision to achieve hemostasis with subsequent application of the extracts and standard drug dermally thereafter followed in every three days interval. At the end of the study (on the 36th day), sample tissues around the scar area were excised and kept in non-heparinized bottle for biochemical evaluation.

In the curative study (which lasted for 63 days) the excised area was left for 1 to 2 weeks until the scar was developed and confirmed through blind confirmation and ultrasound observation. Thereafter, the extracts and standard drug were applied. Sample of tissues were collected at the end of the study from the scar areas which was then kept in the non-heparinized bottle for evaluation of biochemical markers.

Tissue Processing

Ice cold isotonic saline solution was used to rinse the harvested tissues and then homogenized with 0.1M phosphate buffer (pH 7.4) to produce 10 % w/v homogenates. The homogenates were centrifuged at 10000 rpm for 15 min and the supernatants were used for hydroxyproline and histamine estimations.

Biochemical Assay of Hydroxylproline and Histamine

Hydroxylproline and histamine estimation was carried out using ELISA technique principles according to the manufacturers guide. The ELISA principle is that of an antigen-antibody reaction, representing the chemical interaction between antibodies produced by the B cells of leukocytes and antigens. In this study, sandwich ELISA technique was utilized. The assay uses a stationary phase which consists of wells pre-coated with a monoclonal antibody specific to hydroxylproline and histamine in rabbits and these biomarkers were referred to as the antigen. The mobile phase consisted of the antibody linked to an enzyme, Sreptavidin horse radish peroxidase (HRP-Conjugate), and referred to as enzyme conjugate. The sample is added to the coated well followed by the enzyme conjugate to form a sandwich mixture of antibody-antigen-enzyme linked complex. By incubation and washing, unbound enzymes are removed. This is followed by addition of chromogen A (Hydrogen peroxide, which generates reactive oxygen species (ROS) and chromogen B (tetramethylbenzidine) which turns the solution blue. After incubation, addition of a stop solution (2 M H_2SO_4) changes the blue solution into

yellow from oxidation by the ROS previously generated. In the study, standard markers of 50 µL were loaded into the designated standard wells. A total of 10 µL of samples were added to the wells with diluent of 40 µL to all sample wells (except the standard and blank), followed by the addition of 100 µL of HRP conjugate to all the wells except the blank. All samples were mixed, sealed and incubated at 37 °C for 60 min following washing to remove the unbound enzyme and each of the well was then washed five times with 350 µL of wash solution and dried using filter paper. For colour development, chromogen-A (50 µL) was added to all wells, followed by 50 µL of chromogen B mixing with the samples 40 µL which was then incubated at 37°C for 15 min. Each well was added a stop solution (50 µL) after 15 mins to stop the reaction with subsequent measuring of the absorbance using an ELISA microplate reader placed at 450 nm. A regression curve for hydroxyproline and histamine were plotted and their concentration in each sample was extrapolated from the curve based on the absorbance.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) software Version 23 was used to carry out the data analysis and the differences between means were analyzed using One-Way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test. Values of $p<0.05$ were considered statistically significant.

Results

Effect of 35 and 63 Days Dermal Administration of M. cavendishii on Hydroxylproline Level

In preventive study, the dermal administration of aqueous extract of M. cavendishii peel at 1500 mg/kg significantly ($p<0.05$) decreased hydroxyproline concentration when compared to distilled water and triamcinolone groups. However, no significant ($p>0.05$) changes were observed in all other treatment groups (hexane, ethyl acetate and methanol) when compared with either distilled water control or standard group (Table 1). Also in curative study, the dermal administration of M. cavendishii peel extracts (hexane, ethyl acetate, methanol and aqueous) significantly ($p<0.001$) decreased the hydroxyproline concentration when compared with distilled water group. However, this observation was not seen in the hexane extract at doses 375 and 750 mg/kg when compared with distilled water that served as the negative control group (Table 2).

Effect of 35 and 63 Days Dermal Administration of M. cavendishii Peel Extracts on Histamine Concentration in Rabbit's Ear Scar

In preventive study lasting for 35 days, the administration of M. cavendishii peel extracts dermally (n-hexane, ethyl acetate, methanol and aqueous) at 750 and 1500 mg/kg significantly ($p<0.05$) decreased the histamine concentration when compared with distilled water control. A similar decrease in the histamine concentrations were also observed when compared to the standard group (Table 3). In curative study, the administration of M. cavendishii peel extracts dermally (n-hexane, ethyl acetate, methanol and aqueous) at all the tested doses of 375, 750 and 1500 mg/kg produced a significant ($p<0.05$) decrease in histamine concentration when compared with distilled water control. In addition, a significant ($p<0.05$) decreased in histamine concentrations were observed with the highest doses of all the extracts (1500 mg/kg) when compared with the standard (triamcinolone) group (Table 4).

Table 1: Effect of 35 days dermal administration of *Musa cavendishii* peel extracts on hydroxylproline concentration in rabbit's ear scar

Treatments (mg/kg)	Hydroxyproline level (µg/mg tissue)
D/W (1 ml/kg)	0.97±0.03
TCA 40	0.89±0.01
HEMC 375	0.88±0.05
HEMC 750	0.80±0.06
HEMC 1500	0.74±0.02
EEMC 375	0.82±0.13
EEMC 750	0.79±0.12
EEMC 1500	0.75±0.09
MEMC 375	0.88±0.03
MEMC 750	0.77±0.09
MEMC 1500	0.72±0.04
AEMC 375	0.85±0.09
AEMC 750	0.73±0.05
AEMC 1500	0.71±0.05*a

Values are expressed as Mean ± SEM, a: $p<0.05$ as compared with D/W group; *: $p<0.05$ as compared with TCA group - One-way ANOVA followed by Bonferroni's post hoc test. n = 3; D/W: Distilled water; TCA: Triamcinolone acetonide; HEMC: Hexane extract of *M. cavendishii* peel; EEMC: Ethylacetate extract of *M. cavendishi*i peel; MEMC: Methanol extract of *M. cavendishii* peel; AEMC: Aqueous extract of *M. cavendishii* peel.

Table 2: Effect of 63 days dermal administration of *Musa cavendishii* peel extracts on ydroxylproline concentration in rabbit's ear scar

Treatments (mg/kg)	Hydroxyproline level (µg/mg tissue)
D/W (1 ml/kg)	1.08±0.00
TCA 40	0.88±0.02
HEMC 375	1.08±0.00
HEMC 750	1.01±0.00
HEMC 1500	0.97±0.01*c
EEMC 375	0.81±0.00*c
EEMC 750	0.80±0.00*c
EEMC 1500	0.76±0.00*c
MEMC 375	0.74±0.00*c
MEMC 750	0.69±0.01*c
MEMC 1500	0.65±0.01*c
AEMC 375	0.77±0.00*c
AEMC 750	0.72±0.01*c
AEMC 1500	0.68±0.01*c

Values are expressed as Mean ± SEM, a: $p<0.05$ as compared with D/W group; *: $p<0.05$ as compared with TCA group - One-way ANOVA followed by Bonferroni's post hoc test. n = 3; D/W: Distilled water; TCA: Triamcinolone acetonide; HEMC: Hexane extract of *M. cavendishii* peel; EEMC: Ethylacetate extract of *M. cavendishi*i peel; MEMC: Methanol extract of *M. cavendishii* peel; AEMC: Aqueous extract of *M. cavendishii* peel.

Table 3: Effect of 35 days dermal administration of *Musa cavendishii* peel extracts on histamine concentration in rabbit's ear scar

Treatments (mg/kg)	Histamine concentration (µg/mg tissue)
D/W (1 ml/kg)	118.00±0.20
TCA 40	114.80±0.00
HEMC 375	113.17±0.61
HEMC 750	111.03±0.58[b]
HEMC 1500	108.13±1.13[*c]
EEMC 375	113.13±0.61
EEMC 750	111.13±0.52[b]
EEMC 1500	107.43±0.62[*c]
MEMC 375	110.57±1.37[b]
MEMC 750	108.50±1.42[c]
MEMC 1500	104.03±1.98[*c]
AEMC 375	113.53±0.38
AEMC 750	109.07±0.68[a]
AEMC 1500	104.17±1.18[*c]

Values are expressed as Mean ± SEM, a: $p<0.05$ as compared with D/W group; *: $p<0.05$ as compared with TCA group - One-way ANOVA followed by Bonferroni's post hoc test. n = 3; D/W: Distilled water; TCA: Triamcinolone acetonide; HEMC: Hexane extract of *M. cavendishii* peel; EEMC: Ethylacetate extract of *M. cavendishi*i peel; MEMC: Methanol extract of *M. cavendishii* peel; AEMC: Aqueous extract of *M. cavendishii* peel.

Table 4: Effect of 63 days dermal administration of *Musa cavendishii* peel extracts on histamine concentration in rabbit's ear scar

Treatments (mg/kg)	Histamine concentration (µg/mg tissue)
D/W (1 ml/kg)	125.80±0.00
TCA 40	116.20±0.00
HEMC 375	116.17±0.09a
HEMC 750	112.47±0.07c
HEMC 1500	106.50±0.10*c
EEMC 375	115.30±0.06b
EEMC 750	112.60±0.06c
EEMC 1500	106.17±0.03*c
MEMC 375	120.90±0.38a
MEMC 750	111.73±0.24c
MEMC 1500	104.43±0.09*c
AEMC 375	116.17±0.15c
AEMC 750	110.77±0.52b
AEMC 1500	104.17±0.03*a

Values are expressed as Mean ± SEM, a: $p<0.05$ as compared with D/W group; *: $p<0.05$ as compared with TCA group - One-way ANOVA followed by Bonferroni's post hoc test. n = 3; D/W: Distilled water; TCA: Triamcinolone acetonide; HEMC: Hexane extract of *M. cavendishii* peel; EEMC: Ethylacetate extract of *M. cavendishi*i peel; MEMC: Methanol extract of *M. cavendishii* peel; AEMC: Aqueous extract of *M. cavendishii* peel.

Discussion

Traditionally, plants and their products have been used in the management of many illnesses resulting from the scavenging and antimicrobial properties attributed to their various secondary metabolites (Sonam et al., 2017; Ugboko et al., 2020). Traditionally, study had showed that *Musa cavendishii* peel is used in the management of wound healing, ulcers and scars (Singh et al., 2017). According to Sheidu et al., (2021), important plant metabolites such as alkaloids, flavonoids, triterpenes and steroids were present in the peel extracts of *M. cavendishii*, and their biological activities are attributed to some of these phyto-constituents (Singh et al., 2017; Sheidu et al., 2021). The present study evaluated the anti-hypertrophic scar activities of the peel extracts of *M. cavendishii* using rabbit model.

Hydroxylproline comprises approximately 10 % of collagen and it represents the best surrogate for assessing the level of collagen in scar (Cheng et al., 2017). Hypertrophic scar is a type III collagen piled up in the ECM positioned parallel to the epidermal surface containing myofibroblasts, large extracellular collagen filaments and enormous mucopolysaccharides (Slemp and Kirschner, 2006). The present study revealed that there was a dose dependent in the potency of the extracts as used in the treatment of hypertrophic scar and showed significant decreased in collagen level in all the animals treated as seen in both the preventive and curative studies. The decrease in hydroxyproline levels may be associated with inhibition of the continuous synthesis of collagen in the extracellular matrix which in concordance with the works of Phan et al., (2003) and Ho et al., (2006), may be associated with the phenolic compounds present in the *M. cavendishii* peel. Furthermore, alkaloids have been shown to have antiplatelet activity thereby interfering with the different mediators of clot formation that may lead to scar (Ain et al., 2016). Thus, the anti-hypertrophic scar activity of *M. cavendishii* peel could also be attributed to its alkaloidal content.

Mast cells are an important immune cell type in the skin which play an active role in tissue homeostasis, remodeling and repair during wound healing (Komi et al., 2020). They store and release different mediators like histamine, proteases, lipid mediators and cytokines that can enhance acute inflammation, stimulate re-epithelialization as well as angiogenesis, and promote skin scarring during wound healing. Studies have showed that increase in the release of mast cells especially histamine is related to abnormal pathological cutaneous scarring (hypertrophic scar) which occur as a result of excessive itching and increased stimulation of inflammatory cytokines (Wilgus et al., 2020). Histamine induced-itching is triggered by the excitation of a subset of unmyelinated C-fibers (Tani et al,. 1990; Han and Dong, 2014) which results into acceleration of cutaneous wound healing through an enhancement of angiogenesis and, possibly, by facilitating the exudation of molecules required for optimal wound healing from the blood circulation (Thabet et al., 2018; Yang et al., 2019). In this study, the administration of M. cavendishii peel extracts significantly decreased the level of hydroxylproline and histamine accumulation in the hypertrophic scar created in the rabbit ear which was prominent in both the preventive and curative studies. This activity may be attributed to the different phyto-constituents (flavonoids, alkaloids, phenols, steroids and triterpenes) of *M. cavendishii* peel (Sheidu et al., 2021), making it to serve as an antihistaminic agent.

Conclusion

The peel extracts of *M. cavendishii* possess anti-hypertrophic scar activities by decreasing hydroxyproline levels and inhibiting histamine secretion in rabbits ear scar. The results provided some scientific basis for its ethno-medicinal use in wound, ulcers and scars managements. Additional studies are required to isolate and validate the bioactive constituents responsible for the anti-hypertrophic scar activity on other markers associated with hypertrophic scar formation.

Declarations

Conflict of interest

The authors declare no conflicts of interest with regards to this manuscript.

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Consent for publications

All the authors approved the manuscript for publication

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Authors' contributions

ARS, BBM, MGM, and AA conceived and designed the study, ARS, and ABN carried out the data analysis, while ARS drafted the manuscript. All the authors participated in editing, proofreading, and approved the submission of the final manuscript.

Ethical considerations

The experimental protocols were approved by the Ahmadu Bello University Committee on Animal Use and Care with the approval number: ABUCAUC/2018/075.

References

Abubakar A, Ibrahim H, Yusuf KM, Iliya I, Suleiman MM, Eloff NJ. Antioxidant properties of extracts from Tacazzea speculate Oliv. (Periplocaceae). FUW Trends in Science and Technology. 2019: 4(1): 75-7.

Ain Q, Khan H, Mubarak MS, Pervaiz A. Plant alkaloids as antiplatelet agent: drugs of the future in the light of recent developments. Frontiers in pharmacology. 2016;7:292. https://doi.org/10.3389/fphar.2016.00292.

Bodenstein J, Toit KD. The susceptibility of Staphylococcus aureus and Klebsiella pneumoniae to naturally derived selected classes of flavonoids. Antimicrobial agents. 2012. https://doi.org/10.5772/47948.

Cheng X, Shao Z, Li C, Yu L, Raja MA, Liu C. Isolation, characterization and evaluation of collagen from jellyfish Rhopilema esculentum Kishinouye for use in hemostatic applications. PloS one. 2017;12(1):e0169731. https://doi.org/10.1371/journal.pone.0169731.

Daian T, Ohtsuru A, Rogounovitch T, Ishihara H, Hirano A, Akiyama-Uchida Y, et al. Insulin-like growth factor-I enhances transforming growth factor-beta-induced extracellular matrix protein production through the P38/activating transcription factor-2 signaling pathway in keloid fibroblasts. Journal of Investigative Dermatology. 2003;120(6):956-62.

Diller RB, Tabor AJ. The role of the extracellular matrix (ECM) in wound healing: a review. Biomimetics. 2022;7(3):87. https://doi.org/10.3390/biomimetics7030087.

Dong J, Chen L, Zhang Y, Jayaswal N, Mezghani I, Zhang W, Veves A. Mast cells in diabetes and diabetic wound healing. Advances in therapy. 2020;37(11):4519-37. https://doi.org/10.1007/s12325-020-01499-4.

Galli SJ, Metz M, Starkl P, Marichal T, Tsai M. Mast cells and IgE in defense against lethality of venoms: possible "benefit" of allergy. Allergo journal international. 2020;29(2):46-62. https://doi.org/10.1007/s40629-020-00118-6.

Gozali MV, Zhou B. Effective treatments of atrophic acne scars. The Journal of clinical and aesthetic dermatology. 2015;8(5):33-40.

Han L, Dong X. Itch mechanisms and circuits. Annual review of biophysics. 2014;43:331-55. https://doi.org/10.1146/annurev-biophys-051013-022826.

Ho WS, Ying SY, Chan PC, Chan HH. Use of onion extract, heparin, allantoin gel in prevention of scarring in Chinese patients having laser removal of tattoos: a prospective randomized controlled trial. Dermatologic surgery: official publication for American Society for Dermatologic Surgery. 2006;32(7):891-6. DOI: 10.1111/j.1524-4725.2006.32192.x.

Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: an in vitro study. Contemporary clinical dentistry. 2015;6(4):496-9. DOI: 10.4103/0976-237X.169864.

Kim SW. Management of keloid scars: noninvasive and invasive treatments. Archives of plastic surgery. 2021;48(2):149-57. DOI: 10.5999/aps.2020.01914.

Komi D, Khomtchouk K, Santa Maria PL. A review of the contribution of mast cells in wound healing: involved molecular and cellular mechanisms. Clinical

reviews in allergy & immunology. 2020;58(3):298-312. DOI: 10.1007/s12016-019-08729-w.

Kumar PM, Ghosh A. Development and evaluation of silver sulfadiazine loaded microsponge based gel for partial thickness (second degree) burn wounds. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences. 2017;96:243-54. DOI: 10.1016/j.ejps.2016.09.038.

Lateef Z, Stuart G, Jones N, Mercer A, Fleming S, Wise L. The cutaneous inflammatory response to thermal burn injury in a murine model. International journal of molecular sciences. 2019;20(3):538. DOI: 10.3390/ijms20030538.

Lawrence JW, Mason ST, Schomer K, Klein MB. Epidemiology and impact of scarring after burn injury: a systematic review of the literature. Journal of burn care & research: official publication of the American Burn Association. 2012;33(1):136-46. DOI: 10.1097/BCR.0b013e3182374452.

Manna B, Nahirniak P, Morrison CA. Wound debridement. In StatPearls. StatPearls Publishing. 2022.

Marshall CD, Hu MS, Leavitt T, Barnes LA, Lorenz HP, Longaker MT. Cutaneous scarring: basic science, current treatments, and future directions. Advances in wound care. 2018;7(2):29-45. DOI: 10.1089/wound.2016.0696.

Moreno-Sanchez D, Hernandez-Ruiz L, Ruiz FA, Docampo R. Polyphosphate is a novel pro-inflammatory regulator of mast cells and is located in acidocalcisomes. The Journal of biological chemistry. 2012;287(34):28435-44.

Morris DE, Wu L, Zhao LL, Bolton L, Roth SI, Ladin DA, Mustoe TA. Acute and chronic animal models for excessive dermal scarring: quantitative studies. Plastic and reconstructive surgery. 1997;100(3):674-81. DOI: 10.1097/00006534-199709000-00021.

Nabai L, Ghahary A. Hypertrophic scarring in the rabbit ear: a practical model for studying dermal fibrosis. Methods in molecular biology (Clifton, N.J.). 2017;1627:81-9. DOI: 10.1007/978-1-4939-7113-8_6.

Ouyang HW, Li GF, Lei Y, Gold MH, Tan J. Comparison of the effectiveness of pulsed dye laser vs pulsed dye laser combined with ultrapulse fractional CO2 laser in the treatment of immature red hypertrophic scars. Journal of cosmetic dermatology. 2018;17(1):54-60. DOI: 10.1111/jocd.12487.

Phan TG, Amesbury M, Gardam S, Crosbie J, Hasbold J, Hodgkin PD, Basten A, Brink R. B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. The Journal of experimental medicine. 2003;197(7):845-60. DOI: 10.1084/jem.20022144.

Piera L, Szymański J, Juszczak M, Drobnik J. Histamine is involved in the regulation of collagen content in cultured heart myofibroblasts via H2, H3 and H4 histamine receptors. Biomedical reports. 2021;15(2):71. DOI: 10.3892/br.2021.1447.

Rabello FB, Souza CD, Farina Júnior JA. Update on hypertrophic scar treatment. Clinics (Sao Paulo, Brazil). 2014;69(8):565-73. DOI: 10.6061/clinics/2014(08)11.

Schultz GS, Chin GA, Moldawer L, Diegelmann RF. Principles of wound healing. In: Fitridge R, editor. Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists. University of Adelaide Press; 2011.

Seo BF, Lee JY, Jung SN. Models of abnormal scarring. Bio Med research international. 2013;423147. DOI: 10.1155/2013/423147.

Sheidu AR, Maiha BB, Ahmed A, Mohammed GM, Mohammed A, Lawal M. Ultrasonographic technique evaluation of antihypertrophic scar activity of *M. cavendishii* peel extract in New-Zealand rabbit ear scar model. Journal of Current Biomedical Research. 2022;2:198-208. DOI: 10.54117/jcbr.v2i2.15

Sheidu AR, Maiha BB, Ahmed A, Mohammed GM. Successive solvent extraction, phytochemical screening and thin layer chromatography (TLC) profiling of Musa cavendeshii Linn peel. Science Forum (Journal of pure and applied sciences). 2021;21(1):192. DOI: 10.5455/SF.103644.

Sheidu AR, Maiha BB, Magaji MG, Ahmed A, Giaze TR, Nazifi AB. Acute and sub-chronic toxicological profile of Musa cavendishii (Musaceae) Lamb peel extracts in New-Zealand rabbit. Journal of Current Biomedical Research. 2023;3:1219-46. DOI: 10.54117/jcbr.v3i5.3.

Singh B, Singh JP, Kaur A, Singh N. Phenolic composition and antioxidant potential of grain legume seeds: A review. Food research international (Ottawa, Ont.). 2017;101:1-16. DOI: 10.1016/j.foodres.2017.09.026.

Slemp AE, Kirschner RE. Keloids and scars: A review of keloids and scars, their pathogenesis, risk factors, and management. Current Opinion in Pediatrics. 2006;18:396-402.

Sonam M, Singh RP, Saklani P. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. Phytopathology. 2017;9(4):523-527.

Tani E, Shiosaka S, Sato M, Ishikawa T, Tohyama M. Histamine acts directly on calcitonin gene-related peptide- and substance P-containing trigeminal ganglion neurons as assessed by calcium influx and immunocytochemistry. Neurosci Lett. 1990;115:171-176. DOI: 10.1016/0304-3940(90)90450-N.

Thabet AA, Youssef FS, Korinek M, Chang FR, Wu YC, Chen BH, El-Shazly M, Singab A, Hwang TL. Study of the anti-allergic and anti-inflammatory activity of Brachychiton rupestris and Brachychiton discolor

leaves (Malvaceae) using in vitro models. BMC complementary and alternative medicine. 2018;18(1):299. DOI: 10.1186/s12906-018-2359-6.

Thompson CM, Hocking AM, Honari S, Muffley LA, Ga M, Gibran NS. Genetic risk factors for hypertrophic scar development. Journal of burn care & research: official publication of the American Burn Association. 2013;34(5):477-482. DOI: 10.1097/BCR.0b013e3182a2aa41.

Tracy LE, Minasian RA, Caterson EJ. Extracellular matrix and dermal fibroblast function in the healing wound. Advances in wound care. 2016;5(3):119-136. DOI: 10.1089/wound.2014.0561

Tredget EE, Yang L, Delehanty M, Shankowsky H, Scott PG. Polarized Th2 cytokine production in patients with hypertrophic scar following thermal injury. Journal of Interferon Cytokine Research. 2006;26(3):179-189.

Ud-Din S, Foden P, Mazhari M, Al-Habba S, Baguneid M, Bulfone-Paus S, McGeorge D, Bayat A. A double-blind, randomized trial shows the role of zonal priming and direct topical application of epigallocatechin-3-gallate in the modulation of cutaneous scarring in human skin. The Journal of investigative dermatology. 2019;139(8):1680-1690.e16. DOI: 10.1016/j.jid.2019.01.030.

Ugboko HU, Nwinyi OC, Oranusi SU, Fatoki TH, Omonhinmin CA. Antimicrobial importance of medicinal plants in Nigeria. The Scientific World Journal. 2020;2020:7059323. DOI: 10.1155/2020/7059323.

Wilgus TA, Ud-Din S, Bayat A. A review of the evidence for and against a role for mast cells in cutaneous scarring and fibrosis. International journal of molecular sciences. 2020;21(24):9673. DOI: 10.3390/ijms21249673.

Wilgus TA, Wulff BC. The importance of mast cells in dermal scarring. Advances in wound care. 2014;3(4):356-365. DOI: 10.1089/wound.2013.0457.

Wolak M, Bojanowska E, Staszewska T, Piera L, Szymański J, Drobnik J. Histamine augments collagen content via H1 receptor stimulation in cultures of myofibroblasts taken from wound granulation tissue. Molecular and cellular biochemistry. 2021;476(2):1083-1092. DOI: 10.1007/s11010-020-03974-6.

Yang R, Liu F, Wang J, Chen X, Xie J, Xiong K. Epidermal stem cells in wound healing and their clinical applications. Stem cell research & therapy. 2019;10(1):229. DOI: 10.1186/s13287-019-1312-z.

Ye Q, Wang SJ, Chen JY, Rahman K, Xin HL, Zhang H. Medicinal plants for the treatment of hypertrophic scars. Evidence-based complementary and alternative medicine: eCAM. 2015;2015:101340. DOI: 10.1155/2015/101340.