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Original Research

Wound Healing Using Honey & Aloevera Combination on the Skin of West African Dwarf Goats

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Abstract

This study was undertaken to compare the rates of wound healing on the body of West African dwarf goats, using the epidermal wound contraction method, with the topical application of the mixture of Honey and Aloe vera extracts in the ratio 1:1 (50% honey and 50% aloe vera) on the wound sites. Five male adult West African Dwarf (WAD) goats, weighing between 10 and 12 kg, were used. Wound contraction was measured on Days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. Skin biopsies were taken for histopathology on Days 0, 4, 8 and 12 to represent the phases of wound healing- inflammatory, proliferative and maturation. Histology revealed the dynamics of macrophages and inflammatory cells, representing the phases of wound healing. Healing in the experimental animals was concluded on Day 8, as evident by the scab falling off, while it occurred on Day 13 in the control animals. Histopathology showed normal histological features of normal skin in biopsies of day 0 from both experimental and control sites, an earlier inflammatory and maturation phase in the experimental sites' biopsies, as seen in the increased population of inflammatory cells such as macrophages, neutrophils, coupled with massive fibroblastic response, causing faster healing than the wounds at the control sites.

Keywords: Aloe Vera, Honey, Skin, Wound Healing, West African Dwarf Goats.

Introduction

Wound healing is a complex process in which the skin and the tissues under it repair themselves after injury (Nguyen *et al.,* 2009). Wound healing is depicted in a discrete timeline of physical attributes (phases) that constitute the post-trauma repair process. In undamaged skin, the epidermis (the outermost layer) and dermis (the deeper layer) form a protective barrier against the external environment. When the barrier is breached, a regulated sequence of biochemical events is initiated to repair the damage (Rieger *et al.,* 2015). This process is divided into predictable phases, and it's important to know that this classification varies with various schools of thought, where some classify the phases into 3. In contrast, others classify it into 4, viz, blood clotting (haemostasis), inflammation, tissue growth (proliferation), and tissue remodelling (maturation). The 3-phases classification system illustrates that blood clotting may be considered to be part of the inflammation stage instead of a separate stage (as in the 4-phases system.

Haemostasis (Blood Clotting)*:* Within the first few minutes of injury, platelets in the blood begin to stick to the injured site. This activates the platelets, triggering several responses: they transform into an amorphous shape better suited for clot formation and release chemical signals that enhance the clotting process. This results in the activation of fibrin, which forms a mesh and acts as "glue" to bind platelets to each other. This makes a clot that serves to plug the break in the blood vessel, slowing/preventing further bleeding (Rasche *et al.,* 2001).

Inflammation: During this phase, damaged and dead cells are removed, along with bacteria, other pathogens, and debris. This happens through the process of phagocytosis, where white blood cells "eat" debris by engulfing it. Platelet-derived growth factors are released into the wound, which causes the migration and division of cells during the proliferative phase (Midwood *et al.,* 2004).

Proliferation (Growth of New Tissue): In this phase, angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur (Midwood *et al.,* 2004). In angiogenesis, vascular endothelial cells form new blood vessels (Chang *et al.,* 2004). In fibroplasia and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix (ECM) by excreting collagen and fibronectin (Midwood *et al.,* 2004). Concurrently, re-epithelialization of the epidermis occurs, in which epithelial cells proliferate and 'crawl' atop the wound bed, providing cover for the new tissue (Garg, 2000). In wound contraction, myofibroblasts decrease the size of the wound by gripping the wound edges and contracting using a mechanism that resembles that in smooth muscle cells. When the cells' roles are close to complete, unneeded cells undergo apoptosis (Midwood *et al.,* 2004).

Maturation (Remodelling): During maturation and remodelling, collagen is realigned along tension lines, and cells that are no longer needed are removed by programmed cell death, or apoptosis (Midwood *et al.,* 2004).

In categorising wounds, wounds can be categorised as being surgical, traumatic, diabetic, venous, arterial, pressure, or epidermal. Wounds can also be classified according to the level/ degree of contamination, aetiology, duration of wound healing, integrity of the skin, wound depth, morphological characteristics, and according to severity. Scientists like Phillipo (2015) also describe that there are 3 types of wound healing modes, viz: healing by primary intention, healing by secondary intention, and healing by tertiary intention.

Wound healing has been reported to be affected by many factors, mainly: Local and Systemic Factors. Local factors usually comprise tissue ischemia, infection, the presence of a foreign body, surgical technique used, oxygenation, as well as venous sufficiency, among many others. Whereas, factors such as age, gender, sex hormones, stress, ischemia, presence of diseases, medication, nutrition, etc. are systemic factors that affect wound healing. (Jeevan, 2018).

Honey has been documented to have medical applications in the management of wounds and burns (Boekema *et al.,* 2013; Maghsoudi *et al.,* 2011; Gupta *et al.,* 2011; Zbuchea *et al.,* 2014; Saikaly *et al.,* 2017) and for its antibiotic properties (Majtan *et al.,* 2012; Kwakman *et al.,* 2012). With increasing scientific research exploring the applications of Aloe Vera in cosmetics and alternative medicines, Aloe Vera is also currently being used commercially as an ingredient in yoghurts, beverages, and some desserts, but at certain low and acceptable doses. Its use in medicine in the management of wounds is also experiencing soaring emergence and development by many researchers and medical practitioners (Olaifa, 2016; Hashemi *et al.,* 2015; Takzare *et al.,* 2009). The medicinal benefits of both substances —honey and aloe vera, and their relevant antibiotic and wound-healing properties —

were exploited in this study to investigate the ability of their combination to hasten certain wound-healing phases and, hence, to accelerate wound healing.

Materials and Methods

Materials

Animal Management

Five West African Dwarf goats (aged 1 year and with each weight, 10-12kg) were put in different stalls with plastic feeders and drinkers, concentrates, dried cassava peels, grass, de-wormers, antibiotics, needles and syringes.

Physicochemical **-** Stethoscope, clinical thermometer, recording book.

Cellular Dynamics **-** Clean glass slides, cover slips, microscopes, markers, blood samples, Haematoxylin and Eosin (H&E) stain, and biopsy samples.

Wound Creation **-** Methylated spirit, needles and syringes, distilled water, gauze, cotton wool, a pair of surgical scissors, surgical gloves, new scalpel blades, dissecting set, lignocaine/adrenaline, mild toilet soap, cardboards, and cotton wool.

Biopsy Collection **-** Sample bottles, a scalpel blade, formalin, skin, recording book, Haematoxylin and Eosin stain, glass slide, cover slips, and cassettes.

Wound Contraction Measurement **-** Vernier Calliper, recording book.

Other Reagents **-** Formalin.

Methods

Procurement and Breeds of Goats **-** 5 goats (Animals I-V) were obtained from a reputable goat herd in Ibadan. They were of the West African Dwarf breed.

Stabilization **-** The animals, three weeks before the commencement of the experiment, were acclimatised and given well-balanced concentrate, grass and cassava peels were fed to the animals and water was provided ad libitum.

Epidermal Wound Creation **-** The dorsum of the goats was moistened and washed with soap and water, shaved and cleaned with cotton wool moistened with methylated spirit to disinfect the area. The proposed site for wound creation was locally blocked with 2% lignocaine and administered at a dose of 3mg/kg subcutaneously. 3mg/kg of 2% lignocaine was used in caudal epidural block and local infiltration to desensitise the skin and provide the required anaesthesia. Booster injections of up to one-half of the initial dose were administered as needed to ensure that the goats were pain-free during the skin excision procedure. Each marked portion was blocked individually before surgery was performed. A pin-prick non-response indicated effective local anaesthesia following epidural anaesthesia. Four epidermal wounds (1cm x 1cm each) were created on the superficial epidermis of the dorsum of the skin of each goat using a cardboard template, scalpel and thumb forceps. A sharp, sterilised scalpel was used, and bleeding was reduced by the use of pressure gauze and shortening of the surgery duration. Each wound was lavaged with normal saline using a needle and syringe. The day of wound creation marks day 0 of the experiment (Olaifa *et al., 2022)*. The wounds were created as the T full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying tissue.

The wounds were topically dressed generously with the aqueous extracts of *Aloe Vera- Honey* mixture (constituted as 1ml:1ml), dispensed with a syringe, while the control wounds were treated with normal saline daily from day 0 until the wound scabs fell off, indicative that healing had taken place. The wounds were measured daily by placing the Vernier's calliper on the wound edges dorsally and ventrally, and readings were recorded until scabs fell off, marking healing.

The first two wounds were dedicated to the animal's control wounds contraction measurement, while the last two

were dedicated to experimental monitoring of the contraction, after applying the honey-aloe vera mixture. The wounds were labelled for each animal: C1, C2, E1, and E2.

Measurement of Wound Contraction **-** Each wound was measured in centimetres (cm) daily using the length and breadth dimensions of the wound with the aid of a Vernier calliper. The error due to parallax was reduced by ensuring that wounds were measured under adequate illumination, and they were measured by the same person. These dimensions were then used to calculate the wound area in centimetres square (cm^2). This was done daily.

Biopsy Collection and Processing **-** Biopsies were collected from C1 & E 2 of animal model II and C1 & E1 of animal model III on days 0, 4, 8 and 12 (of which both of them had normal saline applied daily onto their C1 & C2 wounds and the honey-aloe vera mixture was applied daily onto their E1 & E2 wounds); and processed histopathologically demonstrating cellular responses of the inflammatory, proliferative and maturation phases of wound healing. The biopsy tissues were fixed in formalin (10%) before arrival at the laboratory for histology.

Preparation of Paraffin-Embedded Biopsies **-** The purpose of fixation was to preserve the skin specimen indefinitely in a life-like state. For normal histological sections, the sample should be transported in a fixative (usually 10% neutral buffered formalin); the volume was about ten times that of the tissue and was labelled accordingly concerning the sex of the animal, site of the biopsy and the date of taking the biopsy. It remained in this preservative for a minimum of 24 hours before processing. Formalin acts as a good fixative medium by forming cross-links between lysine residues in the proteins of the tissues without altering their structure.

The biopsies were processed for 24 hours; the tissues were graded in alcohol and xylene. This involved first replacing the 70% (v/v) ethanol with 90% (v/v) ethanol, before placing it in absolute ethanol. This is then repeated with xylene and liquid paraffin. The skin biopsies were embedded in metal moulds filled with paraffin wax with particular attention to the position of the biopsies to ensure the orientation of the tissues from the epidermis down to the subcutaneous tissues, using ice to hold the tissue in place. The cassette was positioned, and the blocks were left in the ice to set. The mould was removed whilst cold, and the excess wax was trimmed manually using a dissecting blade. The wax blocks were cooled in ice and sectioned at a thickness of 4µm using a microtome. (Olaifa *et al., 2022).*

The sections were floated on a 40^0C water bath and collected on adhesive slides to minimise section loss during heat-mediated retrieval. They were then incubated at 37^0C overnight for 24 hours on slide racks, and kept in a special slides' container at room temperature, to be used later on for H&E staining.

Haematoxylin and Eosin (H&E) Staining **-** The staining method involves the application of haematoxylin, which is a complex formed from aluminium ions and oxidised haematoxylin. These colours the nuclei of cells (and a few other objects, such as keratohyalin granules) blue. The nuclear staining is followed by counterstaining with Eosin, which colours eosinophilic structures in various shades of red and pink. Optimisation may be necessary to achieve staining (haematoxylin may be diluted in water, and eosin may be diluted in ethanol as needed) (Olaifa *et al., 2022).*

H&E Staining of Paraffin Sections **-** Paraffin sections were prepared for H&E staining by mounting super-frost slides, drying on a hot plate, and then immersing in three sets of Xylene for 10 minutes each, followed by three sets of absolute ethanol for 10 minutes and finally rinsed with tap water. The purpose is to remove the wax and dehydrate the sections. Slides were placed into haematoxylin for 5 minutes and rinsed thoroughly under tap water for approximately 4-5 minutes. Excess haematoxylin was removed by adding 1% acid alcohol (1% HCl in 70% (v/v) alcohol) for 5 seconds, followed by a tap water wash.

The pink haematoxylin stain was converted to blue by adding Scott's tap water for approximately 10 seconds until the sections turned blue. The slides are rinsed in tap water before being stained in Eosin (1% (w/v)) for 15 seconds with a subsequent wash in a running water tap for 1-5 minutes. The sections were then dehydrated in two washes of absolute alcohol and in two washes of xylene for 10 minutes each before being mounted in DPX mountant and covered with glass coverslips. (Olaifa *et al., 2022).*

Results and Discussion

Physiological Parameters

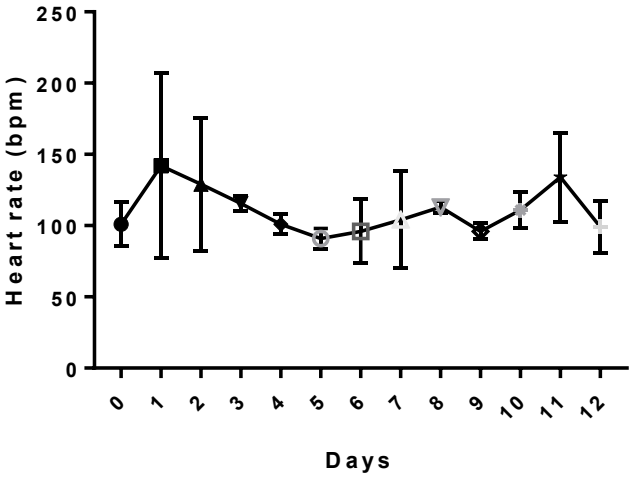


Fig. 1: Shows the heart rates of animals in the study

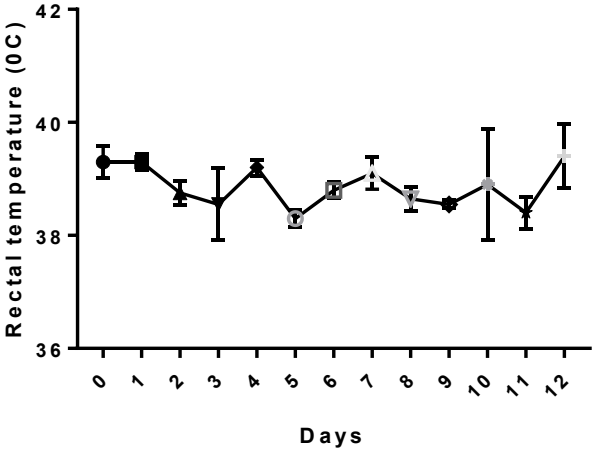


Fig. 2: Shows the rectal temperatures of animals in the study

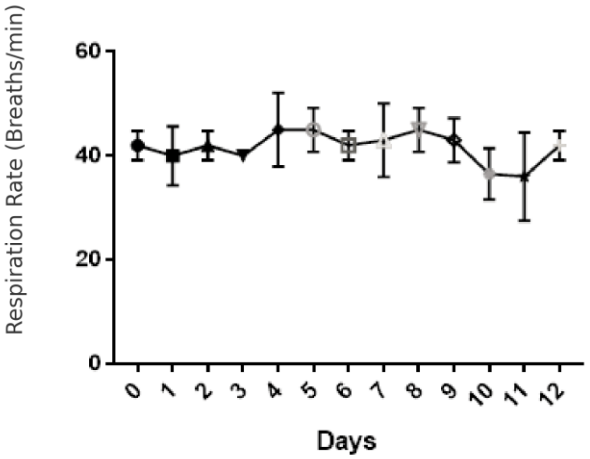


Fig. 3: Shows the respiration rates of animals in the study

Wound Contraction

Table 1: shows the mean control and experimental contraction measurements for animals I-V.

Days	Control (cm2)	Experiment (cm2)
0	100 ± 10.11	100 ± 8.16
1	98.200 ± 12.51	91.732 ± 12.35
2	96.706 ± 12.64	81.772 ± 6.55
3	92.512 ± 4.24	73.612 ± 6.84
4	89.960 ± 6.49	67.442 ± 8.99
5	85.660 ± 8.84	56.462 ± 9.47
6	78.630 ± 11.98	33.222 ± 11.31
7	71.910 ± 11.98	16.912 ± 10.47
8	61.452 ± 11.8	0.00
9	50.508 ± 10.16	0.00
10	37.974 ± 9.85	0.00
11	26.758 ± 6.73	0.00
12	11.297 ± 8.21	0.00
13	0.00	0.00

**P<0.05, Mean ± SEM*

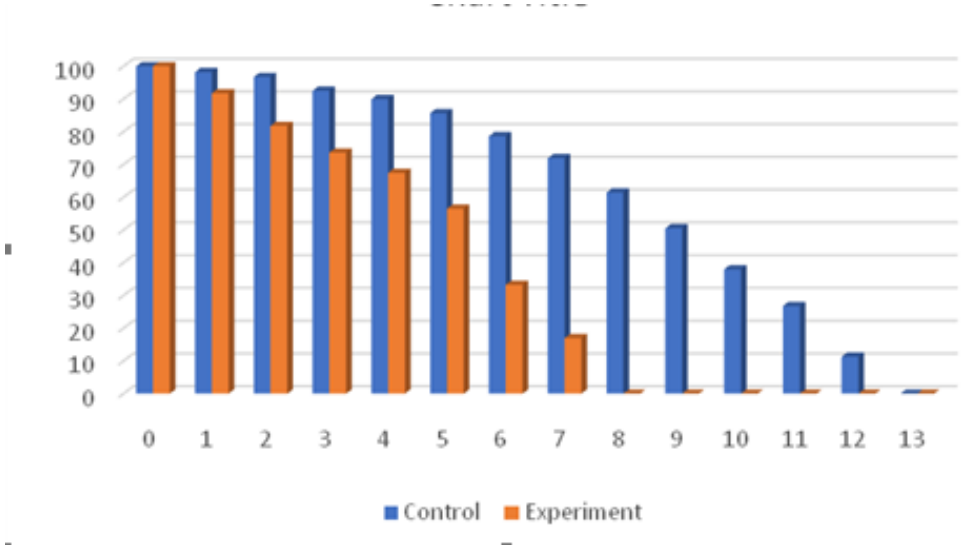


Fig. 4: shows the graph of the wound contraction rate

Vital parameters such as respiratory rate, heart rate and rectal temperature showed little or no significant difference from normal range values, indicating their healthy states throughout the experiment. Healing of the experimental site was completed on Day 8- earlier than the control sites, where healing was completed on Day 13, as indicated by the scab falling off. This is explained by the greater fibroblast infiltration, shortening the inflammatory phase. Concerning the histopathology of the tissue biopsies taken on days 0, 4, 8 & 12, sections from day 0 biopsies showed normal skin epidermal layout. Sections of Day 4 biopsies showed that the biopsies from control sites had cellular characteristics associated with the Inflammatory phase of wound healing (i.e., The epidermis was covered by a few layers of inflammatory cells and marked fibroblastic response), while those from the experimental sites on the same day showed cellular characteristics of proliferative phase (i.e., there is locally extensive fibroblastic response with deposition and maturation of collagen fibres).

Biopsies of Day 8 showed the same cellular changes in both the control and experimental biopsies (i.e., the epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response), also indicative of the Proliferative phase m Biopsies of control sites on Day 12 showed cellular changes typical of the Late Proliferative phase transitioning to the Maturation phase, characterised by re-epithelialization and remodelling of the skin. In contrast, biopsies of the experimental sites on the same day revealed the histological

layout of normal skin, suggestive of the Maturation phase.

It can be deduced then that the healing rates were significantly affected on Days 4 and 12, as seen in the faster transitions to Proliferative and Maturation phases, respectively.

Haematological and Serum Chemistry Values

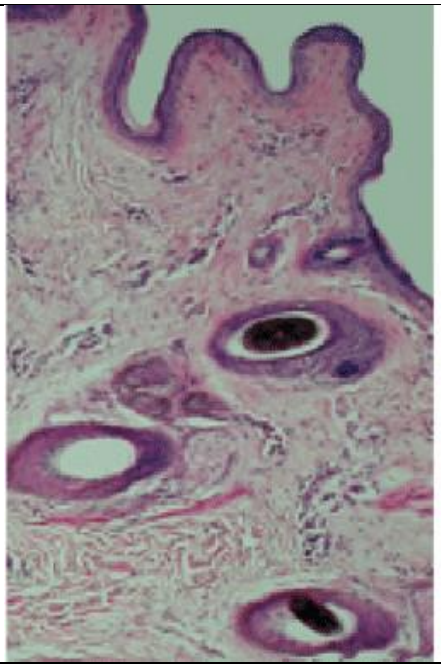
Table 4: shows the Mean physiological parameters' values for animals I-V.

Parameter	Normal Reference Range	Day 0	Day 4	Day 8	Day 12
PCV (%)	22-38	25.2	32.8	26	27.5
Hb (g/dl)	8-12	8.65	9.1	9.5	10.85
RBC ($x10^6$/µl)	8-18	10.61	11.425	14.785	13.705
WBC ($x10^3$/µl)	4-13	12.225	9.975	7.825	6.200
PLATELETS ($x10^3$/µl)	300-600	432	525	486	388
LYMPHOCYTES ($x10^3$/µl)	50-70	62	58	71	69.5
NEUTROPHILS ($x10^3$/µl)	30-48	44.5	36.5	47	36.5
MONOCYTES ($x10^3$/µl)	0-4	1.5	2.5	1..5	2.5
EOSINOPHILS ($x10^3$/µl)	1-8	2	2	2.5	1.5
TOTAL PROTEIN (g/dl)	6.4-7.0	6.75	6.9	7.2	7.2
ALBUMIN (g/dl)	2.7-3.9	2.65	2.9	3.7	3.4
GLOBULIN (g/dl)	2.7-4.1	4.1	4.1	3.1	2.9
A.G RATIO		0.6465	0.6835	0.6055	0.672
CREATININE KINASE (u/l)	0.8-8.9	5.4	5.7	3.9	4.25
GLUCOSE (mg/dl)	50-75	73.5	77	61.5	74
Na^+ (mEq/L)	124-146	124	135.5	141	145
K^+ (mEq/L)	3.5-6.7	4.75	4.7	4.9	4.9
Cl^- (mmol/L)	99-110.3	109	111.5	113.5	115

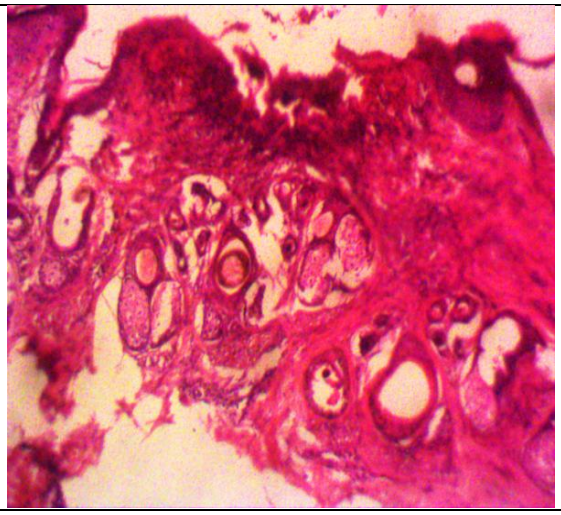
**All reference values are as given by Mercks' Manual*

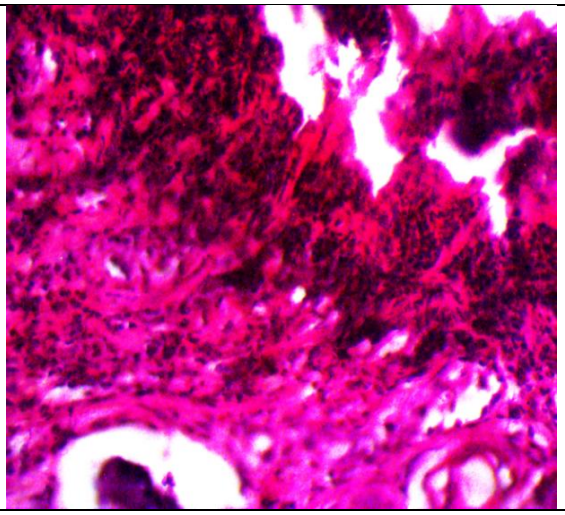
Histopathology

			Healing Score
D4 CIII	Stage II to III	The epidermis is covered by a few layers of inflammatory cells and a marked fibroblastic response	3
D4 EIII	Stage III	There is a locally extensive fibroblastic response with deposition and maturation of collagen fibres	4
D8 CIII	Stage II to III	The epidermis is covered by scab tissue. The lesion consists of tissue debris and inflammatory cells, with a moderate fibroblastic response.	2
D8 EIII	Stage II to IV	The epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response.	2
D12 CIII	Stage II to IV	The epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response.	2
D12 CII	Stage IV	There is re-epithelialization and remodelling of the skin.	5
D12 EII	Stage IV	Normal skin	5
D12 EII	Stage IV	Normal skin	5

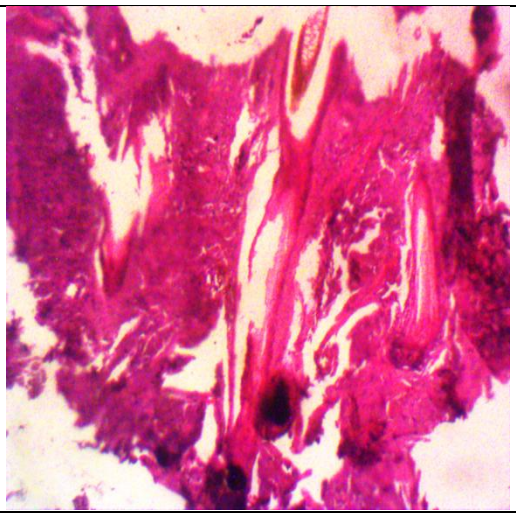


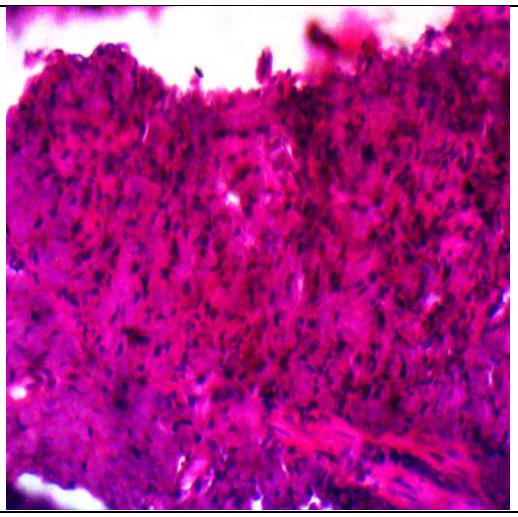
D0: Normal skin histology for all animals on Day 0, showing a thin keratin layer with normal hair follicles



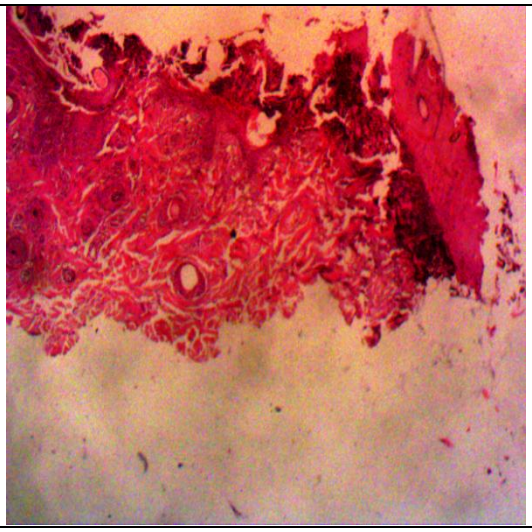
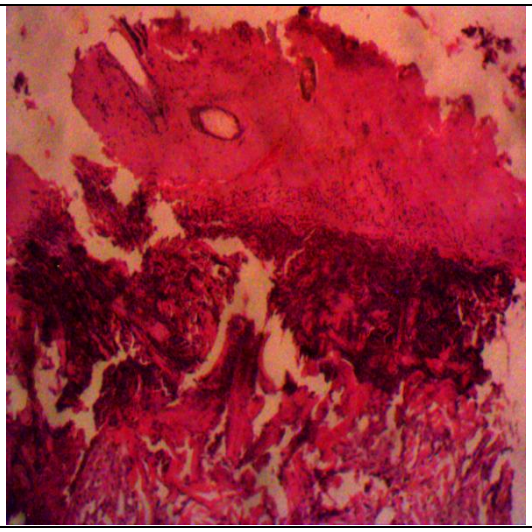


D4 CIII: The epidermis is covered by a few layers of inflammatory cells and marked fibroblastic response.

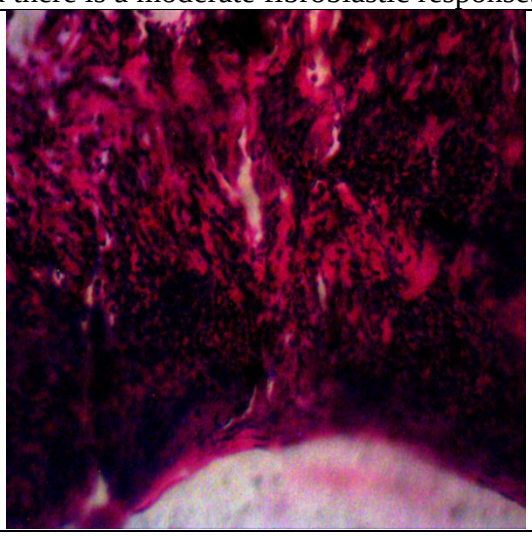
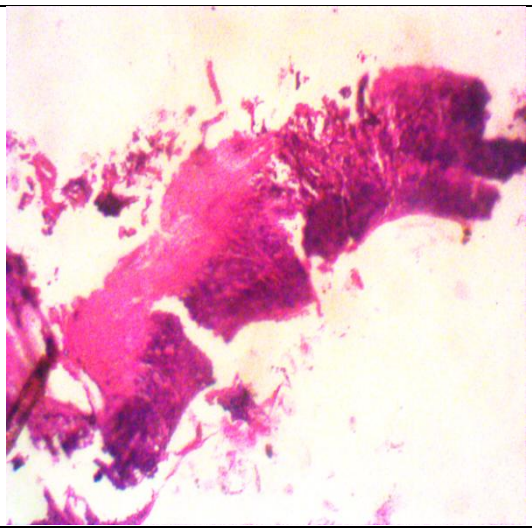




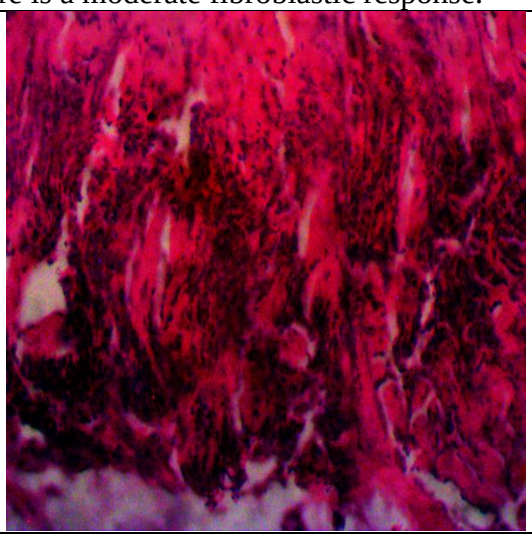
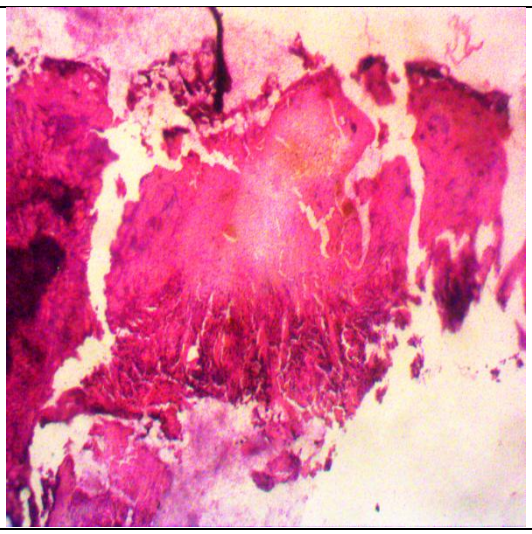
D4 EIII: There is a locally extensive fibroblastic response with deposition and maturation of collagen fibres.

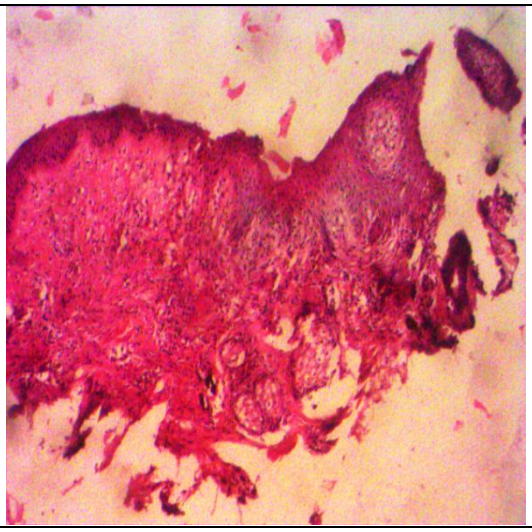
D8 CAIII: The epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response.

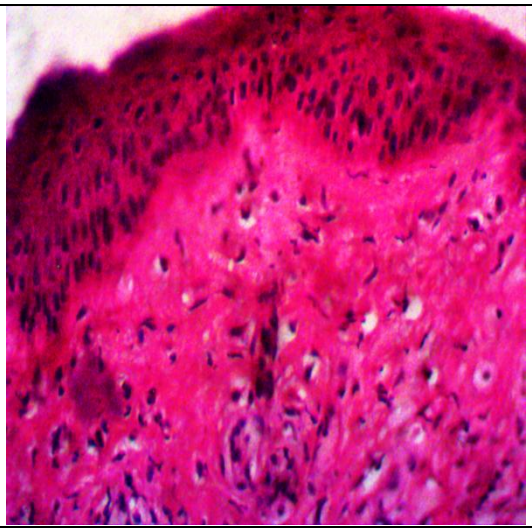



D8 EIII: The epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response.

D10 CIII: The epidermis is covered by scab tissue comprising tissue debris and inflammatory cells, and there is a moderate fibroblastic response.





D12 CAII: There is re-epithelialization and remodelling of the skin

***KEY**: CII: Control of Animal II, CIII: Control of Animal III, EIII: Experimental of Animal II, EII: Experimental of Animal II, D0: Day 0, D4: Day 4, D8: Day 8, D12: Day 12*

Fig 1: Showing Animal III on Day 0

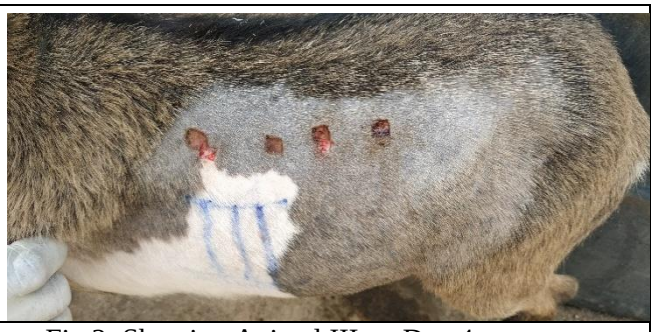


Fig 2: Showing Animal III on Day 4

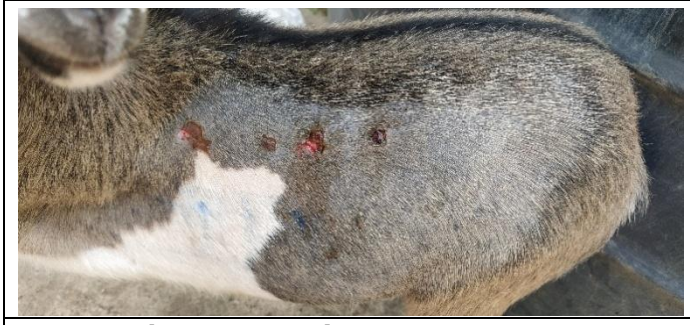


Fig 3: Showing Animal III on Day 8

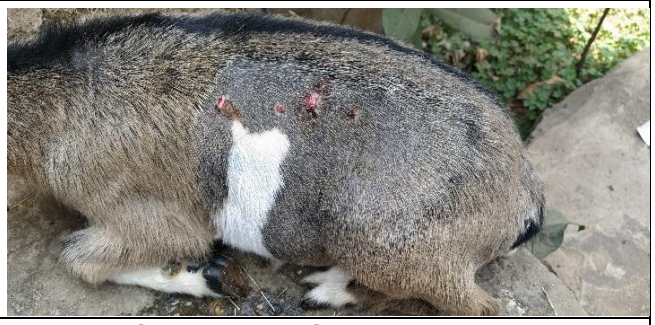


Fig 4: Showing Animal III on Day 12

Discussion

Several factors modify the rates of wound healing, some of which include systemic factors like age, stress, medications, nutrition, gender, chronic diseases, laboratory values and local factors like infection, presence of foreign bodies, obesity, oedema, surgical techniques, hematoma formation and many others, to mention but a few. (Thomas *et. al.*,2011; Jeevan, 2018; S. Guo *et. al.*, 2010).

Younger animals heal faster than older ones; so also, healthy ones with better nutritional status than malnourished ones; and female animals (due to hormonal Influence-Oestrogen) than male animals. (Olaifa, 2017)

Stress is also a factor that impairs healing rates. In this experiment, the physiological/laboratory values were within normal ranges, suggesting a stable milieu in the animals, which could have negatively affected the healing rates.

The laboratory values in Figs. 1-3 and Table 4 were also within physiological ranges, suggesting the absence of underlying disease conditions and supporting the rapid healing in the experimental model compared to the controls.

Throughout healing, the goats showed a significant difference ($P<0.05$) in healing at the Proliferative phase, as the scabs fell off on day 8. This is in line with Olaifa (2016), who worked with aloe vera, applying it to an epidermal wound on rabbits, resulting in healing on day 9. Similarly, Eyarefe et al. (2016) used honey to manage an injection site abscess, resulting in healing by day 18. It is important to note that the more effective outcome of the use of the two substances combined compared to their applications as in the previous works cited earlier with an earlier onset of inflammatory and maturation phase may be due to the chemical properties of the substances used (honey and aloe vera) which include: hygroscopicity, hypertonicity, lower pH, complex chemical composition, presence of antiseptic (H_2O_2), Increased Lymphocytic and Phagocytic activities, Nitric oxide, Nonperoxide activity and antioxidants, anti-inflammatory properties and the presence of Glucomannan which stimulates fibroblasts responses (Surjushe, 2008).

Finally, there will be a need for further studies and designs to investigate whether these properties possessed by the substances used actually are responsible for the faster healing rates, shortened inflammatory and maturation phases, and, if perhaps, there are other properties not yet discovered, but also responsible for the enhanced healing rates. However, the findings here are very unique in that they've never been explored in this substance combination, but rather, in their applications.

Conclusion

From the above discussion, it can be conveniently agreed that the Aloe vera/Honey combination has a valuable chemical composition that readily enhances wound healing. These novel findings may constitute an effective alternative to other wound-healing substances in humans and animals. Moreover, the aspect of economic gains should be considered in animals, where its application on the skin will reduce damage to the hides and skins of sheep and goats, leading to increased profits in the hides and skin market. Also, as wound sites are potential entry sites for pathogenic organisms or toxic substances, it's of utmost importance to endeavour to manage wounds properly and to do that within the shortest possible time in order to prevent secondary bacterial/ viral infections. Also, further designs by modifying the ratio of the substance combination could yield better results.

Contribution by Authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all authors and consented to publication.

Conflict of Interests

There is no conflict of interest.

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