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## PRECLINICAL EFFICACY AND SAFETY OF HERBAL FORMULATION FOR MANAGEMENT OF WOUNDS

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### ABSTRACT:

**Background:** Medicinal plants in Tripathi hills and other developing countries have been scientifically demonstrated to have medicinal benefits but few or none have been translated to products for clinical use. Most herbal products developed by local herbalist and sold to the public are not standardized and lack efficacy and safety data to support use.

**Objective:** to evaluate the medicinal plants for its wound management and test its preclinical safety and efficacy using rat models.

**Methods:** Thirty Wistar albino rats were randomly divided into five groups and wounds were surgically created on the mid dorsal region. The wounds were treated topically and group I served as control group administered with distilled water, group II served as standard, Group III served with MESP, group IV treated with MESI and the group V was treated with METR. The effects of the treatment on the rate of wound closure were assessed.

**Results:** the plant extracts had significantly higher rate of wound closure which was better than distilled water. The significancy was found to be  $P < 0.01$ .

**Conclusion:** In this study, the plant extract showed significant accelerated wound healing in normal Wistar albino rats. The extracts showed good safety profile in experimental animals and better efficacy than standard drug. Among the three plants METR showed more significancy than MESP and MESI. We therefore recommend that the METR can be tried in clinical setting.

**INTRODUCTION:** In India, herbal origin is used for various diseases, and Indian folk medicine is used as prescriptions for therapeutic purposes such as healing of wounds, Inflammation, skin infections, leprosy, diarrhea, scabies, venereal disease, ulcers, snake bite, etc.

For wound management the herbal formulations involved as disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process <sup>1</sup>. Wounds arise due to physical, chemical or microbial agents. Wound is defined as a break in the epithelial of skin or tissue <sup>2</sup>. Wound healing is a complex but dynamic process of restoring damaged tissue that involves several interrelated events. Faster healing of wounds is paramount because the skin is the organ through which the body interacts with the environment.

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Many plants have been found to have wound healing activities owing to the vast array of medicinal compounds synthesized by these medicinal plants<sup>3-5</sup>.

A wound is a disruption of tissue integrity that result in damage and is typically associated with loss of function. Healing is survival mechanism and represents an attempt to maintain normal anatomical structure and function. Wound healing is a process by which tissue regeneration occurs. It is complex, dynamic process of restoring integrity and tissue layer, which involves an array of inter-related and concomitant events. The process of wound repair differs little from one kind of tissue to another and is generally independent of the form of injury.

Although the different steps in the wound healing processes occur in a continuous, integrated manner. It is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purposes. It involves regeneration of specialized cells by proliferation of surviving cells. Wound healing is divided into four sequential, yet overlapping phases, homeostasis, inflammatory phases, proliferative phase, and remodelling phase. Wounds are generally classified as wounds without tissue loss (e.g. in surgery) and wounds with tissue loss, such as burn wounds, abrasions or as secondary events in chronic ailments e.g. venous stasis, diabetic ulcers, or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions.

Proper healing of wound is essential for the restoration of disrupted anatomical continuity and disrupted functional status of the skin. Deficiency of certain vitamins, trace elements and proteins lead to delayed wound healing. Many cytotoxic, immunosuppressant and NSAIDS drugs suppress wound healing. Many herbs have proved to possess significant prohealing properties in different types of wounds. In this review we have made an attempt to give an insight into the different herbs having potential wound healing properties which could be beneficial in therapeutic practice<sup>6</sup>.

However Mother Nature has been kind to us by creating various herbs which assist in the healing process. This treatment provides fibrogenetic and concentration of collagen resulting in faster.

The drugs selected for this work are *Solanum pubescens*, *Sophora interrupta* and *Tabebuia rosea*.

## METHODS:

**Study materials:** All the plants were collected from S.V. University and authenticated by a Botanist<sup>7</sup>, Dr. Madhavachetty and deposited at the Pharmacognosy Laboratory Herbarium with different specimen number. The dry plant materials were powdered and extracted using methanol by continuous hot percolation for 24 hrs. Methanol was then removed using rotary evaporator at low temperature and the semi-solid concentrated used as the test product. The plants used for the study are *Solanum pubescens*, *Sophora interrupta* and *Tabebuia rosea*. *Solanum pubescens* belong to the family Solanaceae was commonly used in India by tribal people for the treatment of liver disorder, diarrheal diseases and cancer. It is commonly called as pajarito. The plant contains phytoconstituents like flavonol-O- methyl ethers, Solanopubamine a steroidal alkaloid and with different pharmacological property<sup>8</sup>.

*Sophora interrupta* Bedd. Belongs to the family Fabaceae and is commonly called as Edwardeia maderaspatana wt and in Telugu "Adavibillu". This is a woody perennial shrubs the leaves are odd – pinnate, leaflets are sub opposite and broadly ovate. More than 15 species is there with long history in Chinese traditional medicine<sup>9</sup>.

*Tabebuia rosea* (Bertol).D.C, belong to the family Bignoniaceae, commonly known as "Pink Trumpet tree" grow up to 15 meter and well known for its beautiful flowers. The Timber is widely used for general construction and carpentry in many European countries it is used in allopathic medicine as potential sources of antiviral, antitumoural and antimicrobial agents<sup>10</sup>.

Tea made from the leaves and bark is known to have a fever reducing effect. The herbal products obtained from the bark of *Tabebuia* trees are called Tahebo, Lapacho, Pandarco and iperoxo. Traditionally, Tahebo has been used for treating ulcers, syphilis, gastrointestinal problems candidiasis, cancer, diabetes, prostatitis, constipation and allergies.

## EXPERIMENTAL DESIGN:

**Excision Model:** An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm<sup>2</sup> diameter homeostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound closure in the first two weeks, were studied by tracing the wound on a transparency paper initially. Then an impression was taken on a millimeter scale graph paper, scar area after complete epithelization and time for complete epithelization in days was evaluated to calculate the degree of wound healing.

The parameters were studied were wound closure, epithelization time and scar features. The observation of the percentage wound closure were recorded on 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> post wounding day and also the epithelization and size and shape of the scar area. The animals were starved for 12 hours prior to wounding. Studies were carried out using ether anaesthetized rats. The rats were divided into five groups (n=6). Animals were depilated at the dorsal thoracic region before wounding. The first Group-I served as control, similar second Group-II served as a positive control and third, fourth and fifth Group III, IV & V served as a Methanolic extract of *Sophora interrupta* (MESI), Methanolic extract of *Tabubeia rosea* (METR), *Solanum pubescens* (MESP), and at a dose of 400 mg/kg, 500 mg/kg, 300 mg/kg respectively, body weight by oral route daily for 20 consecutive days<sup>11</sup>.

**Statistical analysis:** The data for wound contraction rate and epithelisation time were analysed by ANNOVA followed by Dunnet's test. The statistical significance was set for P<0.05.

**Ethical consideration:** This study was cleared by the IACE and the ethics committee number 1447/PO/a/11/CPCSEA the study animals were handled humanely as per the CPCSEA guidelines.

**RESULT AND DISCUSSION:** The results of the present study revealed that animals treated with methanol showed faster rate of epithelialisation in excision wound model.

Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar<sup>12</sup>.

Flavonoids and triterpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation<sup>13, 14</sup>. Drugs which influence one phase may not necessarily influence another. Hence different models are used to assess the effect of various phases, which run concurrently, but independent of each other. Control group wound showed granulation tissue and fibroblast aggregation. Wound healing comprises of different phases such as contraction, epithelialization, granulation and collagenation<sup>15</sup>. It normally involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibres and shrinking, occurring concurrently but independent of one another<sup>16</sup>.

Wound healing effect is also attributed to free radical scavenging activity of Flavonoids<sup>17</sup>. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing onset of cell necrosis, but also by improving vascularity. Lipid peroxidation is an important process in several types of injuries like burns, infected wounds and skin ulcers.

Hence, any drug that inhibits lipid peroxidation is believed to increase strength of collagen fibres, by increasing circulation or by preventing cell damage or by promoting DNA synthesis<sup>18</sup>.

The extract was able to promote epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells. Some ingredients for regulating mechanisms due to which there was acceleration in wound repair. All stages of wound repair process are controlled by a wide variety of different growth factors and cytokines<sup>19</sup>. Beneficial effect of many of these growth factors e.g. Platelet derived growth factors PDGFs, fibroblasts growth factors (FGFs) and granulocytes macrophages colony stimulating factors (GM-CSF) on the healing process have been demonstrated.

In wound healing mechanism following the migration of platelets, the first response cells, neutrophils and macrophages migrate to the wound. Numerous enzymes and cytokines are secreted by macrophages and neutrophils. Among these TNF- $\alpha$  is the one which stimulates the angiogenesis, helps to build up the tissue granulation bed and thus has significant potential to improve the healing process. Plants may exert their effect by modulating the cytokine(s) secretions during different conditions. TNF- $\alpha$  is a major cytokine secreted by macrophages and neutrophils during the inflammation phase.

TNF- $\alpha$ , a macrophage derived cytokine, is also known to play a major role in the inflammatory phase of wound healing by enhancing angiogenesis<sup>20</sup>. During the early phase of wound repair, TNF- $\alpha$  was predominantly expressed in polymorphonuclear leukocytes suggesting a normal function of these cells in the initiation of wound healing<sup>21</sup>. TNF- $\alpha$  inhibits collagen formation and hydroxyproline production which are essential for the final part of proliferation phase in wound healing<sup>22</sup>.

**TABLE 1: EFFECT OF METHANOLIC EXTRACT OF SOLANUM PUBESCENS, SAPHORA INTERRUPTA AND TABEBUIA ROSEA LEAVES ON EXCISION WOUND HEALING**

GROUP	4 days	8 days	12 days	15 days	20 days
Control	28.83±1.887	32.33±1.687	43.83±1.51	52.33±1.764	63.50±1.258
Standard	43.67±1.606**	65.83±1.641**	73.67±1.43**	80.67±1.892**	93.50±1.408**
M.E.S.P(T1)	34.33±1.453*	37.67±1.202*	43.00±1.183*	51.00±1.789*	55.00±1.390**
M.E. S.I(T2)	36.83±2.301*	38.83±1.641*	46.33±1.838*	53.67±1.542*	63.50±1.668*
M.E. T.R(T3)	35.50±2.156*	44.83±1.973**	61.17±1.662**	72.00±1.844**	84.17±2.786**

Each value is the mean  $\pm$  SEM for 6 mice, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnett's test, standard (Morphine 10mg/kg, of B.W), METR- Methanolic extract of *Tabebuia rosea*, dose (500mg/kg of B.W) MESI- Methanolic extract of *Sophora interrupta* (300mg/kg of B.W), MESP- methanolic extract of *Solanum pubescens* (300mg/kg, B.W).

**Statistical analysis:** All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The level of significance was set at P<0.05.

**CONCLUSION:** In this study the plant extract showed significant accelerated wound healing in normal wistar albino rats. The extracts showed good safety profile in experimental animals and better efficacy than standard drug. Among the three plants METR showed more significance than MESP and MESI. We therefore recommend that the METR can be tried in clinical setting.

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