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Research Article

**ASH AS A HEALING MEDIATOR**<sup>1</sup>Hafiz Ahmad Jalal, <sup>2</sup> Hafiz Ahmad Bilal, <sup>3</sup> Hafiz Ahmad Kamal, <sup>4</sup> Ayesha Khalid<sup>1</sup>Avicenna medical college Lahore, Pakistan<sup>2</sup>Mohiuddin Islamic medical college Mirpur khas, Azad Kashmir<sup>3</sup>Avicenna medical college Lahore, Pakistan<sup>4</sup>Frontier medical college Abbottabad, Pakistan**Abstract:**

**BACKGROUND:** Rabbit skin wound model was used for the assessment of the ash as a natural healer of the wounds in this research.

**METHODS:** The production of the ash is made through dried buffalo dung, charcoal and wood and analysis of their contents was made through an atomic absorbed spectrophotometry. We used every type of ash in the wound of rabbit skin as an ointment and assessed the healing process in thirteen days.

**RESULTS:** Our research observed a consistent healing process in experimental wounds more rapid than the sites of control wound. On the eleventh day the healing was complete (charcoal ash) and thirteenth day (wood and dung cake ash).

**CONCLUSION:** A unique property of the ashes is their influence on the sepsis-free and safe healing of the wound in the used model of rabbit skin in this research.

**Keywords:** Ash, Skin Fibroblasts, Rabbit, Anti-bacterial and Ointment.

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**INTRODUCTION:**

Injured tissues can heal with an orderly development of healing process. Issue of wound healing is as old as old is the history of human. Natural products were used in primitive times; the medicines used by those primitive people are objected and challenged by the modern science. At present, the satisfactory medication of the treatment of wounds is still an issue and dilemma. Various ointments and medicines have been introduced to heal humans in wounds and cuts [1].

Healing process is dependent on the suitable combination of the tracing elements acting as cofactors of enzymes, increase the components structure and also repair the damaged tissue, ash provides a suitable combination. It has been proved that certain metals when combined in ash become a quick remedy for the healing of cuts and bruises. If the wound is covered with ash, it gives humid and natural environment to wound and accelerates the wound repair process which is even faster than the advanced medications [2]. The topical application of Bentonite has the ability of various metals to repair the tissue and cofactors of enzymes which are required for wound treatment.

**MATERIAL METHODS AND ANIMALS:**

A rabbit weighing 1000 – 1250 grams was taken for the research purpose, this animal was grown in controlled environment including temperature as (30 + 5°C), humidity as (55% – 60%) and dark cycle of twelve hours as provided in the guidelines of international institutions (AAALAC, 1996). Individual animal was kept in a cage of stainless steel. Fresh tap water and alfalfa was given to these rabbits.

**ASHES:**

After the burning process all the three various ash types were collected including wood chips (dead branches), buffalo dried dung-cake (as produced in the nearby country side) and charcoal (locally supplied).

**ANALYATE:**

We dried the ashes in oven at a temperature of 105°C. Replicate 1.9 – 2.0 grams dried ash samples were noticed for their weight and preserved them in 100 milliliter conical flasks, also processed with nitric acid 5.0 milliliters (added to flask when it was empty). Watch glass covering was placed on the flask and heated through electrical plate for refluxing up to one hour. Another nitric acid five milliliters were treated, two milliliters (35%) hydrogen peroxide was mixed and gentle heating reflux was carried out for

one more hour, volumes were reduced and remaining quantity was 2 – 3 milliliters. Contents were cooled, de-ionized water dilution was done and filtered with the help of What Man No 42 paper, volume was secured through de-ionized water and assessed through an atomic absorption spectrophotometry levels of copper, calcium, manganese, magnesium, iron, potassium, sodium and zinc.

**EXPERIMENTAL DETAILS:**

Surgical ten-millimeter-long incisional full-thickness skin wounds were made with the help of scalpels (No 15) at rabbit dorsal skin which was shaved closely under local anesthesia conditions. Various wounds were color coded by various color markers. Two wounds were measured for the quantity of ash on cranial side an also caudal side wound, other wounds were covered with antibacterial ointment of skin. We filled the wounds with ash and left them for intentional healing process. No dressing was carried out and wounds were open in air. We took the day of operation as reference. Sacrifice of the rabbits was made on day – 1, day – 3, day – 5, day – 7, day – 9, day – 11 and day – 13 after wounding the rabbits. Wound including 4 – 5 millimeters area was lifted through excision and kept in the containers already marked having ten percent formaldehyde as preservative for eosin and hematoxylin histology and staining.

**HISTOLOGY AND MICROPHOTOGRAPHY:**

We adopted manual procedure for the processing of formaldehyde-preserved tissue, which includes dehydration, impregnation, clearing, cutting and embedding followed by section staining carried on the alternate days, we also carried out microscopic pathology.

**STATISTICAL ANALYSIS:**

SPSS – 11 was used for the statistical analysis of the data, Chi Square test and Post ANOVA Hoc were also applied with significant p-value as (0.01).

**RESULTS:**

Day to day physical assessment of the wounds was carried out and followed as given:

**Day One**

No changed was observed persistent gaping was there. However, thick ash crust was visible on the wound surface. Both control and experimental groups showed no change. We observed 3 – 4 mitotic figures in dermal fibroblasts only in the test sites; whereas, no activity was observed in the control. Both the types of wounds control and test we observed number of polymorph nuclear and erythrocytes leukocyte

infiltrates showed inflammatory phase beginning as shown in the figures.

#### Day Three

Wounds were reduced by two millimeters that means from ten to eight millimeters. This change was not observed in the control group. Heavy PMNs and monocytes were observable at sites; however, the it was revealed through test sites about the numerous eosinophils. We also observed increased fibroblasts at test sites.

#### Day Five

Control wound surface was dried and leathery; whereas, ash crust was visible on the test wound decreased by six millimeters. Hardening and Crusting of wound surface was also seen with the loss of progression of the ash crust which also followed growth of the hair on the site of the test.

#### Day Seven

There was a decrease in control wound which was measured as 8 millimeters whereas much decrease was observable at the site of test. Ash thin crust was extruded from test site and dermal fibroblasts were observed with number of mitotic figures with ground matrix forming. Healthy tissue granulation was observed in test and control wound.

#### Day Nine

Another one-millimeter decrease was observed in control wound but scar protrusion was observed more than the actual margins of the wound. It was revealed through the site of the test that status quo in physical shape but through microscopic appearance of the

fibroblasts was mutating in to my fibroblasts as shown in Figures 1, 2, 3 & 4.

#### Day Eleven

Obvious inflammatory decrease was observed in test wound in terms of healing, cater formation was also observed near margins of the wounds. Debris epidermal realignment of the wound was by a retia formation, pit-like configuration and hyperkeratosis as shown in Figure 1, 2, 3 & 4. It was revealed through gross assessment that growth of hair was observable except near the wound. No signs of panniculus carouses muscle repair were there but a disposition of the collagen was observed (pale staining). Count of the cells was 2686 fibroblast / mm<sup>2</sup> through grid and reticule method observed maximum in terms of test wounds.

#### Day Thirteen

We observed pinkish skin on the granulation tissues; however, complete hairs were on the sites of the wound. Almost perfect scar healing was observed through histological appearance in control and test wound. However, the healing was delayed by two to three days in the controls. We observed on wounds a complete lining epidermis normalization; however, scanty monocytes and PMNs were still observable on the deep dermis layer and in and around panniculus carouses muscle. On the 13<sup>th</sup> day six percent decrease was observed in the cell count and count was 2543 (previously 2686). The reason cannot be tracked, more work is required to probe this reason as shown in Figure 1, 2, 3 & 4.

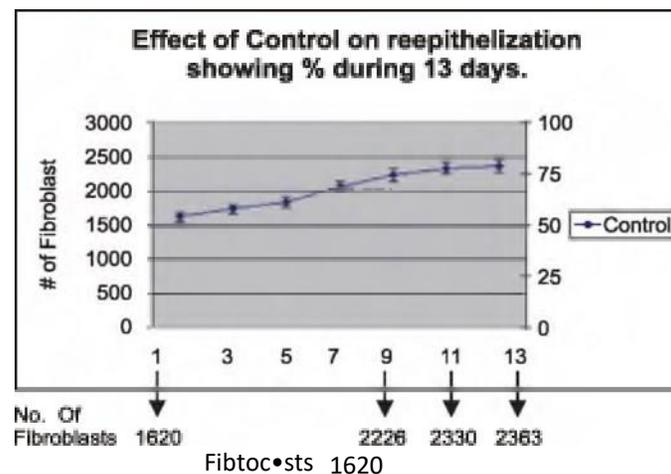


Figure 1. (Indicates the specific day post wounding)

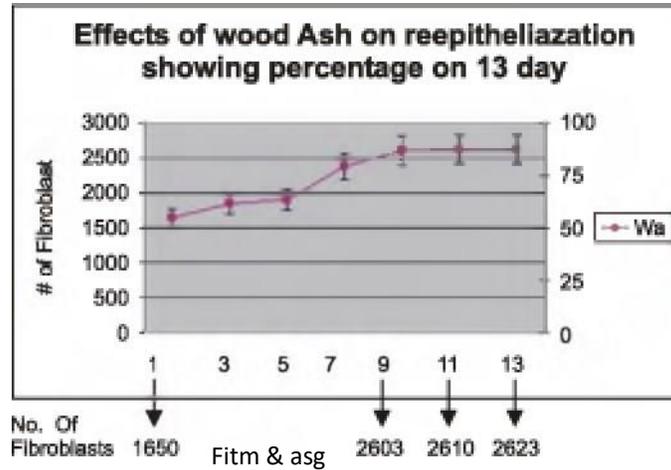


Figure 2. (Indicates the specific day post wounding)

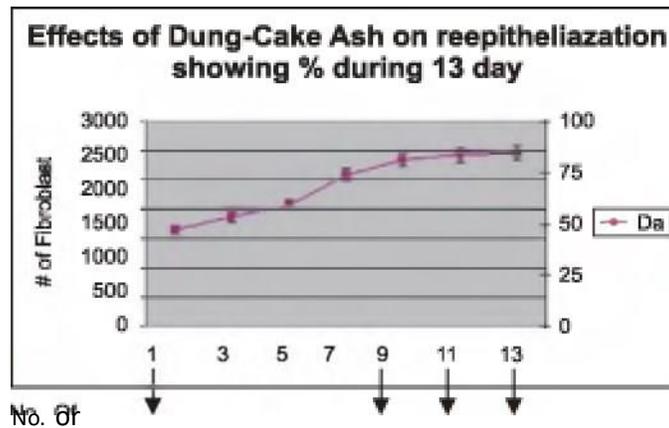


Figure 3. (Indicates the specific day post wounding)

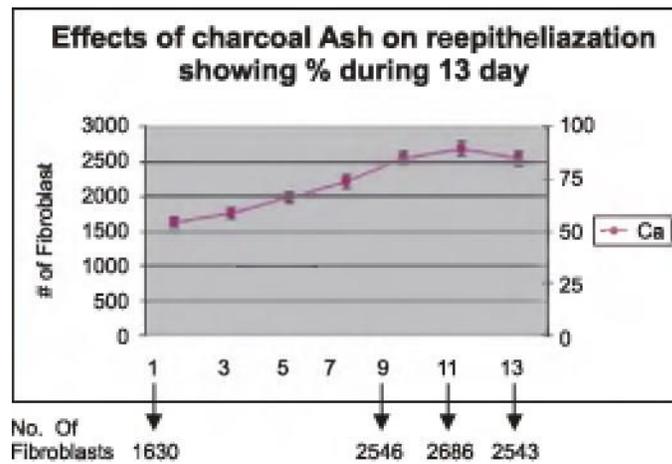


Figure 4. (Indicates the specific day post wounding)

### HISTOLOGICAL FEATURES OF DAY13

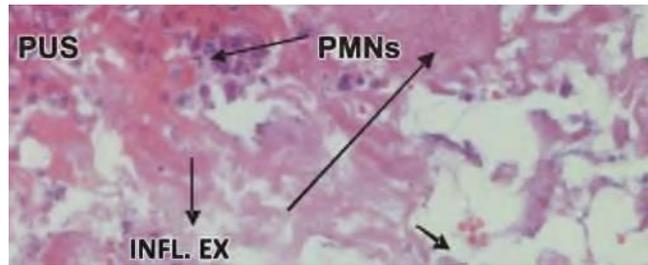


Fig – 5a: Extensive inflammatory exudates with number of polymorph nuclear (PMN) cells, Inflammatory exudates and RBCs

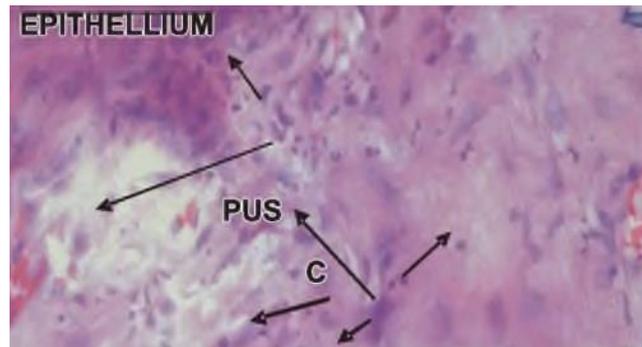


Fig 5b: Edematous epithelium on upper left hand just under the persistent foci of pus and cellular debris. Few vesicular my fibroblast (MF) nuclei are observable along with collagen (C)

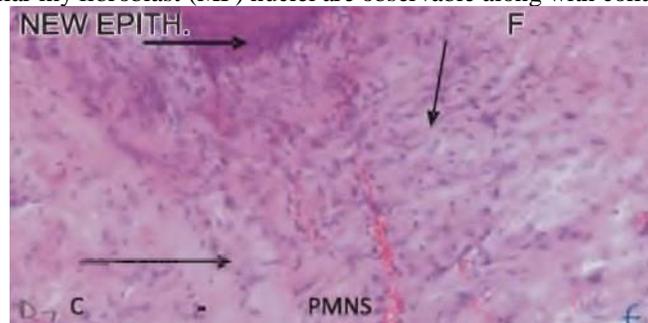


Fig 5f: Reveals an established epithelium with a few fibroblasts (F), my fibroblasts (MF) and PMNs with added signs of Extensive collagen (C) deposition.

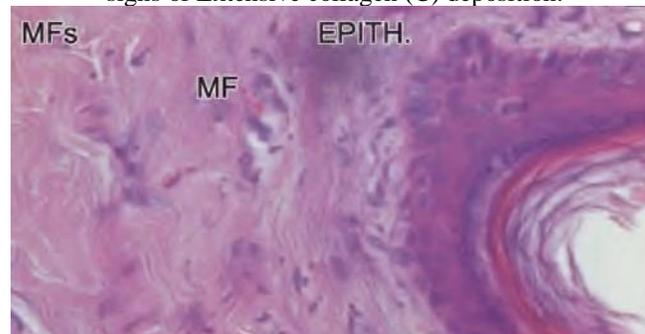


Fig 5h: Almost competently reconstituted epidermis and dermis with hyaline keratin (K) presence, numerous my fibroblasts (MF) and mature collagen (C).

**DISCUSSION:**

In the availability of modern medicine, the complete healing of the wounds is a challenge. We surgically made wounds on the rabbit skin and histological and physical assessments were made accordingly. We assessed a continuous healing process in the test and control wound sites [3].

On the 3<sup>rd</sup> day fibroblasts count was assessed which has been compared with the electric current application which is among the modern methods he 7<sup>th</sup> day results were the same [4]. Ash was superior in its healing features. We found our outcomes same as observed by another author Mohit (2008). Undoubtedly, treatment of the wound through electric application is valuable but no definite [5]. These wound matrix fibroblasts for the cells named as “my fibroblasts,” which concentrate the area of the wound as we observed it on the ninth day of the assessment [6]. Restoring the anatomic extra cellularity was gained through Collagen deposition, same was observed on the eleventh day. Local skin wound healing process recruitment of fibroblasts is from intact adjacent skin dermis to inflammation site [7]. My fibroblasts conversion from fibroblasts is managed through mechanical microenvironment of dermal healing of the wound in the later phases [8]. Our observation may also bring a breakthrough related to the basic wound healing biology along with the healing quality. It is revealed through micro technological advancements that PMNs migration to skin lesions induces a large program of transcriptional activation which regulates the cellular function, fate and also promotes the healing of the wound. We also observed these cell on 1<sup>st</sup> and 3<sup>rd</sup> day in the experimental and control wounds [9].

Sabine (2007) states that repair of wounds in mammals is optimized through dirty condition and where rapid inflammatory responses and multiple redundant compensatory are required. Same notion cannot be implemented in this research as no organic materials are present in the ash [9]. However, centellaasiatica and Aloe Vera, various botanical treatments are widely used for the curative purposes from decades for the repair of wound [10]. Healing confirmation is not available except their biochemical features such as benefits and safeties, advice is also extended to avoid these plants. Our research observed that ash can heal the wounds with effective, cost effective and in a timely manner [11].

Another recommended natural medicine is honey (Prophet PBUH). A regular increase in my fibroblasts and fibroblasts, as a critical healing process component, also promotes physiological repair of the tissue. Through the application of various ashes healing becomes cost effective, rapid and effective [12]. Essential tracing elements are the part of the

ashes its deficiency can lead to various complications on the sound site and in the delayed healing process. Hinz (2007), is of the view that variation from fibroblasts to my fibroblasts is a key marker for tissue repair and healing of the wound [13]. The high my fibroblasts contractile force favors remodeling of the physiological tissue. Healing is an incident of opportunity and time. Possibly, as we observed ashes are productive for the healing of the wounds as through a chemical environment.

**CONCLUSION:**

It is concluded that balanced trace elements are observed in the dried dung-cake of the buffalos and their burnt dried ash can be used as the natural remedy to heal the wound sites. However, more biophysical and pharmacological research studies are required for the establishment of functional and effective significance.

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