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Wound healing activity of Manjishthadi ghrita in albino rats

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ABSTRACT

To treat a wound is to bring out the process of repair of the tissues in the manner most conducive to the present and future welfare of the patients. Better wound healing with minimal scar formation with least pain is the prime motto of every surgeon. The present experimental study was designed to observe the wound healing activity of an innovative compound formulation "*Manjishthadi Ghrita*" using excision, incision wound and dead space models in Wistar strain albino rats. The results of *Manjishthadi Ghrita* were compared with the results of untreated control group and results of betadine ointment, considered as positive control. Effect of wound healing activity was assessed by noting the contraction of wound size, days taken for complete healing, tensile strength assessment of the scar and histopathological examination of the granulation tissue as well as incised skin area of the wound. Test drug application did not affect the complete days taken for complete healing, tensile strength of incised skin and collagen, ground substance formation in the granulation tissue and angiogenesis to significant extent. The present study demonstrates that local application of *Manjishthadi ghrita* did not having significant wound healing activity in experimental animals.

INTRODUCTION

With the advancement of molecular biology and cell culture techniques, objective assessment of tissue repair has become easy with increasing sophistication. Successful advancement in wound healing discipline depends on the effective integration of scientific discoveries and wound care practices. The aim of this study is to increase our basic understanding about the molecular and cellular events of the repair and healing processes and to use this information as the basis for developing new therapy that can minimize the adverse consequences of wounds.

A number of active chemical constituents are attributed to serve the healing activities. Many individual drugs possess some of the required chemical constituents which show only partial effect that is why for a single drug it is difficult to achieve the complete aim of wound management[1]. Hence there is a dearth to find out a rational and optimal healing compound of the group of drugs for the wound management in better way.

Manjishthadi ghrita is an innovative herbal compound formulation which is prepared from seven potent wound healing drugs recommended in *Sushruta Samhita* viz., *Manjishtha* (*Rubia cordifolia* Linn.), *Daruharidra* (*Berberis aristata* DC), *Mocharasa* (*Salmalia malabarica* Linn.), *Dhatakipushpa* (*Woodfordia fruticosa* (Linn.) Kurz.), *Madhuka* (*Madhuca indica* J.F.Gmel) *Lodhra* (*Symplocos racemosa* Roxb.) and *Rasanjana* (*Extractum berberis*). This formulation has shown excellent wound healing activity in post operative wounds of

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piles, fissure, cyst, pilonidal sinus, corn etc., in patients[2]. Hence to provide experimental basis to clinical findings this study was designed to assess the wound healing activity of *Manjishthadi ghrita* in different experimental models. The use of a single model was inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence, three different wound models viz., excision wound, incision wound and dead space wound models were designed in the present study.

MATERIALS AND METHODS

Test drug

The test formulation was prepared in the Pharmacy attached to our institute by adopting standard procedure. Betadine (Manufactured by Win Medicare, New Delhi - Batch PN0039) ointment which contains povidine iodine (5% w/w) was used as standard wound healing drug.

Animals

Wistar strain albino rats of either sex weighing between180-220g were selected from the animal house attached to our institute. They were housed at 22 ± 03°C with constant humidity of 50-70% on a 12 hour natural day and night cycles. They were fed with diet 'Amrut' brand rat pellet food supplied by 'Pranav' Agro Industries, Baroda and tap water was given *ad libitum*. The experiments were carried out in accordance of the Institutional animal ethics committee (Approval number; IAEC 09-10/05MD 02).

Excision wound model

Prior to the operative procedure all the instruments (scissor, forceps, etc.) were autoclaved. The area to be excised (on the back portion of the rat – suprascapular region) was shaved carefully by scissor prior to the procedure without causing any abrasions. The rats were anaesthetized with diethyl ether and they were inflicted with excision wounds as described by Morton and Malone (1972)[3]. The dorsal fur of the animals was shaved and the area of the wound to be created was outlined on the back of the animals with marker using a circular coin. A full thickness of the excision wound of circular area 400-520mm^2 and 2mm depth was created along the markings with a surgical blade. The animals were randomly divided into four groups of six each.

First group (Group A) is served as normal control to which normal saline was applied. To the second group (Group B) plain g*hrita* was applied and is served as vehicle control group. Third group (Group C) was applied with *Manjishthadi ghrita* where as fourth group (Group D) was applied with standard drug daily until complete epithelialization. The wound contraction rate was assessed by tracing the wound on every third day using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. The point at which the eschar fell off without any residual raw wound was considered epithelialization.

Incision and dead space wound model

The effect of test drug on incision and dead space wound was evaluated by noting effect on the formation of granulation tissue in subcutaneously implanted PVC tube. The selected animals were randomly divided into four groups of six each as mentioned in excision wound model.The animals were anaesthetized by inhalation of diethyl ether and a mid line incision of 3cm was made and a tunnel was created subcutaneously in which sterilized PVC tube (length 2 cm. and diameter in 1 mm.) was inserted and the incision was closed with the help of two interrupted sutures at 1cm apart as described by Ehrlich and Hunt (1968)[4]. The test drug was filled in the PVC tube before implantation and also applied locally over the incision wound daily for 12 consecutive days. The sutures were removed on the 8th post wound day and the skin-breaking strength was measured on the 12th day by the method described by Lee (1968)[5]. In brief the anesthetized animal was secured to the table and a line was drawn on either side of the wound 3mm away from the suture line. The line on either side of the suture was

gripped with a forceps one at each end opposed to each other. One end of the forceps was supported firmly, whereas the other was connected to a freely suspended lightweight measuring jar. Water was slowly added continuously till the wound began to gap. As soon as wound gaping appeared the addition of water was stopped. The volume of water was determined and noted as a measure of breaking strength in grams. The PVC tube was taken out from the subcutaneous tunnel by careful dissection and the tissue was collected from the plastic tube and preserved for histopathological study. The tissue of incision wound was also preserved for histopathological study. The histopathological slides of skin and granulation organs were prepared by referring standard procedures and three types of staining was carried out viz., H and E staining[6], Prussian blue[7] and Van-Gieson's[8]. The slides were viewed under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical analysis

Results were presented as Mean ± SEM, difference between the groups was statistically determined by paired and unpaired student's 't' test for paired and unpaired data respectively with the level of significance set at $P<0.05$. The level of significance was noted and interpreted accordingly.

RESULTS

Effect of test drugs on excision wound

In the early days (up to 3 days) moderately higher rate of contraction was observed with betadine treated group where as it was observed slightly less in *Manjishthadi ghrita* applied group. So, not much difference was noted among different groups. Around 28 days were required for complete healing in control group and in betadine treated group 27 days and around 30 days were required in plain *ghrita* and *Manjishtadhi ghrita* applied groups (Table - 1).

Effect of test drugs on incision wound and dead space wound

Application of test drug did not affect the tensile strength of the incision wound to significant extent in comparison to control group. Table – 2 shows effect on test drug on tensile strength of the incision wound. The microscopic examination of incised wound area with different staining showed that *Manjishtadhi ghrita* and betadine both promoted epithelialization. In plain g*hrita* treated group also slight increase in epithelialization was observed. Microscopic examination of granulation tissue did not show increase in proliferation of epithelial tissue in all the treated groups.

DISCUSSION

Wound healing is the summation of a number of processes which follow injury including coagulation, inflammation, matrix synthesis and deposition, angiogenesis, fibroplasia, epithelialisation, contraction, remodelling and scar maturation[9]. The wound healing process completes in three phases. They are the inflammatory, the proliferative, and the remodelling. The inflammatory phase is characterized by haemostasis and inflammation. Collagen exposed during wound formation activates the clotting cascade (both the intrinsic and extrinsic pathways), initiating the inflammatory phase. Release of thromboxane A_2 and prostaglandin 2-alpha, which are potent vasoconstrictors from the damaged cell membrane in the wound after the injury. This initial response helps to limit haemorrhage. After a short period, capillary vasodilatation occurs secondary to local histamine release, and the cells of inflammation are able to migrate to the wound bed. The timeline for cell migration in a normal wound healing process is predictable. Granulation, collagen maturation and scar formation are some of the different phases of wound healing, which run concurrently, but independent to each other[10].

The most important part in excision wound model is the wound closure. The efficacy of

the medication is measured in terms of the rate of wound contraction and duration required for complete epithelialization of the wound. In initial 3 days moderately higher rate of contraction was observed with betadine where as it was slightly less in *Manjishthadi ghrita* group. There after not much difference could be observed among different groups. Approximately 28 days were required for complete epithelialization in vehicle control group and in 27 days in betadine treated group and average 30 days were required in plain *ghrita* and test *ghrita* treated groups. The results thus show that the local application of test *ghrita* do not promote healing of excision wounds. The skin has three main areas epidermis, dermis and hypodermis. Myofibroblasts which are situated in the fascia of the hypodermal layer are reported to play major role in the wound contraction. They have the feature of both smooth muscle and fibroblasts. Though they respond to many therapeutic interventions their response is different from the one observed in smooth muscle fibers[11]. It seems that the plain *ghrita* and *Manjishtadhi ghrita* do not have any influence on these cells to enhance their activity and to promote wound contraction.

In incision wound model effect of local application on two important parameters was assessed. One was the effect on epithelialization of the incisional wound and other was the tensile strength of the scar. The first part of evaluation was done through histological evaluation of the incision wound with different staining methods. The second part was completed through actual measurement of the tensile strength of wound through well established and standardized method. General pattern assessment is possible through standard Hematoxylin and eosin staining but Van-Gieson's stain is helpful in assessing the collagen content and Prussian blue staining which was helpful in assessing the development of ground substance. The microscopic examination showed that *Manjishtadhi ghrita* and betadine both promoted epithelial formation. In these sections comparatively larger epithelial layer was observed in comparison to Van-Gieson's staining did not show any remarkable increase in the collagen content of the skin in plain *ghrita* and *Manjishtadhi ghrita* applied groups. In betadine group slightly higher collagen content was observed. Prussian blue staining showed less intense staining in plain *ghrita* control, *Manjishtadi ghrita* and reference standard were- indicating that they do not promote formation of ground substance to significant extent. From the above it can be suggested that the local application of the test formulation promotes epithelial cells formation in the incision wounds and may not have significant impact on the collagen and ground substance formation. Surprisingly, none of the treatment groups including betadine group could promote tensile strength in comparison to control group. This clearly indicates that they do not have influence on the collagen and ground substance formation.

The effect on dead space wound was evaluated by noting down the effect of drug application (local as well as in embedding tube) on the formation of granulation tissue in the implanted PVC tube. Contrary to the expectation, not much granulation tissue formation was observed in plain *ghrita*, *Manjishradi ghrita* and even betadine applied groups. Hence the emphasis was laid on histological examination of the granulation tissue. H and E staining did not reveal much difference among the groups. Van-Gieson's staining showed higher collagen content in betadine treated group and less in *Manjishtadhi ghrita* treated group. In plain *ghrita* applied group, the Prussian stain intensity was less. The staining intensity was observed higher in Betadine treated group and almost similar to control group in *Manjishtadhi ghrita* treated group. From this it can be suggested that the test formulation has no influence on the collagen and ground substance formation in the granulation tissue. Angiogenesis could not be influenced much. The overall effect is that the test formulation on local application in the dead space wound may not be influenced in wound healing to the significant level. From the present study it can be concluded that local application of test formulation in excision wound, incision wound and dead space wound did not have significant wound healing activity in experimental animals.

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