WOOD ASH AND CHARCOAL ASH, AN INSTRUMENT TO SKIN TISSUE REPAIR IN ACUTE INJURY IN RABBIT MODEL

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Background: The topical administration of ash along with local defence mechanism appears to be superior to other types of therapies as it reduces super infection and enhance epithelization in rabbit skin wound healing. **Method:** Both wood ash and charcoal ash were prepared and collected by complete burning of the parent material i.e. wood chips and dead branches of wattle (Acacia) and charcoal from local supplier respectively. Ashes were analysed and applied besides the Polymyxin B-Bacitracin Zinc ointment as control. **Results:** Healing by both wood ash and charcoal ash (Ca) were significantly better and faster in comparison to control. Healing by wood ash (Wa) was completed by day 13. Some fall in number of fibroblasts were observed in case of charcoal ash after 11th day. **Conclusion:** Ashes accelerated the wound healing process and gave maximum cover as antiseptic and anti infective. Complete recovery was observed within a period of 11th to 13th day.
Keywords: Wood-ash, charcoal ash, skin, Polymyxin B-Bacitracin Zinc.

INTRODUCTION

Since the earliest times in the history of human kind the traditional unscientific and unproven clinical trials of different types of mixtures have been used successfully.[1] This recommends developing a trust in the healing power of nature. The traditional practice of using oils[3] ashes and clay[4] etc is being questioned because these materials have not been scientifically proven and reported. In the present study both ashes were applied to asses the effectiveness of treatment. In addition to traditional agents *viz* Zinc oxide and cod liver oil are practiced as modern alternatives to conventional wound treatments and wound dressings.[3] The choice of Zinc applied topically is being used as it works more effectively.[2,5] To elaborate these claims the study was designed as an a experimental work on the ashes applied to evaluate the speed of wound healing in comparison to application of Polymyxin B-Bacitracin Zinc ointment. Healing process in the surgical cut in full-thickness skin wounds include contraction, formation of granulation tissue as well as re-epithelialization. The knowledge of regulation of the proliferative tissue response allows the development of optimize treatment regimes which accelerate the wound healing process and give maximum cover as antiseptic and anti infective by topical wound application.

In the present study the use of ashes gives broad-based cover as antiseptic and antibiotic successfully to regulate the proliferative phase without any unusual activity. Epidermal cells successfully started dividing after the lag period of seventy two hours at the edges of wound areas and margins gradually contracted decreasing wound surface and ultimately sealed off on the 13th day. All rabbits survived until the end of the study with no signs of pain or discomfort.

MATERIAL AND METHODS

Ash collection and analysis

Both wood ash and charcoal ash were prepared and collected by complete burning of the parent material, i.e., wood chips and dead branches of wattle (Acacia) and charcoal from local supplier respectively. Ashes were analysed by first drying them at 105 ºC in the hot air oven. Replicate 1.9 gm to 2.0 gm samples of dried ash were weighed in to 100 ml conical flasks and treated with 5.0 ml of nitric acid. 5.0 ml of nitric acid was also added to an empty flask. The flasks were covered with watch glasses and their contents were heated to reflux gently on an electrical plate. After refluxing for one hour the contents of the flasks were treated with 5.0 ml more of nitric acid, 2.0 ml of 35% hydrogen peroxide was added and the heating at gentle reflux was continued for another hour. The watch glasses were removed from the flasks and the heating was continued until the volume of their contents was reduced to 2–3 ml. The contents of the flasks were cooled, diluted with de-ionized water and filtered through Whatmann #42 paper into 25.0 ml volumetric flasks and brought to volume with de-ionized water and examined by Atomic Absorption Spectrophotometry (Hitachi Model 180-50) for the Sodium, Potassium, Calcium, Magnesium, Iron, Zinc, Manganese and Copper levels. (Table-1 and 2).

Animals

Adult wild type rabbits (1,000–1,250 gm body weight) were used in this study. They were bred in the animal house of Isra University to maintain same breed. They were housed under controlled

conditions of 30±5 ºC, 55–60% relative humidity and 12 hour day/night cycles as specified in the (AAALAC international institutional animal care and use program guide 1996). The animals were housed individually in stainless steel cages. Alfalfa fresh and tap water were provided ad libatum.

Experimental details

Incesional full thickness skin wounds (10 mm long) were made surgically with #15 scalpels in the closely shaved dorsal skin of rabbits under conditions of local anaesthesia. The rabbits were shaved on the dorsal aspect of their skin after clipping the dense hair. A centimetre scale and skin pen were used to mark four sites in the cranio-caudal direction, two on each side of the spine, each was two centimetre distant on the contra-lateral side and also on the ipsilateral aspect. The area was disinfected using spirit as standard disinfectant. The rabbits were injected with 1.0 mg/ml diazepam IM for relaxation and sedation and the previously marked sites were anesthetized using 2% Lignocaine+0.00001% adrenaline (w/v) taken in 1.0 cc syringes having needle gauge of 29. Each site of proposed incision was injected with this local anaesthetic subcutaneously taking precaution that none of the deeper structures or blood vessels was penetrated. The rabbits were allowed 10 minutes per animal for the drugs to take effect. The incision on each site was made as cleanly as possible and deep enough to cut through the panaculus carosus muscles however the sub-cutaneous tissue covering the lumbo-dorsal fascia was spared. Four different coloured permanent markers were used to colour code the different wounds by smudging the hair on cranial aspect of each wound. Both ashes were smeared on the two different wounds on the cranial side and one wound on the caudal side, the remaining wound was covered liberally with Polymyxin B-Bacitracin Zinc ointment. The ashes were deliberately filled into the wounds, so that the cut edges could not be approximated and the wounds were left to heal by secondary intention. The wound in every case was left open to air and without any dressing material to access the barrier function. Taking the operation day as zero (d 0), three rabbits for each ash were sacrificed on days 1, 3, 5, 7, 9, 11 and 13 post wounding. The entire wound area along with 45 mm of the surrounding skin was lifted by excision and placed within previously marked containers, in 10% formaldehyde as preservative for haematoxylin and eosin staining and histology.

Histological procedure and microscopy

The manual procedure was adopted to process the formaldehyde preserved tissue. Which include dehydration, clearing, impregnation, embedding, cutting followed by staining of section carried out on alternate days.

RESULTS

Physical and microscopic examination of post wound sites:

By day 1

There was no change in the control wound which was gapping. However the test sites showed a thin crust of ashes on the wound surfaces. No decrease in wound area was observed at this stage in both control and experimental wounds. Three to four mitotic figures were observed in dermal fibroblast in the test sites only whereas control wound showed no such activity.

In both types of wounds (i.e., test and control) there is presence of numerous RBC and polymorphonuclear (PMNs) leukocyte infiltrates which indicates beginning of inflammatory phase.

By day 3

The test wounds have reduced in length from 10 mm to 8 mm, this decrease was not evident in control wound. Both wound sites still show heavy PMNs and monocytes however the test sites reveal presence of numerous Eosinophils. The increase begins in the number of fibroblasts is also witnessed at the test sites at this time.

By day 5

The surface of the control wound appears leathery and dried out where as the test wounds still retain a crust of ash on there surface, which have now decreased to 6 mm in length. Crusting and hardening of the wound surface is noted in the test wounds with progressive loss of superficial ash crust followed by prominent hair growth in the test sites.

By day 7

The control wound measures 8 mm from the original 10 mm but the test sites have decreased another one mm, i.e., a total of 5 mm or half the initial size. The thin crust of ashes has been extruded from the test sites and the dermal fibroblasts show numerous mitotic figures with appearance of ground matrix being laid down.

Both the control and test wound show presence of healthy granulation tissue.

By day 9

The control wounds have decreased by another 1 mm but there appears to be protrusion of the scab above the actual wound margins. The test sites reveal a status quo in physical appearance but in microscopic appearance the fibroblasts appear to be mutating into myofibroblasts.

By day 11

Healing in the test wound from day 11 on words was marked with obvious signs of decrease in inflammatory cells in and near the wound margins with a very pronounced reduction in the crater formation and wound debris. Epidermal realignment is by a pit like configuration, retae formation and hyperkeratosis is visible.

The gross examination revealed a very transparent pinkish skin with furry appearance (Hair) on the entire surface of wounds except the very centre which still exhibits reddish pale granulation tissues. There is no evidence of repair of panaculous carosus muscle but collagen deposition is seen (pale staining). The cell count by reticule and gird method revealed 2686 fibroblast/mm^2, this number was maximum for test wounds.

By day 13

By this time the control wound displayed a thin pinkish skin over the granulation tissue however the test sites have now been completely covered by hair bearing skin. The histological appearance of both to test and control wounds was characteristic of near perfect healing. However there was a lag of 2–3 days for the control sites.

The test wounds showed complete normalization of the lining epidermis however there was still presence of scanty Monocytes and PM Eosinophils in the deeper layer of dermis and around and in the panaculous carosus muscle.

The control wound displayed the same configuration but with the note worthy exception of presence of foci of PMNs and cellular debris underneath the completely reconstituted epidermis which for the most part was oedematous.

In the case of charcoal application the maximum number of 2686 fibroblast was observed on 11th day. There after by 13th day the number was decreased by about 6% that is up to 2543. The reason is not known and is to be worked out. (Figure-2 and 4). However in case of wood ash application the maximum number of fibroblasts was observed on day 13 (Figure-3 and 5).

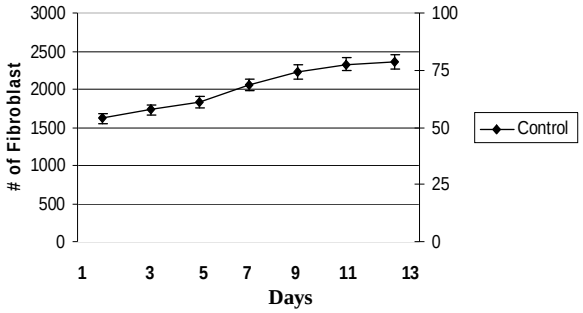

Figure-1: Effect of Polymyxin B-Bacitracin Zinc ointment on re-epithelization

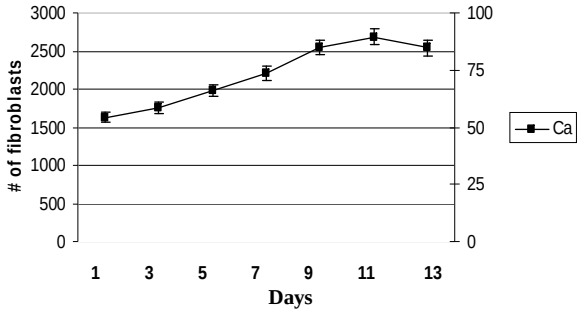

Figure-2: Effect of Charcoal ash on re-epithelization

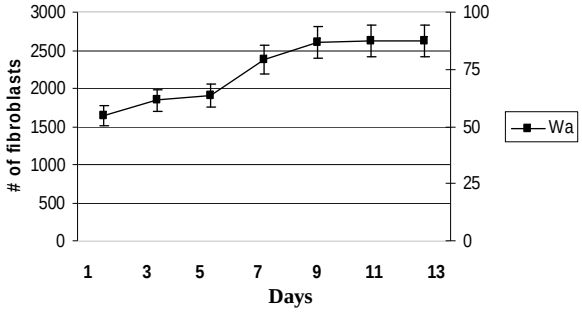

Figure-3: Effect of wood ash on re-epithelization

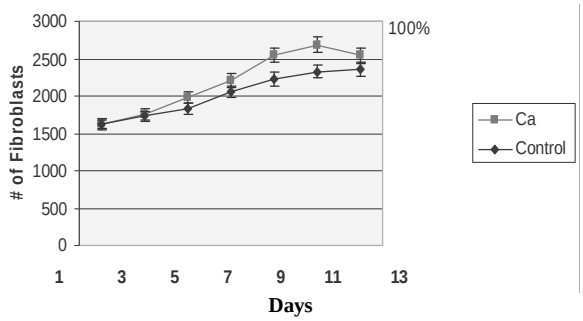

Figure-4: Comparison of Effect of Charcoal ash with control on re-epithelization

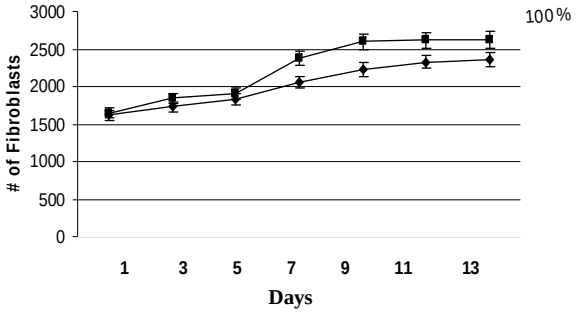

Figure-5: Comparison of Effect of Wood ash with control on re-epithelization

Table-1: Relative concentrations (ppm) of trace elements in charcoal Ash

Na+	K+	Ca++	Mg++	Fe++	Zn++	Mn++	Cu++
0.25	1.0	5.0	1.0	1.0	0.5	1.0	0.7

Table-2: Relative concentrations (ppm) of trace elements in wood Ash

Na+	K+	Ca++	Mg++	Fe++	Zn++	Mn++	Cu++
0.25	1.2	4.9	1.95	0.9	0.25	1.3	0.32

Table-3: Showing comparative effect of Polymyxin B-Bacitracin Zinc ointment and Charcoal ash on skin healing after 13 days

Application of treatment	Day 1	Day 11	Difference	Recovery %
Control group No. of Fibroblasts	1620	2330	756	
Experimental group (Ca) No. of Fibroblasts	1630	2686	1056	71.6

Table-4: Showing comparative effect of Polymyxin B-Bacitracin Zinc and wood ash on skin healing after 13 days

Application of treatment	Day 1	Day 13	Difference	Recovery %
Control group No. of Fibroblast	1620	2363	743	
Experimental group (WA) Fibroblast	1650	2623	973	71.16

Note: 71.07 Overall % recovery above the control

DISCUSSION

Since long before the development of even the most rudimentary language comes in to existence to describe it, the experimental health remedies such as herbs[17], clays[4] ashes[18] etc. have been in use for almost all kinds of animals and human ailments undoubtedly the applications gave relief to some extent. These with the passage of the time and success, became known as traditional remedies and even today these simple and natural products are being used world wide under the novel name of 'Alternative Medicine'.[17]

Herbal medicine in skin wound healing has been used for folk remedies. Improved wound healing is reported from topical application of centella asiatica and Aloe Vera.[15–17] Both herbs stimulate the production of collagen. Possibly in the present study besides the increase in the number of fibroblast, the contents of ash might be concurrently facilitating the synthesis of collagen.

Egyptians claim that many of these medicaments are scientifically proved and hence are being used without hesitation.[1]

Ashes are one of just such a product that has been in use since the times of the Prophet (S.A.W.S).[7,13]

Recent studies have demonstrated that concentration of one or more trace elements and minerals change in wounds to reflect their requirements in metaloenzyme complexes in sequential events in the wound healing cascade.[10] In the present study an attempt has been made to prove and high light the action of wood and charcoal ashes on the healing of cutaneous wounds; scientifically in animal model is an attempt to reflect human wound healing problems. Fibroblasts proliferate dramatically and are crucial for promoting wound healing.[12]

Skin wound healing is a natural restorative response to tissue injury. Wound healing requires interaction between a variety of cells including Fibroblasts and Myofibroblasts.

Since fibroblasts, a critical component of granulation tissue lay down a network of collagen fibers needed to repair the tissue surrounding the neo-vascularization, of the wound.[8] Hence in this study we have concentrated on the number of these cells, as it gives a clear picture and guide line of the progress of wound healing.

The myofibroblasts are often implicated. This in turn possibly triggers a large array of growth factors *viz* FGF, PGDF, IGF etc.[11] released at the wound site act as potent stimulators of new wound matrix contraction and hypothetically eliciting signals for granulation tissue contraction in vivo in wound models.[6] Many of the growth factors present at wound site can act either as mitogens or as chemotactic factors are presumed to be necessary for wound healing. Presence of recruited inflammatory cells is also observed in this study.

The crust formation by the ashes provides and facilitates a conducive internal environment to heal the wound normally in presence of trace elements in ashes.

Especially substantial concentration of Zinc present in ash and in appropriate combinations may enhance enzyme activity, DNA synthesis, cell division, protein synthesis (necessary for tissue regeneration) and repair of skin. Since this element is 50% higher in skin in normal conditions.[14] Zinc demand is also higher from the time of wounding, till early inflammatory phase, on the 5th day.[14]

Undoubtedly this supports our present study as it is all the time available in the crust; which may be utilized continuously during sequential changes.

The resident dermal fibroblasts begin to proliferate and then 3–4 days after the wound insult they begin migration into the provisional matrix of the wound clot under the crust and lay

down collagen rich matrix as witnessed on 3rd day in present study; as lag period is required by fibroblasts to emerge from quiescence.

The progressive loss of crust initiate regeneration of hair is observed on day 5 experimental sites possibly in response to the signals from underlying wound tissue. While in controls the wound appears leathery and dried out.

Proliferative fibroblasts with help of underlying contractile tissue make easier the margins to contract. The continuous process goes on till 11^{th} to 13^{th} day and there after seals off the wound. The contraction[6] process is bit faster at experimental sites than the control one. Martin (1997) has reported that in collagen gel model the wound fibroblasts remain active before, during and after contraction. These observations support the present visual and microscopic study where fibroblasts constantly increase and numbers rise up to 2686. (Table-3 and 4). In comparison to control the maximum number is 2330.

Further study is required to know how and what types of signal growth factor and elements in particular present in ashes synchronize in skin wound healing process that multiples the number of fibroblasts. Also to know why specially in charcoal ash treatment stops or reduces the fibroblast count in order to help clinicians to deal with any problems there in.

CONCLUSION

This is the first clinical study that has perfectly assessed appropriate combination of elements in the ashes. We conclude from present study that ashes exert clear and substantial effects on rabbit skin wound healing. Therapeutically the topical application of ashes is safe and cheaper in comparison to presently available ointments in the market. The successful potentiality of ashes is that it contains an appropriate combination of trace elements and metals required to heal the skin injury naturally.

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