EFFECT OF *TERMINALIA CHEBULA* AND *TERMINALIA BELERICA* ON WOUND HEALING IN INDUCED DERMAL WOUNDS IN RABBITS

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Summary

A study was conducetd to know the efficacy of a paste prepared from *Terminalia chebula* and *T. belerica* on wound healing. Thirty rabbits were divided into two groups (Group A and Group B) having fifteen animals each. An equi-demensional full-thickness excision wounds were created on the back of the each animal. Each group was again divided into 3 subgroups for 4, 8 and 12 days post wounding. Group A was treated with NSS (control) while Group B was treated topically with herbal paste preparation *(Terminalia chebula* and *Terminalia belerica)* @500mg/animal. The parameters observed were hydroxyproline, Uronic acid and DNA content of the granulation tissues. Herbal paste preparation showed significant ($P<0.05$) improvement on maturation, wound contraction and epithelialization in Group B on day 4 followed by day 8 and day 12 respectively. Therefore it may be concluded that the paste obtained from *Terminalia chebula* and *Terminalia belerica* offers a distinctive advantage in wound healing.

Key words: *Terminalia chebula, Terminalia belerica,* Wound healing, Induced dermal-wound, Granulation tissue.

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Introduction

Despite recent advances in antimicrobial chemotherapy and wound management, several types of wounds and ulcers still prove recalcitrant to treatment. Thus, wound healing continues to be one of the major public health problems in the world and wound management still remains a matter of research. The complex cascade of cellular and biochemical events that occurs after injury determines the successful outcome of wound repair. Fibroblasts are the primary synthetic element in the repair process and are responsible for production of large quantities of collagen. They also help in the production of other matrix constituents including hyaluronic acid, fibronectin and glycosaminoglycan[1]. Repair by connective tissue involves four important steps, (i) migration and proliferation of fibroblasts, (ii) deposition of extracellular matrix (iii) formation of new blood vessels (angiogenesis) and (iv) maturation and organization of the scar, also known as remodeling. In recent years, considerable interest in the use of alternative therapies and natural remedy is attracting the attention of many researchers. *Terminalia Chebula,* an evergreen plant has been extensively used in ayurveda, unani & homoeopathic medicine and has become cynosure of modern medicine. *T. Chebula* possesses a wide variety of activities like antimicrobial[2], antioxidant[3], antiviral[4], anticarcinogenic[5], hypocholesterolemic[6], radioprotective[7], antispasmodic & antipurgative[8]. Fruit[9] and leaf[10] extract of this plant also have shown a good amount of wound healing potentiality with improved rate of contraction and a decreased period of ephithelialization. Much of the effectiveness of *T. chebula* in many of its medicinal uses is attributed to its antibacterial activity. *T. chebula* has been shown to be effective against bacterial strains including those that have become resistant to synthetic antibiotics.

Terminalia belerica, a deciduous tree belongs to the family Combretaceae which is available throughout the Indian forests and plains. Its fruits are extensively used in the treatment of diarrhea, dysentery, cough, hepatic diseases and leprosy[11-13]. Wound healing activity of Terminalia belerica fruits was showed by Chaudhary[14]. But there are hardly any literatures which showed the combine therapy of these two fruits.

Considering the above, present work was undertaken to explore the mechanism of action of *T. chebula* and *T. belerica* on wound healing in rabbits.

Material and methods

Preparation of paste

The dried fruits of *Terminalia chebula* and *Terminalia belerica* were collected and crushed thoroughly in a grinding stone separately. When they were converted into fine powder, 500 gms of each of Harida and Bahada were mixed and the 2500ml of water was added to boil for 30 mins. Then the mixed pasty preparation was kept overnight at room temperature and was allowed to cool. Now, the herbal pasty preparation was ready for application.

Animals

Clinically healthy male and female rabbits approximately 30-35 weeks of age and weighing between 900-1200 gm were used in this study. Throughout the period of experiment, the animals were caged individually in galvanized rabbit cage containing sterile paddy husk bedding. The temperature of the experimental animal room was maintained at 22°C (±3°C) and artificial lighting facilities, *ad libitum* drinking water and standard pelleted feed (Hindustan Lever) were provided. The animals were acclimatized in experimental room for 7 days prior to commencement of the experiment. Each galvanized cage was disinfected with hot water and phenol before using. Each day, floor of the experimental room was cleaned and disinfected with phenol.

Study designs
The animals were randomly divided into group A and B containing 15 rabbits each. Each group was again divided into 3 sub groups consisting 5 animals each for 4, 8 and 12 experimental days. Group A received NSS topically (control) while the herbal paste (*Terminalia chebula* and *Terminalia belerica*) was applied topically to each animal of group B.
The study was approved by the Institution animal ethics committee, West Bengal University of Animal and Fishery Sciences, Kolkata-37, India.

Wound model
The animals were anaesthetized locally with lignocaine hydrochloride (Xylocain® 2%) at 1ml/animal surrounding the area. A surgical wound in the form of a skin incision was made by cutting 1 square inch (2.54 cm x 2.54 cm) piece of skin from the dorsal area just behind the shoulders with clean conditions. All wounds were of full thickness extending down to sub-cutaneous tissue.

Dosing Schedule
Treatment was started 24 hours after induction of wound in group B. A sufficient amount of herbal paste preparation @500 mg/animal was applied over the lesion. Wounds were air exposed and treated once a day at a particular time. But only normal saline solution (NSS) was applied to animals of group A (Control).

Sampling
All the 5 animals in each sub group were anesthetized on 4, 8 and 12 days post wounding. Granulation tissues were removed, (great care was taken to include only granulating tissue in the assay) weighed and then analyzed for Hydroxyproline[15] , DNA[16] and uronic acid[17] .

Statistical analysis
The results were expressed as Mean ± Standard error (S.E.). The data were analyzed statistically using general linear model with univariate data in SPSS 10.0 version of software.

Results

Hydroxyproline content (mg/100 mg dry tissue) in granulation tissue of experimental groups in respective 4, 8 and 12 days of treatments were 1.55±0.05,0.99±0.22 and 0.62±0.11 compared to 0.55±0.05, 0.45±0.05 and 0.40±0.03 respectively in control animals(Figure 1).

DNA content (mg/g tissue) in granulation tissue of experimental group in 4, 8 and 12 days of treatments were 5.84±0.48, 4.92±0.50 and 3.17±0.37 while in case of control group these were respectively 4.08±0.41, 2.19±0.34 and 1.28±0.23 (Figure 2).

Uronic acid content(µg/100mg tissue) in granulation tissue taken from induced wound of rabbits in 4,8 and 12 days of treatment were 7.01±0.63,3.87±0.48 and 3.15±0.44 in experimental group while these were 2.86±0.40,2.85±0.37 and 0.99±0.16 respectively in control group (Figure 3).

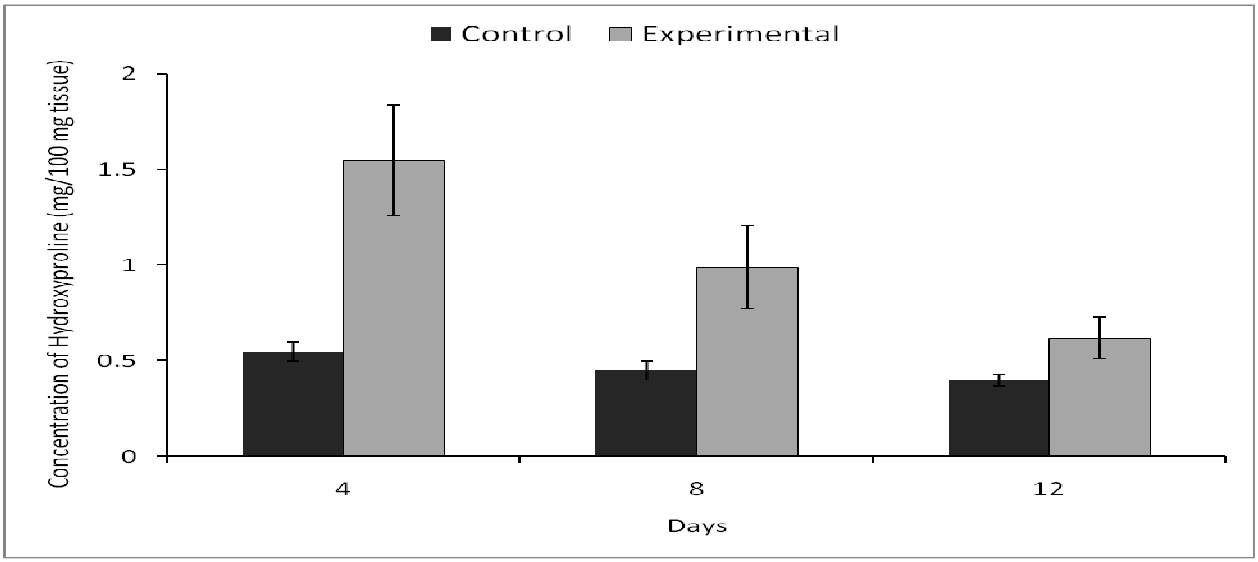

Fig 1. Effect of paste prepared from *T. chebula* and *T. belerica* on collagen (hydroxyproline) content (mg/100 mg tissue) in granulation tissue taken from induced wound of rabbits following consecutive daily application @500 mg/wound on different days (n=5).

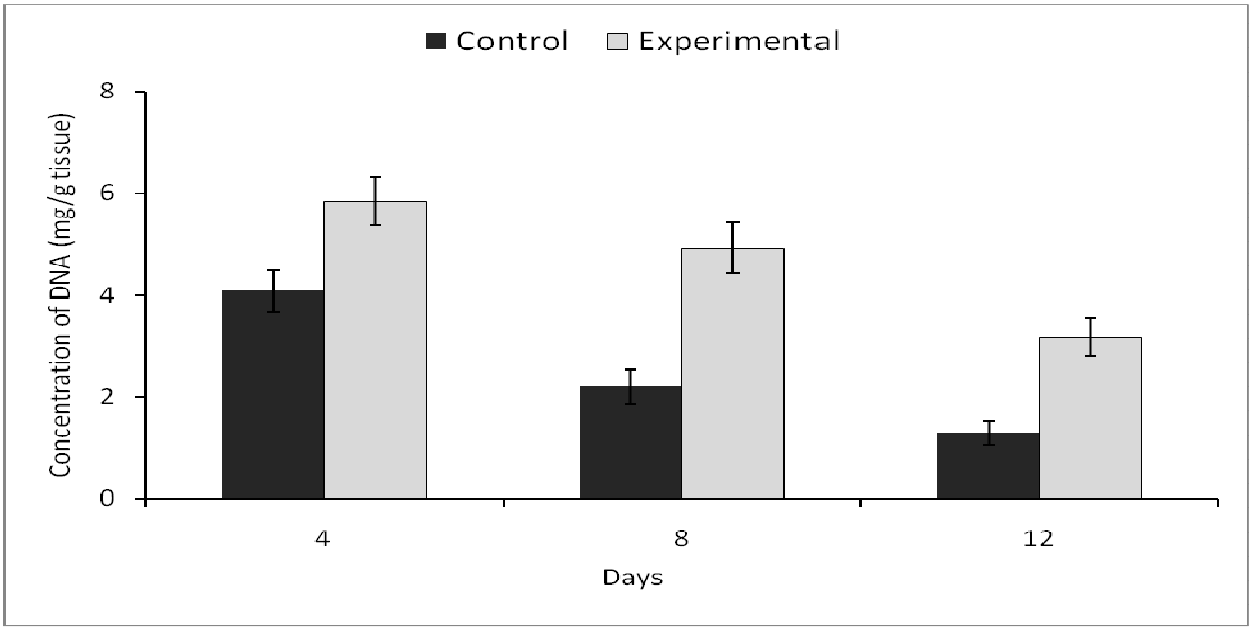

Fig 2. Effect of paste prepared from *T. chebula* and *T. belerica* on DNA content (mg/g tissue) in granulation tissue taken from induced wound of rabbits following consecutive daily application @500 mg/wound on different days (n=5).

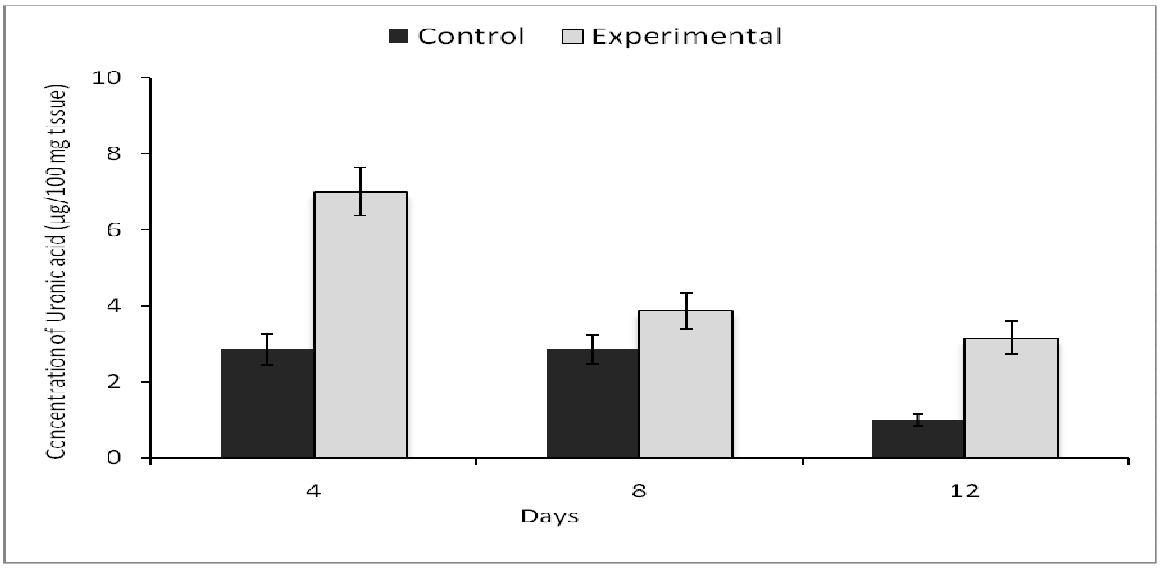

Fig 3. Effect of paste prepared from *T. chebula* and *T. belerica* on uronic acid content (µg/100 mg tissue) in granulation tissue taken from induced wound of rabbits following consecutive daily application @500 mg in rabbits on different days (n=5).

Discussion

Wound healing is a complex but orderly phenomenon involving a number of processes which include migration and proliferation of both epithelial and connective tissues, synthesis of extracellular matrix (ECM) proteins, remodeling of connective tissue and parenchymal components and collagenization and acquisition of wound strength.

Synthesis of collagen is initiated by DNA transcription from specific gene coding for the polypeptide chains. This is followed by synthesis of alpha chains on ribosomes. The alpha chains then come off the ribosome into the cisternae of the rough endoplasmic reticulum (RER). Thereafter they undergo a series of biochemical modifications; one important change being the hydroxylation of the amino acid proline. This provides collagen with its characteristic high content of hydroxyproline and provides tensile strength to healing wounds since collagen content was always higher in the combined treated groups. The activation of collagen synthesis was globally more efficient than catabolism. Wound remodeling is however, an important feature of the healing process.

Increased level of hydroxyproline suggests higher collagen content in the granulation tissue in experimental animals treated with paste of *Terminalia spp.* Since fibroblasts are responsible for the synthesis of collagen in the newly formed granulation tissue, one would expect that any increase in fibroblasts proliferation would more or less result in an increase in collagen

deposition[18]. The beneficial effect of herbal paste on scar management appears to be the stimulation of naturation of the scar by the production of type 1 collagen and the resulting decrease in the inflammatory reaction and myofibroblast production[19-21]. Combined application with *Terminalia chebula* and *Terminalia belerica* significantly increased the cells proliferation in nearly formed granulation tissues as evidenced by higher levels of DNA and corroborated the findings of Suguna et al.[10]. The DNA level reflecting fibroblasia showed maximum concentration on 4 day post wounding. An early and rapid fibroblastic and angiobalstic activity was also observed in *Terminalia chebula* applied wounds. It might be expected that combined treatment may stimulate the growth factors resulting in increased DNA synthesis in competent cells. Under certain conditions, fibroblasts can differentiate into a cell type structurally and functionally similar to smooth muscle and that this cell, the 'myo-fibroblast', plays an important role in connective tissue contraction.

Glycosominoglycans are made up of repeating disaccharides containing uronic acid and hexosamine. These are the first components of extracellular matrix (ECM) to be synthesized during wound healing and form the template for collagen and elastin deposition. The higher level of uronic acid in combined *T. chebula* and *T. Belerica* treated animals indicates an increased synthesis of glycosaminoglycans. Since fibroblasts are responsible for the synthesis of glycosaminoglycans, it is expected that its synthesis doesn't occur until fibroblasia is established. It is the fat that glycosaminoglycans, especially hyaluronic acid and small proteoglycans, play a major role in the healing process and contribute to the organization and strength of the fibrillar network of the wound[22]. Glycosaminoglycan which is a part of proteoglycan retain fluid in the tissue and their major function is to maintain the normal shape and volume of connective tissue. Thus, they regulate the structure and permeability of connective tissue and subsequently modulate cell growth.

In conclusions, the paste obtained from *T. chebula* and *T. belerica* stimulate fibroblast function, enhance synthesis of glycoseminoglycans and deposition of collagen and offers a distinct advantage to wound healing and as such, may be a useful adjuvant in wound healing.

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