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Research Article

Evaluation of Topical Application of Aloe Vera Gel and Honey for Acceleration of Cutaneous Wound Healing in Goat Model

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ABSTRACT

In the present study, the effect of topical application of Aloe vera, honey and combination of Aloe vera and honey (A+H) on cutaneous wound healing in goat model was investigated. A total of 20 goats were selected having body weight 8-12 kg, age 9-18 months and randomly divided into group A, H, A+H and C. Following local anaesthesia, a 1.5×1.5 cm^2 excised wound along with the latissimus dorsi muscle were made on the skin of these goats using a template. The wounds of Group A, H, A+H and C were treated topically with Aloe vera, honey and Aloe vera + honey and sterile normal saline respectively. The efficacy of the treatment was evaluated in terms of bacterial load, tissue response in healing by histopathology and wound contraction rate. The bacterial load was measured by growing them on agar medium and means of colony forming unit (CFU). The wound area in each group was measured using a Vernier calliper scale which was used to trace the wound area. The bacterial load (CFU) appeared lower in every sampling in goats of Group H followed by those of Group A and A+H. Highest level of bacterial infectivity (1700-1980 cfu/100µl nutrient agar) in group C and lower count in Group H (250-362 /100 µl nutrient agar) was seen in the wound following day 3 of treatment. Biopsy examination of Group A skin revealed that the topical application of Aloe vera lowered tissue reaction following day 7 of wounding and enabling rapid repairing of tissue with complete epithelialization over the wound following 14 days of wounding. Clinically the wounds showed various degree of healing in group A, H, A+H goats following day 14 of wounding compared to the existence of raw and unhealed wound in Group C goats. Complete centripetal contraction of wound was obtained in group A, A+H, H at 17, 19, 21 days respectively. The results reveal that topical application of Aloe vera alone appeared best in the wound healing process followed by Aloe vera + honey and honey respectively.

Introduction

Wound healing is the sequential cellular and biochemical event underpinning for the repair of injured tissue of the wound (Gonzalez et al., 2016). These processes involve the interaction between several mediators like extracellular matrix (ECM) molecules, platelets, inflammatory cells, growth factors, cytokines, and chemokines (Pereira, et al., 2013). Wound healing process is divided into four stages including hemostasis, inflammation process, proliferation, and remodeling (Guo and DiPietro, 2010; Opneja et al., 2019).

Hemostasis is initiated by rapid recruitment of platelets through fibrin clot formation during normal healing process at the moment the tissue is injured (Opneja et al., 2019). The inflammatory phase involves different series of events such as platelet accumulation, coagulation and leukocyte migration. Re-epithelialization, angiogenesis, fibroplasia, and wound contraction are events that occur for tissue formation. Remodeling phase may be continued for one month, and the dermis produces collagen and matrix proteins which weave into its previous phenotype (Castilo-Briceno et al., 2010). Interruption of any kind among these stages of healing lead to delayed wound healing. The events of each phase must happen in a precise and

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regulated manner. Interruptions, aberrancies, or prolongation in any of the stages can result in delayed wound healing or a non-healing chronic wound (Guo and DiPietro, 2010).

Normally in an acute wound, the process of healing is predictable but in chronic wounds, the repair process is disrupted by constrains like as infection or low immunity (Dat et al., 2012). In the absence of effective microbial decontamination such wounds enter a state of prolonged inflammation. Bacteria and endotoxins can lead to pro-inflammatory cytokines elevation (interleukin-1 and tumor necrosis factor–α) leading to a chronic state of infection and failed to heal the wound (Guo and DiPietro, 2010). This has imposed a huge health and financial burden in both the developed and undeveloped world. Therefore, research on deriving alternative, cost effective wound healing agents from traditional plant-based medicines is a developing area in modern biomedical sciences (Hosseinkhani et al., 2017). In recent times, there has been an upsurge in incidences of preferences to the use of traditional and alternative medicine in wound management due to the emergence of antibiotic resistance and a decrease in newer antibiotics (Dorai, 2012).

Aloe vera is a perennial, succulent, drought-resistant plant that exhibits many pharmacological characteristics to promote wound and skin burns healing (Chowdhuri et al., 2018). Many of the medicinal properties of Aloe vera are ascribed due to numerous active ingredients including anthraquinones, polysaccharides, alkylbenzenes, dehydrabietic acid derivatives, salicylic acid, lectin, carotenoids, lignin, saponins etc. that attribute for its high therapeutic value (Wynn, 2005). Antibacterial property of aloe vera is because of anthraquinones that behave like tetracycline by blocking the ribosomal A site, thus, interrupting bacterial protein synthesis (Radha and LaxmiPriya, 2017). The anti-inflammatory activity of aloe vera gel may be due to inhibition of cyclooxygenase and hence of the prostaglandin biosynthetic pathway (Lindsey et al., 2002).

Honey has long been used as ancient herbal preparations in wound healing and has gained popularity with the increase of antibiotic resistance (Mandal and Mandal, 2011). In vitro and in vivo data supporting honey's effectiveness in treating wounds and as a natural broad-band antibacterial agent has recently made a value in coming back of honey in clinical medicine particularly in developing countries (Minden-Birkenmaier and Bowlin, 2018). Honey is a highly concentrated viscous solution of floral sugars and proteins, enzymes, and amino acids obtained from the honeycomb (Gethin et al., 2008). Honey can reduce healing time exerting a dual effect on the inflammatory response. It suppresses the production and proliferation of inflammatory cells at the wound site preventing a prolonged inflammatory response, and it stimulates pro-inflammatory cytokine production thus enables normal healing to occur (Khan et al., 2007; Molan, 2002; Steinhorn et al., 2011). Studies using herbal and traditional medicine like *Aloe vera* and honey have been documented in wound care management from different continents. However, sufficient scientific evidences and conducting clinical trials using traditional and alternative medicine in wound therapy holds good promise in the future. Therefore, the study was set out to analyze the therapeutic and synergistic efficacy of honey and *Aloe vera* gel on experimentally induced wound in goats.

Materials and Methods

Animal Ethics

All the authors have agreed for authorship, read and approved the submission for publication. As the study was conducted onto the goats, without involvement of deadly pathogen, and health issue was great concern as live goats were used. The cutaneous wound creation and biopsies were carried out under local anaesthesia with less sufferings as required by the ethical concern, Animal Welfare Committee, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Mymensingh-2202, Bangladesh.

Study area and animals

This research was conducted onto the live goats at the Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh during a period between February to August 2022.

Experimental design

With the approval and following the guidelines and recommendations of Animal Ethics Committee of the Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Science, Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur, twenty goats were bought from Farmhat, Dinajpur. Body weight of these goats ranged from 8-12 kg and age 8-18 months. They were randomly divided into four groups i.e., group A, H, A+H and C with five goats in each group. The wounds of Group A, H, A+H and C were treated topically with Aloe vera, honey and Aloe vera + honey and sterile normal saline respectively. The animals were kept under standard housing conditions with no restrictions on water and food. The goats were kept in quarantine for two weeks before the onset of the study. All goats were dewormed with Fenbendazole @5mg/kg body weight

(BolusFenazol Vet™, Acme Laboratories, Dhaka, Bangladesh) prior to the experimentation.

Preparation of Aloe vera gel (AVG), Honey and combined Aloe vera and Honey topical application

Aloe vera plants were collected from local nursery. The fresh leave was properly cleaned with water and air dried. The green portion of the leave was peeled off with knife. The gel was carefully scooped with knife. The gel was then placed into a ceramic mortar and grinded with pestle to make homogenize. One teaspoon African honey and one teaspoon fresh *Aloe vera* gel was placed into a mortar and mixed with pestle thoroughly. Irradiated honey was collected from South Africa by personal contact to get pure honey without any adulteration.

Creation of wound

The operation site selected was five centimetres away from vertebral column. The operation sites were clipped with sharp scissors and washed with soap water to reduce microbial load in the wound area. A razor blade was used to cleanly shave the residual hair to reveal the underlying skin. The operation sites were drapped with sterile drapper and rubbed with Povidone-Iodine 10% (Povin Vet™ 10%, Opsonin Pharmaceuticals Ltd, Bangladesh). Infiltration of local anesthesia was done following inverse 'L' blocks at the operation site by using lidocaine hydrochloride 2% & Epinephrine 0.005% (Jasocaine-A™, Jayson Pharmaceuticals Ltd. Bangladesh). A 3 cm^2 (1.5 cm × 1.5cm) template was placed on the shaved area. Full thickness of skin was removed from the marked region, using surgical blade. First tracing the area, gently lifting the skin with toothed tissue forceps and separating it from the underlying subcutaneous tissue. Antiseptic were constantly applied around the wound area using sterile gauze.

Post-operative care

A combination of Streptomycin and Penicillin (Streptopen ™, Renata Ltd) at the rate of 1ml/ 10 kg body weight was administered through intramuscular route for five days to reduce the risk of infection. An analgesic and antipyretic Meloxicam @ 0.5 mg/kg (Melvet ™ Acme Laboratories) was given through the intramuscular route for three days. An antihistaminic (Pheneraminemaleate, Antihista vet™, Square Pharmaceuticals Ltd) @ 1mg/kg body weight was given through intramuscular route for five days. Washing of the wound surface was done with sterile gauge and normal saline (0.9%). Therefore AVG, Honey, and combination of Honey and AVG (1:1) was applied on the wound surface twice daily until healing.

Assessment of wound healing

The wound areas of animals were measured everyday using Vernier caliper and tracing paper. The measured wound areas were determined in mm^2. Wound contraction rate was calculated by using the following formula (Attah et al., 2016).

Percentage Mean Centripetal contraction (PMCC)

$$\frac{Wound\ Area in\ PSD\ 0 - Wound\ Area in\ PSDx}{Wound\ Area in\ PSD\ 0} \times 100$$

Where, PSD= Post-Surgical Day, X= any given day following the surgery

Antimicrobial study

Swabs were collected from the raw wound surface following 1, 5, 10 and 15 days of wounding. After initial debridement of wound with sterile gauze and normal saline (0.9%) the superficial debris was removed. Then the commercially available sterile cotton buds were rubbed on the wound surface to collect deep tissue microorganisms. To isolate bacterial contaminants in the wounds nutrient broth and nutrient agar plates were used (Saha et al., 2020). To prevent contaminating fungal growth in nutrient broth and nutrient agar plates, 0.05mg/ml Fungi zone (amphotericin B, Thermo Fisher Scientific INC, NY) was used. A sterile cotton bud was gently rubbing over the surface of the wound and then transferred the swab tip onto a 2ml cryotube containing1ml sterile nutrient broth. Then the cryotube was shipped to the laboratory within an hour. After that the nutrient broth containing swab was vortexed shortly and 100µl, 50µl and 10µl nutrient broth/ agar plate were spread out using glass spreader. The agar plates were incubated at 37˚C overnight to observe bacterial growth and counting the total number of bacterial colonies grown on to the agar plates. In order to accurately count the colonies, magnifying glass was used.

Tissue biopsy/ Histopathology

Tissue biopsies were collected at day 7, 14 and at the day of complete wound healing for better understanding the tissue response following experimental wound healing processes. Before tissue collection, the area was locally anesthetized with 2% lidocaine HCl (Jasocaine A, Jayson Pharmaceuticals, Bangladesh). Tissue samples were collected from the full skin thickness of the entire wound area as well as from the surrounding normal tissue. The collected biopsies were fixed in 10% buffered neutral formalin and were processing tissues for sectioning and staining with hematoxylin and eosin using standard protocol (Luna, 1968).

Data analysis

The generated data were analyzed by SPSS version-20 software by using one-way ANOVA accordance with the principles of Complete Randomized Design (CRD). All values were expressed as Mean±SD and significance was determined when P <0.05.

Results

Area of wound contraction

Table1 represents the area of wound contraction of different groups at different days of experimentation. The extent of wound area was reduced to 0 in *Aloe vera* treated group (day17) followed by combined *Aloe vera* and honey treated group (day 19) and honey treated group (day 21).

Table 1. Effects of *Aloe vera*, honey and combined *Aloe vera* and honey onto the wound area at different days of the experiment measured by Vernier calliper

Groups	Mean ± SD of wound area at different days (mm²)						
	Day 0	Day 5	Day 10	Day 14	Day 17	Day 19	Day 21
A n =5	331.4 ± 20.87	213.2 ± 12.19	134.6 ± 9.68	45.6 ± 3.58	0	0	0
H n =5	310.6 ± 38.56	234.6 ± 28.1	166.8±21.43	117± 16.82	72.4 ± 10.62	38.4 ± 3.29	0
A+H n =5	296 ± 19.73	201.8 ± 16.31	132.2 ± 8.14	70.4± 3.58	34.8 ±5.02	0	0
C n =5	317.2 ± 27.43	282.6 ± 25.92	234.4 ± 23.54	187.8 ± 15.66	158.8 ± 13.97	125.6 ± 12.89	81 ± 6.36
P value	0.2763	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

The value of P is 0.00 (P<0.001) which indicates that the result is highly significant both within the group and between the group.

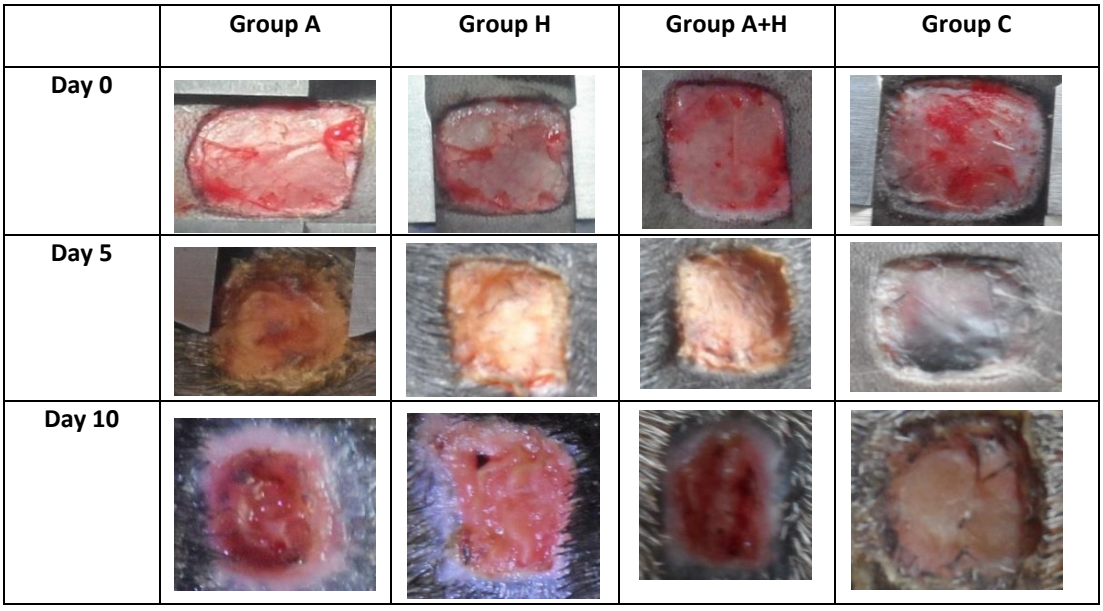
Percentage mean centripetal contraction (PMCC) rate

The percentage of wound contraction was greater in *Aloe vera* treated group and exhibited 100% wound contraction following17 days of wounding. Significant wound contraction was observed in all treated groups from 14th day onwards and achieved 100% contraction in wounds treated with combined *Aloe vera* and honey in 19 days followed by wounds treated with honey only at 21 days as shown in Table 2.

Table 2. Percentage mean centripetal contraction (PMCC) rate of wounds at different days

	Mean ±SD, %						
Groups	Day 0	Day 5	Day 10	Day 14	Day 17	Day 19	Day 21
A	0	35.64 ± 1.29	59.40 ± 0.99	80.18 ± 0.78	100	100	100
H	0	24.41 ± 2.06	43.32 ± 0.57	62.37 ± 1.68	76.68 ± 1.71	87.59 ± 0.57	100
A+H	0	31.87 ± 0.95	55.32 ± 1.06	76.18 ± 1.13	88.26 ± 1.30	100	100
C	0	10.94 ± 0.44	26.17 ± 0.99	40.78 ± 0.92	49.93 ± 1.18	60.44 ± 0.97	71.30± 0.99

The value of P is 0.00 (P<0.001).



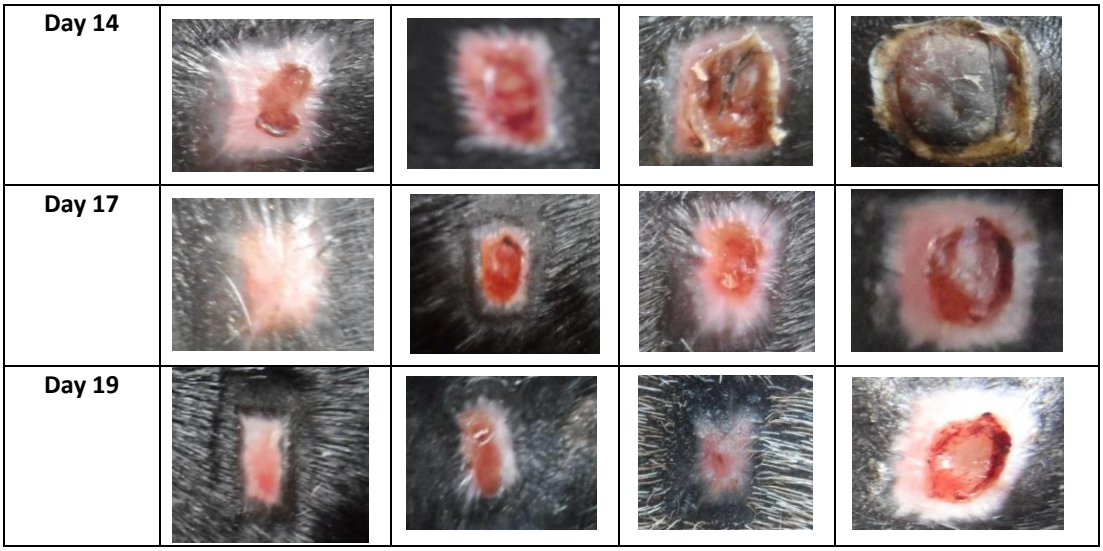


Figure 1. Photographic representation of wound contraction area on different post excision days of aloe vera, honey, combined honey and aloe vera and untreated control goats.

Microbiology of the wound

Table 3 showed the bacterial load of the wounds of different groups. Highest level of bacterial load (1700-1980 CFU/wound) was seen in control group and lower count in honey treated group H (250-362 CFU/wound) following day 3 of experiment. The bacterial loads of Group A, H and A+H appeared lower in study day 15 whereas the wounds of group C left infected and showed fewer tendencies to heal.

Table 3. Colony forming unit of bacterial colony per open wound as collected by rubbing sterile cotton bud (1ml nutrient broth)

Groups	Day 3	Day 5	Day 10	Day 15	Remarks
H	250-362	230-255	110-135	18-26	Showed healing
A	455-564	333-442	178-225	65-93	at various
A+H	271-383	258-237	135-143	37-45	degrees
C	1700-1980	1680-1850	1525-1835	1279-1496	Incomplete healing

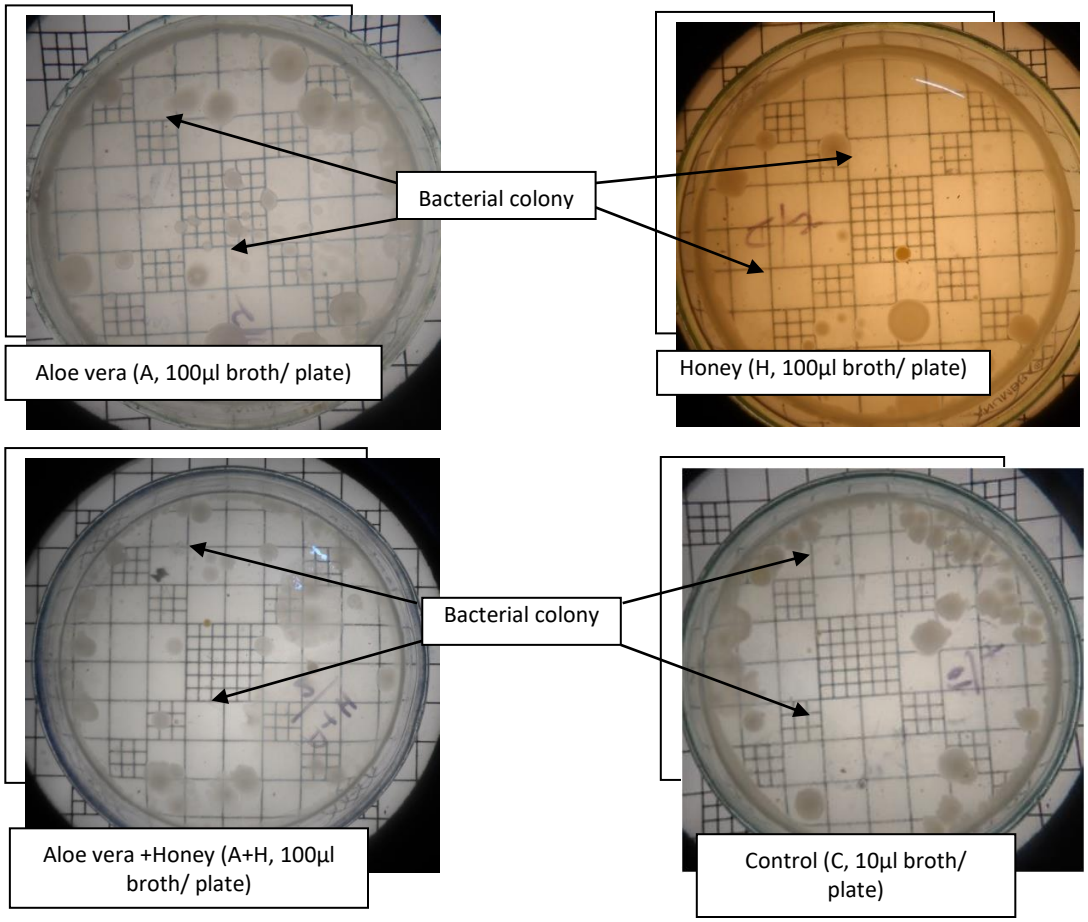


Figure 2. The bacterial colony in primary culture of nutrient agar observed in samples collected from the open wound of goats treated with *Aloe vera* (A), Honey (H), Combined *Aloe vera* and honey (A+H) and untreated control (C) at day 15 of wounding.

Biopsy examination of experimental wound healing:

Histoarchitecture of wound biopsies at days 7 of experimentation; Biopsy of experimental cutaneous wound of goats treated with *Aloe vera* showed early scab formation (Figure 9A) over the cut surfaces. Scab formation over the injured skin was also seen in the fresh honey (H) and *Aloe vera* plus honey (A+ H) treated groups of goats but the scabs appeared heavier in A+H treated groups (Figure 9). The scab was infected in untreated control groups and dissolved (Figure 9C, circle) at time of processing the wound. The cells infiltrated in the injured tissues as seen following day seven of experiment were mostly neutrophils in untreated skin. The reactive cells in other groups of goats following day 7 of experiments were mostly macrophages and lymphocytes. Hemorrhages in tissues was much higher in untreated groups of goats but was scanty in other groups of goats.

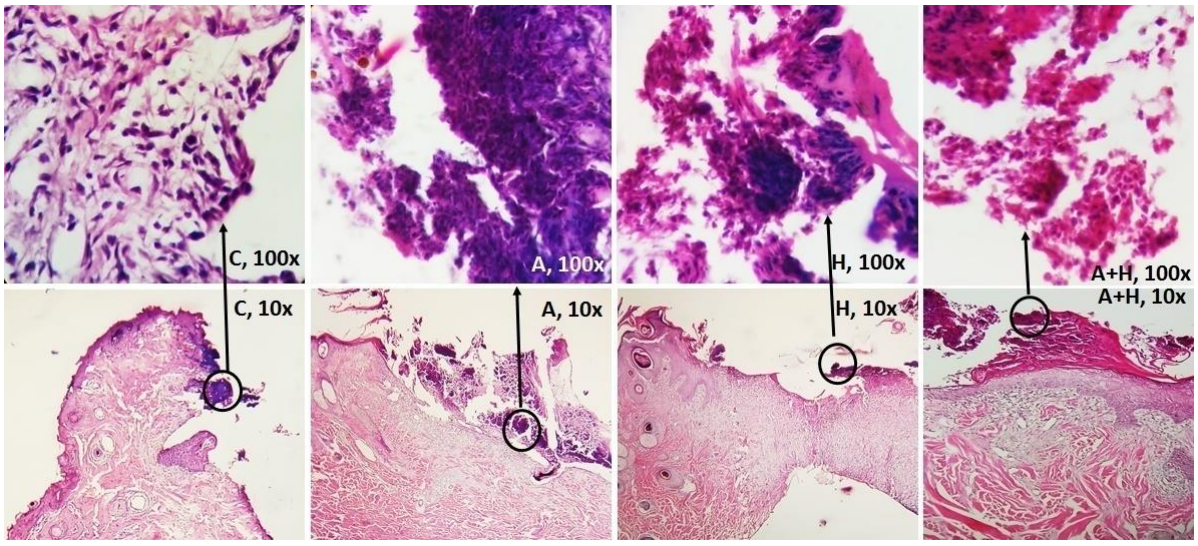


Figure 3. Biopsy of experimental cutaneous wound following day 7 of experimentation

Tissue debris over the wound (scab, black circle) formed early in the *Aloe vera* treated wound (A, black circle) followed by *Aloe vera* plus honey (A+H, circle) and honey alone (H, circle). The tissue debrises were not formed over the injured wound in untreated control cases. The control section (C, 100x) showed cellular debris in the scab consisting of neutrophils, tissue debris and fibroblasts. The reactive cells in scab mostly involved in *Aloe vera* treated injured skin were keratinocytes (darkly stained cells), tissue debris, monocytes, fibroblasts and lymphocytes (Figure 9A, 100x). The reactive cells in scab mostly involved in Honey treated injured skin were keratinocytes (darkly stained cells, moderate quantities), tissue debris, monocytes, fibroblasts and lymphocytes (100x). The reactive cells in scab mostly involved in *Aloe vera* + honey treated injured skin were tissue debris, monocytes, fibroblasts and lymphocytes (A 100x) with scanty of keratinocytes.

Histoarchitecture of wound biopsies at days 14 of experimentation; Cutaneous biopsy collected following day 14 of experiments showed unhealed and injured cut surface of skin from the untreated control goats (Figure 10C). Thicker scabs over the injured skin were seen in honey treated wounds (H). The scab over the healed tissues was scanty in *Aloe vera* (Figure 10, A) and *Aloe vera* plus honey (Figure 10, A+H) treated skin. Keratinization over the injured skin was seen in *Aloe vera*, honey and *Aloe vera* plus honey treated groups of goats (red arrow). The reactive cells seen in the injured tissues of untreated goats were predominantly neutrophils with fewer fibroblasts. The tissue debris onto the surface of wounds was scanty in Aloe Vera, honey and *Aloe vera* plus honey treated groups. The reactive cells onto the margin of injured skin were mostly macrophages and lymphocytes. Complete keratinization was yet to achieve in any of the other groups of goats following day 14 of experimentation.

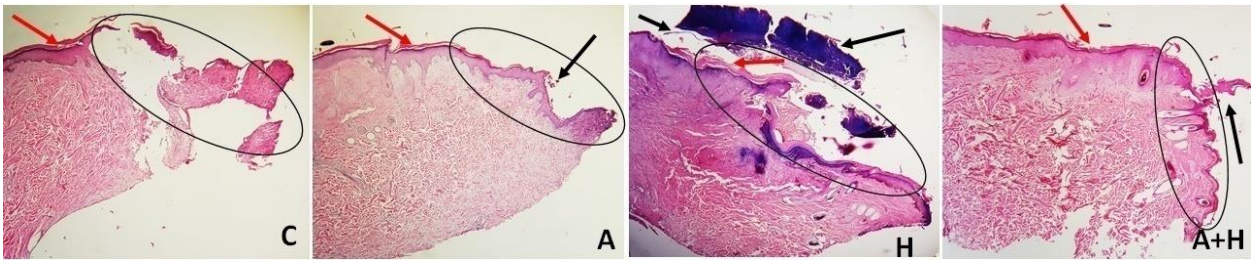


Figure 4. Biopsy of experimental cutaneous wound following day 14 of experimentation

Figures showed biopsy of experimental cutaneous wound obtained from the untreated control (C), wound treated with *Aloe vera* (A), Honey (H) and *Aloe vera* plus honey (A+H) at time of complete wound healing. Tissues of untreated control did not show any tendency of healing until day 21 of wounding. Dermal and epidermal healing in *Aloe vera* treated wound showed a state of complete recovery with scanty of scab (black

arrow) over the injured skin (A, circle) following day 17 of wounding. The dermal and epidermal healing was nearly completed in goats wound treated with *Aloe vera* plus honey (A+H) following day 19 of wounding. The dermal and epidermal healing was not completed in Honey treated wound (H) until day 20 of wounding and there was scab over the injured tissues (black arrow). Red arrow indicates the keratinized epidermal tissue over the injured skin indicating a state of healing. At higher magnification (100x) neutrophilic infiltration was seen in Group C goats until day 21 of wounding. The tissue debris in the scab was minimum in Group A, H and A+ H groups of goats following day 17, 21 and 19 of wounding respectively. There was epithelialization and keratinization along with the healed tissues and under the scab. Epithelialization and keratinization onto the surface of untreated wounds were lacking and the underlying tissues containing tissue debris, neutrophils, and fibrous connective tissues.

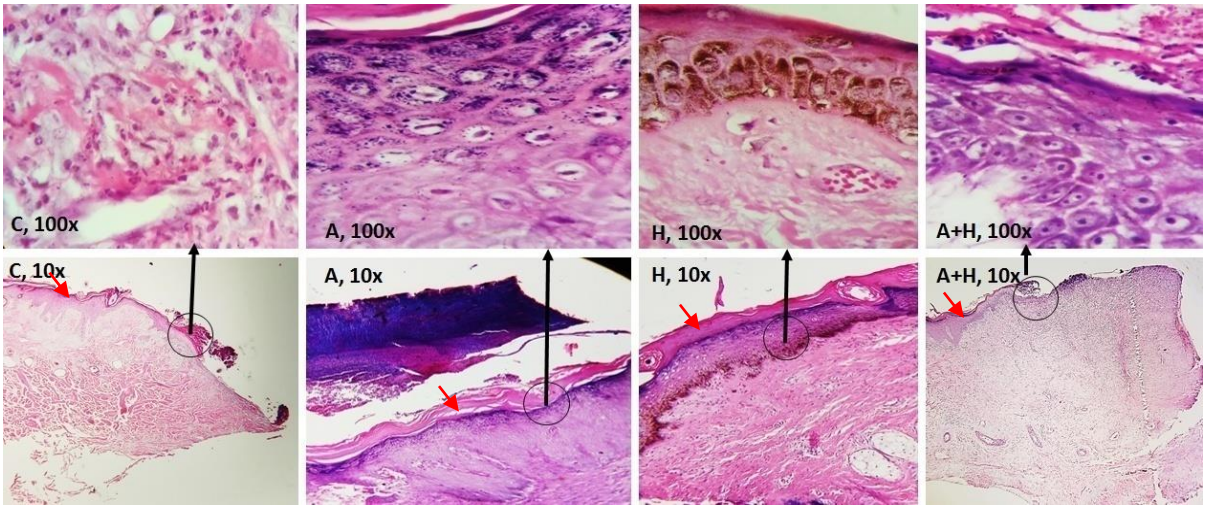


Figure 5. Histoarchitecture of wound biopsy at days of wound healing

Biopsies stained with H&E (4x) and obtained from the untreated control (C) tissues, wound treated with *Aloe vera* (A), Honey (H) and *Aloe vera* plus honey (A+H). Tissues of untreated control skin (C, circle) showed raw surface consisting of fibroblasts and lack of keratinization over the injured skin (C, 10x, black arrow). Arrow (C, 100x) showed the junction of injured skin with huge tissue debris, neutrophils and fibrous connective tissues. Experimental dermal wounds treated with *Aloe vera* (A, circle) showed a state of complete healing with epithelialization (keratinization, red arrow) over the injured skin on day 17 of wounding. Wound (H) treated with honey showed complete epithelialization and keratinization over the injured tissues on day 21 of wounding. The dermal and epidermal healing was nearly completed in goats wound treated with *Aloe vera* and honey (A+H, circle) of day 19 of wounding. Red arrow indicates the keratinized epidermal tissue over the experimental wounding. The biopsies in the upper row (Figure 5) showed tissue response and state of healing at higher magnification (100x).

Discussion

Contraction of wound area and wound healing

Results of this study showed that the wound area of the experimental animals did not increase in size on the post wounding days following treated with *Aloe vera* gel, honey, combined *Aloe vera* and honey topically. Also, the wound did not show the notable signs of inflammation that attributing the anti-inflammatory effects of *Aloe vera* and honey. The finding is in agreement with earlier reporters (Surjushe et al., 2008; Tomblin et al., 2014). *Aloe vera* inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid and thus can inhibit inflammatory process (Surjushe et al., 2008). *Aloe vera* contains substance like enzymes, glycoproteins, growth factors, vitamins and minerals. *Aloe vera* contain glucose-6 –phosphate and manose-6-phosphate (Subramanian, et al., 2006). Among them mannose-6-phosphate is the important structural constituent that plays vital role to promote wound healing through an anti-inflammatory process (Davis, 1994).

Animals were treated with *Aloe vera* gel had a greater wound contraction rate, as well as rapid wound closure followed by combined *Aloe vera*& honey and honey alone treated group. Control group had a highly significant slow wound contraction rate compared to the treated group ($P<0.001$). Previous study showed that *Aloe Vera* gel treated wound had a greater wound

contraction rate with rapid wound closure (Attah et al., 2016). *Aloe vera* gel not only increases the wound collagen rate but also changes the composition of collagen as well as collagen crosslinking. As a result, it accelerates wound healing. Similarly, it has been reported that topical application of *Aloe vera* promote immune system against vast ranges of bacterial infection including *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Mycobacterium tuberculosis, Streptococcus pyogenes, Serratia, and Staphylococcus aureus* (Yari et al., 2018). Contraction of wound depends on myofibroblast proliferation and its subsequent connection to extracellular matrix (Dreifke et al., 2015).

Aloe vera gel significantly increased the rate of wound contraction, epithelialization, and maturation and was pioneer compared to other treatments in promoting healing of cutaneous wound in goat. The study revealed epithelialization onto the dermal tissue at day 14 of wounding. Complete wound contraction and healing was accomplished by day 17 in *Aloe vera* treated group. The report is in compliance with previous researchers (Vázquez, 1996; Oryan 2016; Khorasani et al., 2009). Topical application of *Aloe vera* modulated the inflammation, increased the rate and quality of fibroplasia by enhanced collagen and glycosaminoglycans production, and improved the remodeling stage of the healing tissue (Oryan, 2016).

Acceleration of wound healing through the topical application of honey was found in this study. This result is similar with the findings of previous researchers (Cooper, 2007; Vijaya et al., 2012) who reported that topical use of honey reduces microbial load, formation of thin scar and complete keratinization within 21 days of wounding. Honey has an important role in modulating the inflammatory response through specific pathway and enabling wound healing (Tomblin et al., 2014). In this study, we noticed that the scabs formed by the honey-treated wounds were thinner compared to the other treatment group. Thicker scab was noticed in normal saline treated group followed by *Aloe vera* treated groups. The moist environment created by the honey due to its viscosity and high sugar content found to confer thin scar formation. The thinner scabs form during the healing process appeared as smaller barriers for the epithelialization to occur, and thus accelerate open wound healing (Khoo et al., 2010). Acidification has been shown to promote wound healing by causing oxygen release from haemoglobin during topical application of honey (Leveen et al., 1973). Moreover, different antioxidants present in honey included flavonoids, monophenolics, polyphenolics and vitamin C and these are very vital in tissue regeneration processes (Schramm et al., 2003). Un-boiled commercial honey seems to accelerate wound healing when applied topically due to its energy-producing properties, its hygroscopic effect on the wound, and its bactericidal properties (Bergman et al., 1983). This study also showed a link between honey application and wound healing in experimental trials.

Microbial Load

The antibacterial activity of honey is related with the production of hydrogen peroxide. Honey contains an enzyme called glucose oxidase which breaks down sugars and generates hydrogen peroxide. Hydrogen peroxide is produced in tissues is a kind of bleach, associated with the antimicrobial activity and thus enabling wound healing by combating wound microbes (Mandal and Mandal, 2011; Takzaree et al., 2017; Yaghoobi & Kazerouni, 2013). This study agreed with the findings of Khoo et al., (2010) who mentioned that honey decrease microbial growth over open wound areas thus promote wound healing. Low pH (3.0 to 5.0) and high free acid content of honey may assist wound healing (Medhi et al., 2008).

Biopsy examination

In this study, the reactive cells seen in untreated group following seven days of wounding was mostly neutrophil on the other hand reactive cells mostly seen were macrophage and lymphocytes in *Aloe vera* treated groups. Prakoso et al., (2018) reported that *Aloe vera* may shorten healing period by increasing CD4+ and decreasing CD8+ lymphocytes. CD4+ lymphocytes induce keratinocytes to release IL-1 in the wound area. Keratinocytes have a potential role on epithelization, proliferation, and maturation of epidermis (Lugo, 2011). IL-1 has been released by keratinocytes induces endothelial cells to form angiogenesis and fibroblast to form extracellular matrix (Marmaras et al., 2012). T lymphocytes have an important role in both normal wound healing and in the formation of scars (Martin& Muir, 1990). Enhance rate of lymphocytic infiltration was seen in tissues of honey treated groups.

The experimental wound healing began with the reduced edema, and fibrin clot together with the enhance infiltration of macrophages, fibroblasts, and large blood vessels as observed in the *Aloe vera* treated wounds compared to the controls. This observation suggested that *Aloe vera* could have enhanced the rate and quality of the inflammatory phase of wound healing (Vázquez, 1996). There was an increase in the number of blood vessels in the treated tissues indicated the angiogenic activity of *Aloe vera* at earlier stages of wound healing which may contribute a better perfusion of nutrients and cytokines following enhance circulation in the injured area. Angiogenic activity of *Aloe* vera has

previously been reported by Lee (1995). The *Aloe vera* exerted its beneficial effect by increasing the wound contraction and epithelialization, which resulted in a faster wound closure, and by decreasing the size of scar tissue formation through accelerating the organization and remodeling of tissues at the site of wounding (Oryan, 2016).

Up to now, 75 constituents were found in *Aloe vera* including 20 minerals, 20 kinds of amino acid, 12 kinds of vitamins and water (Jafarzade et al., 2014). This phytogel contains glucomannan, that stimulate receptors of fibroblast growth factor and proliferation which in turn increases the production of Collagen and collagen cross linking. As a result, *Aloe vera* accelerates processes of wound healing by enhancing collagen formation and interfering growth of microbes. There are several experiments that reported *Aloe vera* and its most important component, acemannan promote fibroblasts proliferation. Thus *Aloe vera* may have contributed wound healing by promoting tissue response through the stimulation of insulin-like growth factors II, which binds to the same receptor on fibroblast (Davis et al., 1989).

Honey is reported to have an insulin-like property, stimulate cell proliferation and angiogenesis in wound bed furthermore; thus honey contributed an additional role in wound healing (Takzaree et al., 2017; Yaghoobi & Kazerouni, 2013). Honey had a wider antibacterial activity than *Aloe vera* gel, thus provide better wound healing response in caprine model (Oghenemaro, 2018). This study provide evidence that topical application of Aloe vera alone appeared to show highest response in terms of wound healing process followed by Aloe vera + honey and honey alone respectively.

Conclusion

Experimental dermal wounds treated with *Aloe vera* showed a rapid response of complete healing with epithelialization compared to other treatment groups. However, *Aloe vera* plus honey, *Aloe vera* or honey, any of the formulations can be used in native condition to cure the open wound in goats.

Authors contribution

Rima UK and Islam MI were developed the concept and designed the experiment. Roy KK and Kar M conducted the animal experimentation and performed the laboratory test. Rahman AME contributed to collecting ingredients for the experiment and to record the data. Rima UK and Roy KK evaluated the result, analyzed data statistically and contributed to writing the manuscript. Rima UK and Hossen MI contributed to revising manuscript critically for important intellectual content. All authors read the article and approved the final version to be published.

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Competing interests

The authors have declared no affiliations with or involvement in any organization or entity with any financial and non-financial interest in the subject matter or materials stated in this manuscript.

Ethical statement

All the authors have agreed for authorship, read, and approved the submission for publication. As the study was conducted onto the goats, without involvement of deadly pathogen, and health issue was great concern as live goats were used. The cutaneous wound creation and biopsies were carried out under local anaesthesia with less sufferings as required by the ethical concern, Animal Welfare Committee, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh.

References

Attah, M.O., Jacks, T.W., Jacob, A., Eduitem, O. and John, B. 2016.The effect of *Aloe vera* (linn) on cutaneous wound healing and wound contraction rate in adult rabbits. *Nova Journal of Medical and Biological Sciences*, 5: 3. https://doi.org/10.20286/JMBS-050307Corpus ID: 79030998

Bergman, A., Yanai, J., Weiss, J., Bell, D. and David, M. P. 1983. Acceleration of wound healing by topical application of honey: an animal model. *The American Journal of Surgery*, 145(3): 374-376.

Castillo-Briceño, P., Arizcun-Arizcun, M., Meseguer, J., Mulero, V. and García-Ayala, A. 2010. Correlated expression profile of extracellular matrix-related molecules during the inflammatory response of the teleost fish gilthead seabream. *Developmental & Comparative Immunology*, *34*(10): 1051-1058.

Choudhri, P., Rani, M., Sangwan, R.S. Kumar, R., Kumar, A and Chhokar, V. 2018. De novo sequencing, assembly and characterisation of Aloe vera transcriptome and analysis of expression profiles of genes related to saponin and anthraquinone metabolism. *BMC Genomics* 19, 427. https://doi.org/10.1186/s12864-018-4819-2

Cooper, R., 2007. Honey in wound care: antibacterial properties. *GMS Krankenhhygiene Interdisziplinar*, 2(2): Doc51. PMID: 20204083; PMCID: PMC2831240.

Dat, A.D., Poon, F., Pham, K. B. and Doust, J. 2012. Aloe vera for treating acute and chronic wounds. *The Cochrane Database of Systemic Review,* 15(2):CD008762. https://doi.org/10.1002/14651858.CD008762.pub2

Davis, R.H., Leitner, M.G., Russo, J.M. and Byrne, M.E. 1989. Wound healing. Oral and topical activity of Aloe vera. Journal of the American Podiatric Medical Association. 79(11):559-62. https://doi.org/10.7547/87507315-79-11-559

Davis, R. H., Donato, J., Hartman, G. M. and Haas, R. C.1994. Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera*. *Journal of the American Podiatric Medical Association,* 84(2): 77-81.

Dorai, A.A. 2012.Wound care with traditional, complementary and alternative medicine. *Indian Journal of plastic surgery*, 45(2):418-24. https://doi.org/10.4103/0970-0358.101331

Dreifke, M.B., Jayasuriya, A.A. and Jayasuriya, A.C. 2015. Current wound healing procedures and potential care. *Materials Science and Engineering: C*, 48(1):651-62. https://doi.org/10.1016/j.msec.2014.12.068

Gethin, G.T., Cowman, S. and Conroy, R.M. 2008. The impact of Manuka honey dressings on the surface pH of chronic wounds. International Wound Journal,5:185–194. https://doi.org/10.1111/j.1742-481X.2007.00424.x

Gonzalez, A.C., Costa, T.F., Andrade, Z.A. and Medrado, A.R. 2016. Wound healing - A literature review. *Anais Brasileiros de Dermatologia*, 91(5):614-620. https://doi.org/10.1590/abd1806-4841.20164741

Guo, S. and Dipietro, L. A. 2010. Factors affecting wound healing. *Journal of Dental Research*, 89(3):219-29. https://doi.org/10.1177/0022034509359125

Hosseinkhani, A., Falahatzadeh, M., Raoofi, E. and Zarshenas, M. M., 2017. An Evidence-Based Review on Wound Healing Herbal Remedies From Reports of Traditional Persian Medicine. *Journal of Evidence-based Complementary & Alternative Medicine*, *22*(2): 334–343.

Jafarzade, H., Arabi, M., Najafi, N. and Ahadi, A. 2014. Effect of *Aloe vera* gel on TGF-β gene expression in incisional skin wound in BALB/c mice. *Journal of Gorgan University of Medical Science*, 16(3): 16-23.

Khan, F.R., Ul Abadin, Z. and Rauf N. 2007. Honey: nutritional and medicinal value. *International Journal of Clinical Practice,*61(10):1705–1707.

Khoo, Y.-T., Halim, A. S., Singh, K.-K.B. and Mohamad, N. A. 2010. Wound contraction effects and antibacterial properties of Tualang honey on full-thickness burn wounds in rats in comparison to hydrofibre. *BMC Complementary and Alternative Medicine,* 10(1): 48.

Khorasani, G., Hosseinimehr, S. J., Azadbakht, M., Zamani, A. and Mahdavi, M. R., 2009. Aloe versus silver sulfadiazine creams for second-degree burns: A randomized controlled study. *Surgery Today*, 39(7):587-591. https://doi.org/10.1007/s00595-008-3944-y

Lee, M.J., Yoon, S.H., Lee, S.K., Chung, M.H., Park, Y.I., Sung, C.K., Choi, J.S. and Kim, K.W. 1995. In vivo angiogenic activity of dichloromethane extracts of Aloe vera gel. *Archives of Pharmacal Research*, 18:332-335

Leveen, H. H., Falk, G., Borek, B., Diaz, C., Lynfield, Y., Wynkoop, B. J. and Christoudias, G. C.1973. Chemical acidification of wounds.An adjuvant to healing and the unfavorable action of alkalinity and ammonia. *Annals of Surgery,* 178(6): 745.

Lindsey, K.L., Jäger, A.K. and Viljoen, A.M. 2002. Cyclooxygenase inhibitory activity of Aloe species. *South African Journal of Botany*, 68: 47–50

Lugo, L. M., Lei, P. and Andreadis, S. T. 2011. Vascularization of the dermal support enhances wound re-epithelialization by in situ delivery of epidermal keratinocytes. *Tissue Engineering Part* A, 17(5-6): 665-675.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw Hill Book Co., New York, USA

Mandal, M.D. and Mandal, S. 2011. Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(2):154-60. https://doi.org/10.1016/S2221-1691(11)60016-6

Marmaras, A., Lendenmann, T., Civenni, G., Franco, D., Poulikakos, D., Kurtcuoglu, V. and Ferrari, A. 2012.Topography-mediated apical guidance in epidermal wound healing. *Soft Matter,* 8(26): 6922-6930.

Martin, C. and Muir, I.1990. The role of lymphocytes in wound healing. *British Journal of Plastic Surgery,* 43(6): 655-662.

Medhi, B., Puri, A., Upadhyay, S. and Kaman, L. 2008. Topical application of honey in the treatment of wound healing: a metaanalysis. *Journal of Medical Education & Research,* 10(4): 166-169.

Minden-Birkenmaier BA and Bowlin GL. 2018. Honey-Based Templates in Wound Healing and Tissue Engineering. *Bioengineering (Basel),* 5(2):46. https://doi.org/10.3390/bioengineering5020046

Molan, P. C. 2002. Re-introducing honey in the management of wounds and ulcers – theory and practice. *Ostomy Wound Manage*, 48(11):28–40.

Oghenemaro, E. F., Johnson, J., Itohan I.M., Richard, S. and Michael, O. 2018. Antimicrobial activity of aloe vera gel and honey against bacteria isolates from wound aspirates. *International Journal of Pharmaceutical Sciences and Research*, 9(11): 4890-4893.

Opneja, A., Kapoor, S. and Stavrou, E.X. 2019. Contribution of platelets, the coagulation and fibrinolytic systems to cutaneous wound healing. *Thrombosis Research*,179:56-63.

Oryan. A, Mohammadalipour, A., Ali, M. and Tabandeh, M.R.2016. Topical Application of Aloe vera Accelerated Wound Healing, Modeling, and Remodeling. *Annals of Plastic Surgery*. 77(1):37-46.

Pereira, R. F., Barrias, C. C., Granja, P. L. and Bartolo, P. J. 2013. Advanced biofabrication strategies for skin regeneration and repair. *Nanomedicine,* 8(4): 603-621.

Prakoso, Y. A., SetiyoRini, C. and Wirjaatmadja, R., 2018. Efficacy of *Aloe vera*, Ananascomosus, and Sansevieriamasoniana Cream on the Skin Wound Infected with MRSA. *Advances in Pharmacological Sciences*, Apr 19;2018:4670569. https://doi.org/10.1155/2018/4670569

Radha, M.H. and Laxmipriya, N.P. 2015. Evaluation of biological properties and clinical effectiveness of Aloe vera: a systematic review. *Journal of traditional and complementary medicine*, 5(1):21–6

Saha, S., Malaker, R., Sajib, M., Hasanuzzaman, M., Rahman, H., Ahmed, Z. B., Islam, M. S., Islam, M., Hooda, Y., Ahyong, V., Vanaerschot, M., Batson, J., Hao, S., Kamm, J., Kistler, A., Tato, C. M., DeRisi, J. L., and Saha, S. K. 2020. Complete Genome Sequence of a Novel Coronavirus (SARS-CoV-2) Isolate from Bangladesh. *Microbiology Resource Announcements,*9(24), e00568-20. https://doi.org/10.1128/MRA.00568-20

Schramm, D. D., Karim, M., Schrader, H. R., Holt, R. R., Cardetti, M. and Keen, C. L. 2003. Honey with high levels of antioxidants can provide protection to healthy human subjects. *Journal of Agricultural and Food Chemistry*, 51(6): 1732-1735.

Steinhorn, G., Sims, I.M., Carnachan, S.M., Carr, A.J. and Schlothauer, R. 2011. Isolation and characterisation of arabinogalactan-proteins from New Zealand kanuka honey. *Food Chemistry,*128(4):949–956.

Subramanian, S., Kumar, D.S., Aruselvan, P. and Senthikumar, G.P. 2006. In vitro antibacterial and antifungal activities of ethanolic extract of aloe vera leaf gel. *Journal of Plant Sciences*. 1(4): 348-355. DOI: 10.3923/jps.2006.348.355

Surjushe. A., Vasani, R. and Saple, D.G. 2008. *Aloe vera*: a short review. *Indian Journal of Dermatology*. 53(4):163–166.

Takzaree, N., Hassanzadeh, G., Rouini, M. R., Manayi, A., Hadjiakhondi, A. and Zolbin, M. M. 2017. Evaluation of the effects of local application of thyme honey in open cutaneous wound healing. *Iranian Journal of Public Health*, 46(4): 545-551.

Tomblin, V., Ferguson, L.R., Han, D. Y., Murray, P. and Schlothauer, R. 2014. Potential pathway of anti-inflammatory effect by New

Zealand honeys. *International Journal of General Medicine*, 7: 149–158.

Vázquez, B., Avila, G., Segura, D. and Escalante, B. 1996. Antiinflammatory activity of extracts from *Aloe vera* gel. *Journal of Ethnopharmacology,* 55(1): 69-75.

Vijaya, K. K. and Nishteswar, K. 2012. Wound healing activity of honey: A pilot study. *Ayurveda*, 33(3): 374.

Wynn, R.L. 2005. Aloe vera gel: Update for dentistry. *General Dentistry*,53(1):6–9

Yaghoobi, R. and Kazerouni, A. 2013. Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: A review. *Jundishapur Journal of Natural Pharmaceutical Products,* 8(3): 100-4.

Yari, R., Khodadadi, I., Aliyari, F. and Saremi, Z. 2018. Influences of combining nano zinc, honey and *Aloe vera* to accelerate healing the wounds caused by third–degree burn in male balb/c mice. *Journal of Basic Research in Medical Science,* 5(1):38-46.