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Original Research Paper

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HISTOPATHOLOGICAL AND BIOCHEMICL STUDY OF ALKALI BURNS (KSHARA DAGDHA VRANA) IN ALBINO RATS

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ABSTRACT Acharya Sushrutha mentioned alkali (kshara) as a substance which does debridement (ksharana) and cutting properties (kshanana) and mentioned its properties like excision (chedana), incision (bhedana), scrapping (lekhana), purification (shodhana), healing (ropana) , cauterization(dahana)etc.

How a strong alkali, after injuring tissue, can itself induce healing needs investigation. With this objective, this experimental study on Albino rats was planned to observe and measure alkali induced tissue injury and its subsequent healing. Findings were compared with healing pattern of topical chemical tissue injury and healing using 2N-NaOH (2normal saline sodium hydroxide).

The parameters for assessing tissue injury and subsequent healing were total hematology, C-reactive protein, total protein, tissue hydroxyproline and histopathological study. The study revealed that the rate and extent of healing post-alkali (trial group) injury is much higher compared to chemical injury induced by 2N-NaOH (standard). Lesions treated by applying alkali leave wounds and these wounds need not be treated by other healing medicine. The alkali itself has property which leads to healing of its own wounds.

KEYWORDS : Alkali, Wister Albino rats, Sodium hydroxide.

INTRODUCTION

Alkali (kshar) is described as superior among all surgical and supplementary surgical procedures in ayurveda1. The samhita describes two types of alkali – topical (pratisaaraneeya) and internal administration (paneeya)2. Further, among three types mild (mridu), moderate (madhyama) and strong (teekshna), strong alkali can be used as topically but never for internal administration.

Strong alkali is a paste prepared by filtering ash of specific plants in water or cow's urine, then boiled with hot limestone with final additives of specified herbal pastes. The resultant herbomineral paste, alkali, is extremely corrosive and usually strongly alkaline. For instance, the alkali prepared for this study had a pH of 12.41.3 whereas the 2N-NaOH procured for this study had a pH of 11.90.4

The sharp and penetrating properties of alkali is responsible for its excision, incision, scrapping, digestion or suppuration, dissolving, purification, healing, drying, contraction and other properties6. What is notable here is that after treating a lesion with alkali separate healing measures are not required, the alkali itself induces healing.

Earlier studies in this topic have shown encouraging results. A study carried out in 20087 showed the following: the histological study showed on 3rd day mild inflammatory infiltrate comprising of polymorphs, monocytes and plasma cells are evident in deep dermis, the hair follicles shows atrophy. On 7th day the inflammatory cell extends up to the subdermis with dense layout of fibroblasts, the fibroblasts are plump with spindle shapes and mature into dense collagenous fibers. On 14th day there is marked healing process and regeneration of hair follicles with evacuation of inflammatory cells and maturation of fibroblasts in to dense collagenous fibers, the granulation tissue in the deeper dermis of the wound is gradually replaced by fibrous tissue. On 21st day marked regeneration of hair follicles evident with total replacement of the dermis, inflammatory cells are very sparse and epidermis show focal feature of hyperkeratosis and mild parakeratosis.

A clinical study conducted in 20128 showed the following: A clinical study of 20 patients with diabetic foot was randomly selected and achyranthus aspera alkali was applied at the site. The study shows itching and smell were reduced markedly, whereas pain and inflammation reduced slightly. The wound size, discharge, and granulation tissue reduced markedly whereas tenderness and surrounding area of ulcer reduced slightly. Significant results were seen in wound.

This study seeks to investigate whether an alkali, after injuring tissue, induces healing in the injured area; and whether the healing induced is quicker than other chemical injury. This study, therefore, is planned to investigate healing patterns in alkali induced tissue injury vis-à-vis its healing effect. The results will be compared with the healing pattern of chemical tissue injury and control.

MATERIAL AND METHODOLOGY

OBJECTIVES OF STUDY

1. To describe of vrana its nidana, purvarupa, lakshana, chikistsa and upadrava in Ayurvedic literature.
2. To conduct a literature review of kshara and its preparation of tikshna pratisaaraniya kshara as described in sushrutha samhita.
3. To evaluate healing patterns of kshara dagdha vrana and compare with healing patterns in chemical induced tissue injury, using following parameters:

a. Biochemical changes of kshara dagdha vrana on the skin of Albino rats.
b. Hydroxyproline estimates.
c. Histopathological changes of kshara dagdha vrana on the skin of Albino rats.

Study design and methodology:

This is a controlled standardized experimental study on Wister albino rats. Rats will be grouped into control, standard and trial.

Rats of control group will receive topical normal saline application. Standard group will receive topical sodium hydroxide application. Trial group will receive topical strong alkali application.

DRUGS USED IN THE STUDY: 1. Achyranthus aspera (apamarg) alkali – prepared as per 11th chapter of sutra sthan. 2. Sodium hydroxide – readily available in open market. Instruments like disposable 1ml syringe, needle, blade, pen marker, Cotton, glows, applicator, petty dish, sterile container, seizer.

Experiment animals Wistar strain albino rats of either sex or albino rat were obtained from animal house attached to department of Pharmacology, SDM Centre for research in Ayurveda and Allied sciences Udupi. The experimental protocol was approved by the institutional animal's ethics committee under the reference no. SDMCRA/ IAEC-2013-14 ST 10. The animals were fed with normal rat diet and water ad libitum throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided has the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%.

Inclusion criteria: a) Healthy wistar albino rats of either sex with average weight of 180-220gm were selected randomly for the study.

Exclusion criteria: a) which does not fulfill the above criteria b) Infected. c) Pregnant. d) Those healthy wistar albino rats which are under other experiments will be excluded.
EXPRIMENTAL PROCEDURE: 42 Wister Albino rats of either sex weighing 180 - 220g will be selected and grouped into 7 different groups, each with 6 rats.

Normal saline group (Control) in the experimental set up, the normal saline groups were serving as a control group. There the application of normal saline done on the predetermined area for 1 day, 7 days and 14 days. After topical application rats were sacrificed under deep ether anesthesia after 24 hours, 7th day and 14th day respectively. Test groups (strong Achyranthus aspera alkali) Test group rats were anaesthetized under ketamine (50mg/kg) and xylazine (3mg/kg). The anaesthetized rats were shaved on dorsal region 5cm below the neck region. Using permanent marker 2x2 cm circular mark was done. Using fine needle nick (technical procedure used for achieving fine bleeding on prick) was given. The test drug Achyranthus aspera alkali were applied on a marked area and allowed for 180 seconds. After 180 seconds the area were washed with lemon juice. In 1 day, 7 days and 14 days study after topical application of test drug Achyranthus aspera alkali, rats were sacrificed under deep ether anesthesia after 24 hours, 7th day and 14th day respectively.

Standard group (2N-NaOH) in 1 day, 7 days and 14 days study, the Standard drug 2N-NaOH were applied on a predetermined area allowed for 180 seconds. After 180 seconds the area were washed with lemon juice and after topical application of standard drug 2N-NaOH, rats were sacrificed under deep ether anesthesia after 24 hours, 7th day and 14th day respectively.

In all groups, on the day of sacrifice, blood were drawn from rats retro orbital vein puncture and sent for biochemical investigation and the skin tissue carefully excised and sent for histopathological examination.

Histopathological studies – stored in a mixture of 30ml formalin& 270 ml distilled water in different sterile containers. Hydroxyproline content estimation – stored in about 2-3 ml NS solution in petri dishes which were deep frozen to – 200C. The obtained results of the test groups were compared with that of the standard and control groups.

Statistical analysis of biochemical and hydroxyproline data will generated by One way ANOVA followed by Dunnets multiple comparison "t" test as post hoc test if $p<0.05$ using statistic software of graph pad prism 4.0 version.

Wound healing Parameters a) Biochemical parameter: Total hematology, Total protein and C-reactive protein. b) Hydroxyproline estimation in skin tissue. c) Histopathological examination of part of skin.

RESULT

With respect to the primary objective of the study which was to assess healing patterns following alkali induced tissue injury compared to healing patterns following tissue injury by standard chemical (2N-NaOH), this study showed the following:

1) TC:- In 1st day, 7th day, trial group showed higher in total count compared to standard and control group. On 14th day study trial group showed lower total count compared standard group, which indicated that inflammatory reaction subsided in trial group.
2) CRP:- Higher levels on 1st day, 7th day and 14th day in trial group indicated stronger inflammatory process compared to standard and control.
3) TP:- On 7th day and 14th day study trial group showed higher level compared to standard and control group, this shows higher release of TP due to tissue destruction.
4) Hydroxyproline:- On 7th day and 14th day study hydroxyproline content was higher in trial group compared to standard and control, which indicated that collagen synthesis and wound healing were better in trial group.
5) Histopathological study: Histopathological study slides revealed, chronologically in 1 day study, after 24 hours shows crust formation, epithelial layer disruption in the epidermis, cell infiltration, edema, loss of collagen fibers, overall local tissue injury observed more and on 7th day, 14th day study shows almost a normal picture. Dermis, epidermis and hypodermis presented normal profile. Inflammatory cells were almost absent in most of the sections. Epidermal layer was continuous and well developed observed and overall more of tissue repair process in trial treatment group compared to standard group. In contrast to this epithelialization was impaired and there was tissue loss in standard treated group compared to trial group. Overall among five parameters, all five parameters showed better healing patterns in alkali induced tissue injury when compared to standard chemical tissue injury.

CONCLUSION

This controlled, standardized experimental study has shown that topical use of strong alkali,causes healthy tissue injury, induces excellent subsequent healing at the site of injury, a phenomenon not seen at same degree in standard chemical induced injury. The rate and extent of healing post alkali injury is much higher than chemical injury induced by 2N-NaOH.
The study demonstrates that strong alkali is a complex, comprehensive topical Ayurvedic therapeutic tool and not a simple corrosive. Though this study has been carried out by causing injury to healthy tissue (kshanana), the findings can be extrapolated to lesions treated with topical alkali use also.
It is, however, important to emphasize that the study should not be interpreted to use alkali as a healing agent in clean wound, which it is not. Sushrutha has not made any such description. Further studies can focus on destruction followed by healing effects of topical use of alkali on skin lesions,

effects when applied for different time durations and clinical studies.

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