ORIGINAL ARTICLE

ASH AS A UNIQUE NATURAL MEDICINE FOR WOUND HEALING

Din Muhammad Shaikh and Hamid Zia Shaikh

ABSTRACT

BACKGROUND: The use of ashes as a natural medicine for wound healing was evaluated in surgically induced wounds in the rabbit skin wound model.
METHODS: Ashes were prepared from dried buffalo dung, wood, and charcoal and their contents were analyzed with atomic absorption spectrophotometry. Each type of ashes was used as an experimental application on surgically induced skin wounds in the rabbit; an antibacterial ointment was used as a control. The healing results were evaluated over approximately 13 days.
RESULTS: Consistent healing was observed in all the experimental wound sites, which was comparatively more rapid than the control wound site. The healing was deemed complete on eleventh day only with charcoal ash whereas for dung-cake ash and wood ash, the completion time was approximately 13 days.
CONCLUSION: Ashes have unique properties to influence and enhance safe and sepsis-free wound healing in the rabbit skin wound model.

Key Words: Ash, Rabbit, Skin Fibroblasts, Anti-bacterial ointment.

INTRODUCTION

Healing involves an orderly progression of events to re-establish the integrity of the injured tissue. The problem of wound healing is as old as mankind itself. In ancient times, the use of natural products produced some successful results; hence, these are still being used by general practitioners and practitioners of alternative and complementary medicine in spite of the fact that these medications are constantly being challenged by the scientific community.[1-4] At present, clinicians still face the dilemma of determining satisfactory treatment of wounds. Presently in the treatment of cutaneous wounds in advanced medical practice, the application of different ointments and medicines has not shown a consistent and satisfactorily complete cure.

Wound healing in the skin depends upon the availability in appropriate combinations of trace elements, which act as enzyme cofactors and enhance the structural components in tissue repair.[5] Ashes contain most of the essential and trace elements in theropriate combinations. Analysis of different ashes revealed that they have an appropriate combination of metals required to heal the wound quickly; covering with ashes provides a natural, humid wound environment to accelerate the healing and repair process much more swiftly than most of the modern topical medicaments.[6,7] Bentonite applied topically has demonstrated that the presence of essential trace metals enhances wound healing and reflects their requirement as cofactor for metaloenzymes.[8]

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MATERIAL AND METHODS

ANIMALS:

Adult wild type rabbits (1000 1250 gm body weight) were used in this study. They were bred in the animal house of Isra University and were housed under controlled conditions of 30 $\pm$ 5°C, 55-60% relative humidity, and 12 hour Light/ Dark cycles as specified in the AAALAC international institutional animal care and use program guide, 1996. The animals were kept individually in stainless steel cages. Fresh alfalfa and tap water were provided ad libatum.

ASHES:

The three types of ashes were collected through complete burning of the parent material: wood chips and dead branches of wattle (acacia) taken from the vicinity of Isra University; dried buffalo dung cakes procured from the village adjacent to the University; and charcoal from a local supplier.

ANALYATE:

All three varieties of ash were dried at 105°C 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 1 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 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, brought to volume with de-ionized water, and examined by atomic absorption spectrophotometry for calcium, copper, manganese, iron, magnesium, sodium, potassium, and zinc levels.

EXPERIMENTAL DETAILS:
Incisional 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 anesthesia. The rabbits were shaved on the dorsal aspect of their skin after clipping the dense hair. Four different colored permanent markers were used to color code the different wounds by smudging the hair on the cranial aspect of each wound. Ashes were smeared on the two wounds on the cranial side and one wound on the caudal side, the remaining wound was covered liberally with an antibacterial skin ointment. The ash was 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 the air and without any dressing material to access the barrier function. Taking the operation day as zero (d o), three rabbits were sacrificed on days 01, 03, 05, 07, 09, 11, and 13 post wounding. The entire wound areas along with 4-5 mm of the surrounding skin were lifted by excision and placed in previously marked containers, in 10% formaldehyde as preservative for hematoxylin and eosin staining and histology.

HISTOLOGY AND MICROPHOTOGRAPHY:
The manual procedure was adopted to process the formaldehyde-preserved tissue, this included dehydration, clearing, impregnation, embedding, and cutting followed by staining of the section carried out on alternate days. Microscopic photography was also carried out.

STATISTICAL ANALYSIS:
The results were analyzed using Statistical Package 11. The tests applied were chi-square and Post Hoc ANOVA; the p-value of =0.01 was considered to be significant.

RESULTS

Physical and microscopic examinations of wound sites were carried out on a day-to-day basis as follows:

By day 1
There was no change in the control wound that was gaping. However, the test sites showed a thick crust of ashes on the wound surfaces. No decrease in wound site was observed at this stage in both control and experimental groups. Three to four mitotic figures were seen in dermal fibroblasts in the test sites only, whereas the control wound showed no such activity. In both wound types (i.e. test and control), the presence of numerous erythrocytes and polymorphonuclear (PMNs) leukocyte infiltrates indicated the beginning of the inflammatory phase (Figures 5a, 5c, 5e, 5g).

By day 3
The test wounds had reduced in length from 10 mm to 8 mm; this decrease was not evident in the control wound. Both wound sites still show heavy PMNs and monocytes; however, the test sites revealed the presence of numerous eosinophils. An increase in the number of fibroblasts was also noted at the test sites at that time.

By day 5
The surface of the control wound appeared leathery and dried out while the test wounds retained a crust of ash on their surface, which was decreased to 6 mm in length. Crusting and hardening of the wound surface was observed in the test sites with progressive loss of superficial ash crust followed by prominent hair growth on the test sites.

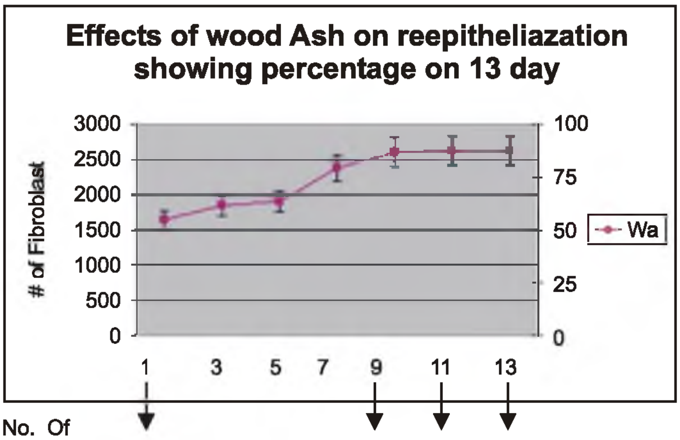
By day 7
The control wound measured 8 mm from the original 10 mm but the test sites had decreased another millimeter, i.e. a total of 5 mm or half of the size of the initial sites. The thin crust of ashes extruded from the test sites and the dermal fibroblasts showed numerous mitotic figures with appearance of ground matrix being laid down. Both the control and test wounds showed the presence of healthy granulation tissue.

By day 9
The control wound decreased by another 1 mm but there appeared to be protrusion of the scar above the actual wound margins. The test sites revealed a status quo physically but in microscopic appearance, the fibroblasts appeared to be mutating in to myofibroblasts (Figs. 1, 2, 3, and 4).

By day 11
Healing in the test wound from day 11 onward was marked with obvious signs of decrease in inflammatory cells in and near the wound margins

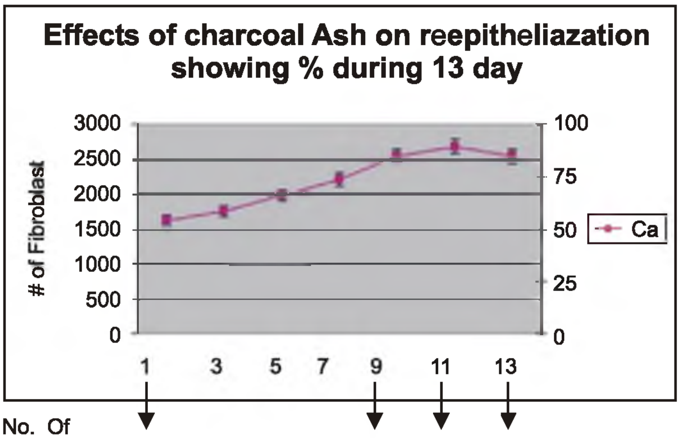
with a very pronounced reduction in the crater formation. Wound debris epidermal realignment was by a pit-like configuration, retia formation, and hyperkeratosis was visible (Figs. 1, 2, 3, 4). The gross examination revealed a very transparent pinkish skin with furry appearance (hair) on the entire surface of wounds except at the very center, which still exhibited reddish pale granulation tissues. There was no evidence of repair of panaculus carosus muscle but collagen deposition was seen (pale staining).
The cell count by reticule and grid method was 2686 fibroblast /mm^2, which was the maximum for test wounds.

By day 13
By this time the control wound displayed a thin pinkish skin over the granulation tissues; however, the test sites were completely covered by hair-bearing skin. The histological appearance of both the test and control wounds was characteristic of near-perfect healing. However, there was a lag of another 2 to 3 days for the control sites.
The wounds showed complete normalization of the lining epidermis; however, there was still the presence of scanty monocytes and PMN eosinophils in the deeper layer of the dermis and in and around the panaculus carosus muscle.
The control wound displayed the same configuration but with the noteworthy exception of the presence of foci of PMNs and cellular debris underneath the completely reconstituted epidermis which for the most part was edematous.
In the case of charcoal application, the maximum number of 2686 fibroblasts was observed on the eleventh day. Thereafter on the thirteenth day, their numbers decreased by about 6% to 2543. The reason is not known and is to be worked out (Figs. 1, 2, 3, and 4).



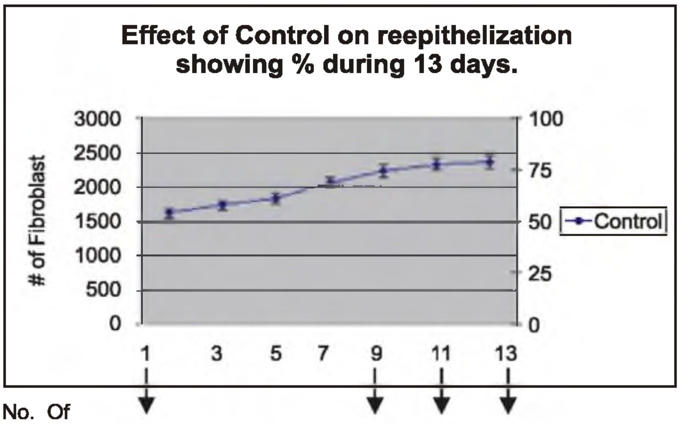
No. Of Fibroblasts 1650 2603 2610 2623

Figure 2. (Indicates the specific day post wounding)



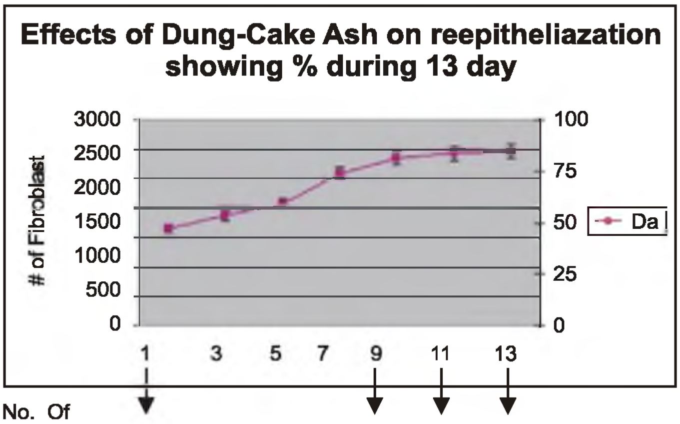
No. Of Fibroblasts 1630 2546 2686 2543

Figure 3. (Indicates the specific day post wounding)



No. Of Fibroblasts 1620 2226 2330 2363

Figure 1. (Indicates the specific day post wounding)



No. Of Fibroblasts 1657 2843 2923 2960

Figure 4. (Indicates the specific day post wounding)

HISTOLOGICAL FEATURES OF DAY 01

Fig 5a shows extensive inflammatory exudates with numerous polymorphonuclear (PMN) cells, RBCs, and inflammatory exudates.

FIG 5A: CONTROL DAY 01

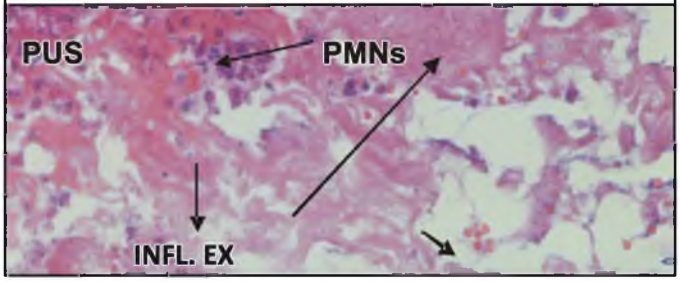


Fig 5c reveals moderate granulation tissue with scant neovascularization. The section also reveals numerous PMNs and fibroblasts.

FIG 5C WOOD ASH DAY 01

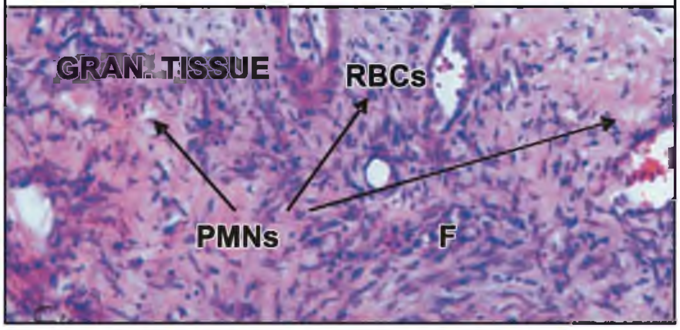


Fig. 5e A moderate to large amount of granulation tissue with numerous PMNs and fibroblasts (F). There is also a high element of neovascularization containing a large amount of RBCs.

FIG 5E CHARCOAL ASH DAY 01

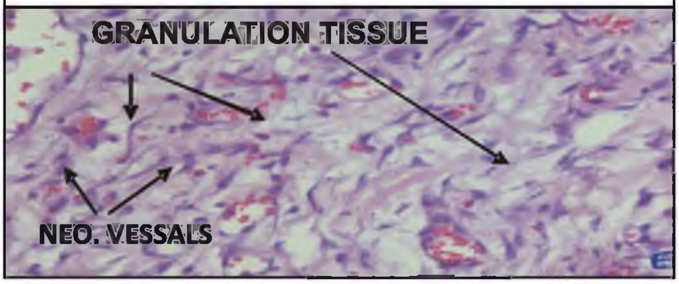
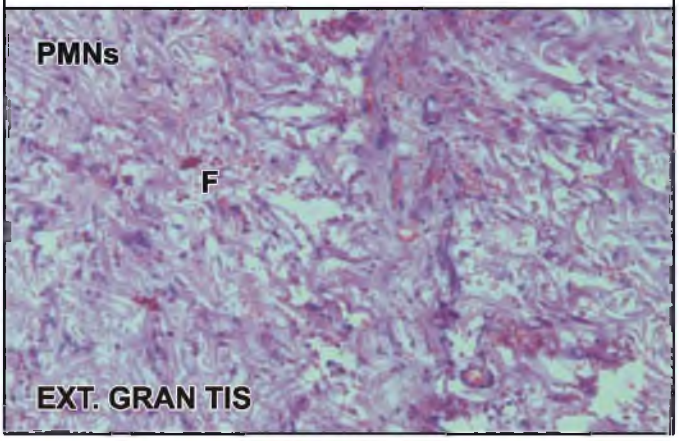


Fig 5g Displays an extensive amount of granulation tissue with numerous PMNs; RBCs Fibroblasts and new vessel formation is also predominant.

FIG 5G DUNG- CAKE ASH DAY 01



HISTOLOGICAL FEATURES OF DAY13

FIG5B CONTROL DAY 13

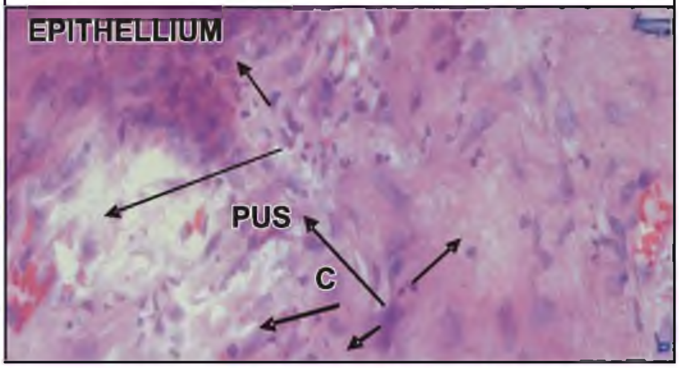


Fig 5b shows edematous epithelium at the upper left hand immediately beneath which are persistent foci of cellular debris and pus. Few vesicular myofibroblast (MF) nuclei are visible along with collagen(C) deposition.

FIG 5D WOOD ASH DAY 13

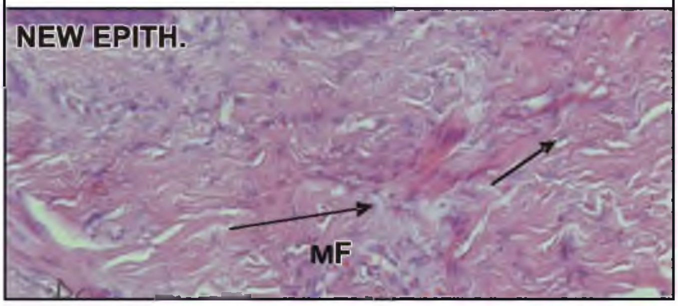


Fig 5d reveals established epithelial elements, extensive collagen (C) deposition, and presence of a large number of myofibroblasts (MF).

FIG 5F CHARCOAL ASH DAY 13

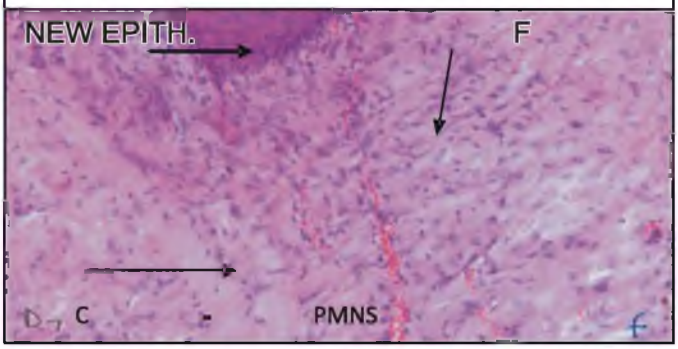


Fig 5f again reveals an established epithelium with a few fibroblasts (F), PMNs, and myofibroblasts (MF). Extensive collagen (C) deposition is also visible.

FIG 5H DUNG-CAKE ASH DAY 13

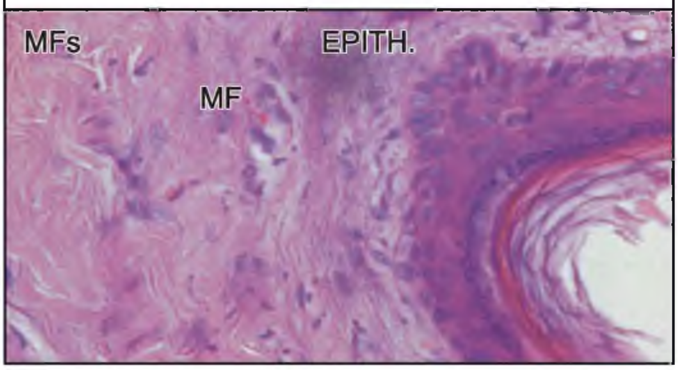


Fig 5h shows an almost competently reconstituted dermis and epidermis with presence of hyaline keratin (K), mature collagen (C), and numerous myofibroblasts (MF).

DISCUSSION

The complete tissue repairing process is still a continuous challenge despite the popular topical application of ointments of varying compositions as they can cause severe morbidity. In the present study, wounds were surgically induced on the skin of rabbits and observations (physical and histological) were made on alternate days as shown in Figures1-4 and Figure 5a-h, to assess the continuity of the healing process both in the control and at the test sites.

The initial increase in the count of fibroblasts was observed on the third day, while in comparison, the application of electric current (one of the modern techniques) has been reported to produce the same results on the seventh day, suggesting the supremacy of ashes with their unique properties as natural medicine. Our observations are consistent with the recent findings of Mohit Kapoor et al 2008, who has forwarded the notion that the recruitment of fibroblasts can start at day 3 if the appropriate co-factor is available.[9] Undoubtedly, the electrical application is being considered valuable but no definite scientific validation has been reported; hence, it is not in common use.[10] Moreover, the interaction between the size of electrodes and that of the wounds is also not consistent.

These fibroblasts of the wound matrix develop into cells called "myofibroblasts," responsible for contraction of the wound area as recorded on day 9. Collagen deposition, which is responsible for restoring the anatomic extracellularity, was also seen on the eleventh day post wounding.[6] Local recruitment for skin wound healing of fibroblasts is from the dermis of the intact adjacent skin to the site of inflammation.[11] Conversion of myofibroblasts from fibroblasts is controlled by the mechanical microenvironment of the later phases of dermal wound healing.[12,13]

The present findings may lead to an important breakthrough concerning the basic biology of wound healing in the skin besides the quality of healing.

Micro technology has recently revealed that migration of PMNs to skin lesions induces a large transcriptional activation program that may regulate cellular fate, function, and promote wound healing.[14] These cells were also observed in the present study in the control and experimental sites on days 1 and 3, respectively. (Fig. 5a, c, e, and g).

Sabine et al (2007) have pointed out the notion that in mammals wound repair has evolutionarily been optimized for speed of healing under dirty conditions where multiple redundant compensatory and rapid inflammatory responses are needed. This notion cannot be applied in the present study, as the ashes do not contain organic materials etc. responsible for infection/immunity. However, aloe vera and centella asiatica, botanical medicines, have been widely used for decades for a host of curative purposes including facilitation of wound repair.[1, 4, 15] No confirmation of healing based on their biochemical properties is reported other than their safety and benefits. The patients are also being advised to avoid the use of these plant parts from safety point of view. More data is required to determine their clinical effectiveness. In comparison, our study of ashes has the wide-range effect of improving wound healing in a timely, safe, and effective manner.

Honey,[7] another natural medicine, used since the days of The Prophet (S.A.W.W). These properties of ashes are being verified through tissue culture media.

The continuous increase in fibroblasts and myofibroblasts, a critical component of the healing process, promotes physiological tissue repair.[12] This is shown in Fig. 5a-h, and suggests that the application of the different ashes used in this study to induced wounds had identical, cost-effective medicinal properties in skin wound healing.

These observations are partly consistent with the findings obtained with treatment with microamperage electrical stimulation (MES).[10] Since ashes contain most of the essential and trace elements in appropriate combinations, the deficiency can lead to different complications at the wound site viz. infections and delay in healing and repair.[6,5]

B. Hinz (2007) has pointed out that differentiation from fibroblasts to myofibroblasts plays a key role during wound healing and tissue repair. The high contractile force of myofibroblasts favors physiological tissue remodeling.[13]

The healing is a matter of time and opportunity. Possibly, in our study, ashes provide the same effect and maintain a favorable chemical microenvironment.

CONCLUSION

We conclude that there is mounting evidence that the finely tuned balance of trace elements and metals in appropriate combination found in ashes from dried buffalo dung is a unique natural medicine for skin wounds. However, more pharmacological and biophysical studies are needed to establish its effectiveness and its functional significance.

REFERENCES

1. Mackay D, Miller AL. Nutritional support for wound healing. Alter Med Rev; 2003; 8(4): 359-377.
2. Chithra P, Sajithlal GB, Chandrakasan G. Influence of Aloe Vera on the healing of dermal wounds in diabetic rats. Ethno Pharmacol; 1998; 59: 195-201.
3. Chang K, Whitaker DC. The impact of herbal medicine on dermatologic surgery. Dermatological Surg; 2001; 27: 759-763.
4. Berger MM, Binnert C, Chiolero RL, Taylor W, Cayeux MC, Benathan M, Shenkin A, Tappy L. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. Am J Clin Nutr; 2007; 85: 1301-1306.
5. Hamid ZS, Shaikh DM. 2009. Wood ash and charcoal ash, an instrument to skin tissue repair in acute injury in rabbit model. Pak J Physio; in press PJP
6. Hamid ZS, Shaikh DM. Topical application of dung cake ash an innovative therapy in skin wound healing in rabbit model. Pak J Physio vol. 4 numer 2008. 2 Pp 24-29.
7. Emami-Razavi SH, Esmaeili N, Forouzannia Sk, Amanpour S, Rabbani S, Alizadeh AM, Mohagheghi MA. Effect of bentonite on skin wound healing: Experimental study in the rat model. Acta Medica Iranica; 2006; 44(4): 235-240.
8. Kapoor M, Liu S, Huh K, Parapuram S, Kennedy L, Leask A. Connective tissue growth factor promoter activity in normal and wounded skin. Fibrogenesis & Tissue Repair; 2008, 1:3
9. Bayat M, Asgari-Moghadam Z, Maroufi M, Rezaie FS, Bayat M, Rakhshan M. Experimental wound healing using micro amperage electric stimulation in rabbits". J of Rehab Research & Development, 2006, 43(2): 219-226
10. Desmouliere A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the myofibroblast. Wound Repair and Regeneration; 2005, 13:7-12.
11. Hinz B, Pittet P, Smith-Clerc J, Chaponnier C, Meister J. Myofibroblast development is characterized by specific cell-cell adherens junctions. Molecular Biology of the Cell; 2004; 15, 4310-4320.
12. Hinz B. Formation and function of myofibroblast during tissue repair. J Investigative Dermatol; 2007; 127, 526-537.
13. Prasad AS. Zinc in human health: Effect of zinc on immune cells. Mol Med; 2008; 14(5-6): 353-357.
14. Werner S, Krieg T, Smola H, Keratenocyte-fibroblast interactions in wound healing. J Investigative Dermatology, 2007; 127, 998-1008.
15. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms". J Investigative Dermatol; 2007; 127, 514-525.