Journal of Traditional and Complementary Medicine 7 (2017) 519–525

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Wound healing potentials of *Thevetia peruviana*: Antioxidants and inflammatory markers criteria

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
17 December 2016
Accepted 11 January 2017
Available online 14 February 2017

Keywords:
T. peruviana
Wound healing
Antioxidants
Anti-inflammation
Antimicrobial

ABSTRACT

Thevetia peruviana is a medicinal plant used in the treatment of external wounds, infected area, ring worms, tumours etc. in traditional system of medicine. The aim of the study was to evaluate the wound healing potentials of *T. peruviana* leaves hexane (LH) and fruit rind (FW) water extracts and to prove the folkloric claims. The antimicrobial, antioxidant and anti-inflammatory potentials could be important strategies in defining potent wound healing drug. Based on these approaches the current study was designed using incision, excision and dead space wound models with the biochemical, antioxidant enzymes and inflammatory marker analysis. The fruit rind water extract showed highest WBS of 1133 ± 111.4 g. The extracts in excision model retrieved the excised wound i.e. complete healing of wound at day 14. The hydroxyproline content of FW and LH treated dry granuloma tissue was increased to 65.73 ± 3.2 mg/g and 53.66 ± 0.38 mg/g, accompanied by elevations of hexosamine and hexauronic acid with upregulation of GSH, catalase, SOD, peroxidase and the down regulation of the inflammatory marker (NO) and oxidative stress marker (LPO) in wet granulation tissue was documented. Conclusively, both the extracts showed enhanced WBS, rate of wound contraction, skin collagen tissue development, and early epithelisation. Therapeutic wound healing effect was further proven by reduced free radicals and inflammatory makers associated with enhanced antioxidants and connective tissue with histological evidence of more collagen formation. The present research could establish *T. peruviana* as potential source of effective wound healing drugs.

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1. Introduction

Healing of wound is the sign of growth and an important biological phenomena. Whether acute or chronic wounds can compromise an individual's wellbeing, self-image, workability and freedom.[17] The impact of wounds on physical, social and financial sectors of a person's life necessitates good wound management not only for the individual but also for the community.[12] The long history of wound care is practiced in, Tibb-e-Nabawi (SAW) in Arabic, use of smoke and hot meat burns in African, Medical Qi Gong and Feng Shui in Chinese and Varna Shodan and Varna Ropan in Indian systems of medicines.[3] The disruption of cellular and structural integrity of the skin tissue strata is called as wound and restoration of the same is healing. The variance in the number of phases and phase description diverges from author to author. In general four phases of wound healing are portrayed as hemostasis, inflammation, granulation and remodelling.

Plants have served as the healing agents for ages, like *Ficus bengalensis*, *Curcuma longa*, *Centella asiatica*, *Aloe vera*, *Cynodon dactylon*, *Rubis cordifolia*, *Ficus recemosa*, *Glycyrrhiza glabra*, *Berberis aristata*, are few medicinal plants, explored for their phyto-constituents and extensively been used in modern medicines too.[12] Beholding the present consequences the phyto-components could provide an excellent fountainhead to develop new wound healing drugs which will be more efficacious, safer and affordable for patients.

There are serious factors that postpones the process of wound healing by means of persuading tissue damage such as, repeated injury, infection, oxygenation, free radical generation. Hence, to tackle the above mentioned situations the use of a drug having

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2017.01.005

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antimicrobial, antioxidant and anti-inflammatory potentials could be an important tactic in healing of wounds. The ethno-medical uses of *Thevetia peruviana* is evident in treating the external wounds, infected area, ring worms, tumours etc., the use of grinded leaves of *T. peruviana* in ethno-veterinary medicine is the evidence for its plenteous use for healing of wounds.[22,25,44]

The plant has stolen the limelight with the existence of novel components having excellent therapeutic values such as cardiovascular effects, anticancer, antimicrobial, antioxidant, immunomodulatory and anti-inflammatory activities.[38] However, the lack of systematic information on wound healing potentials, despite the presence of voluminous reports regarding the pleotropic properties of the plant has called for the scientific validation of wound curing ethno medical claims of *T. peruviana*. To fulfil the lacuna between the folkloric use and scientific exploration, the wound healing efficacy of *T. peruviana* has been evaluated in the present study based on the above mentioned strategy.

2. Materials and methods

2.1. Plant material collection

Unripe fruits and leaves of *T. peruviana* (S) were collected from the surroundings of Kuvempu University, Shankaraghatta, Shimoga Dist., Karnataka, India. Plant identification was carried out by referring Flora of the Presidency of Madras (Gamble, 1915–1935).[8] The plant was further authenticated by Prof. V. Krishna, Taxonomist, Dept. of Biotechnology, Kuvempu University. The specimen was deposited at the Department of Biotechnology. As per the WHO published manual namely 'Quality control methods for herbal materials' the quality control of any herbal drug is based on three important criteria i.e. botanical identification, phytochemical screening, and content standardization.[34,36] In the present study all the above mentioned criteria are fulfilled to control the quality of the drugs.[36]

2.2. Chemicals

Hexane, chloroform, ethanol, sodium carbonate, NBT, EDTA, hydroxylamine hydrochloride, hydrogen peroxide, sulphosalicylic acid, DTNB, KCl, ferric chloride, HCl, TCA, TBA, BHT, naphthylethylene diamine dihydrochloride, sodium nitroprusside, sulphanilic acid, NaOH, H_2SO_4, para-dimethyl amino-benzaldehyde, n-Propanol, Na_2CO_3, Borax, carbazole etc. were purchased from Sigma–Aldrich, Merck and Himedia. All the chemicals and solvents used were of analytical grade.

2.3. Soxhlet extraction

Successive extraction was done using 500 g of powdered material of leaves and fruit rind material in soxhlet apparatus. The solvent hexane (2L, 50 °C˜15 cycles) was used for leaves extraction (LH), and for fruit rind material after successive extraction with hexane (2L, 50 °C˜15 cycles) chloroform (2 L, 45 °C˜15 cycles) and ethanol (2 L, 70 °C, ˜15–17 cycles) the cold water extraction (FW) was performed using 9:1 ratio of water and ethanol for 24 h. The LH and FW extracts were concentrated *in vacuo*. The yield of each dried extract was calculated. The available reports suggest that the hexane extract predominately contains most of the nonpolar components of plant metabolites like oils, fats, waxes. Currently, the study was designed to investigate the therapeutic potentials of extracts containing sole nonpolar and high polar phytochemicals. Therefore, the leaf hexane extract was selected for its nonpolar phytochemicals as the leaf material is evidenced with high content of latex, oils and fats.[36] The fruit rind of *T. peruviana* is evidenced with high carbohydrate content,[36] which are reported for wound healing[31] and hence it was selected for extraction with water: ethanol (9:1) as this combination of solvents dissolves most of the high polar components.[5]

2.4. Animals

Male Wister albino rats weighing 150–230 g were utilised, maintained under standard laboratory conditions (12 h light/darkness; at 25 ± 3 °C) with standard animal diet and water available ad libitum. The Institutional Animal Ethical Committee permitted the studies under the certification (Ref. No. NCP/IAEC/CL/247/2013-14).

2.4.1. Incision wound model

Incision wound model was taken up to evaluate the wound healing potentials of *T. peruviana* extracts in terms of tensile strength methods of Lee 1968 and Murthy *et al.*, 2013 were employed.[18,24] Twenty four (24) rats weighing 150–190 g were evenly distributed into four groups of six animals each grouping was done in following fashion: Control (ointment base), Positive control (povidone-iodine), LH and FW 5% w/w ointments [5 g of extract was mixed with 100 g of cream base (white petrolatum) until a uniform preparation is attained to prepare 5% w/w extract ointment] respectively.[11] Animals were anaesthetized with ketamine (30 mg/kg, i.p.), 6 cm paravertebral incisions were made through the full thickness of the skin. Wounds were sealed with interrupted sutures 1 cm apart, the extract ointment was topically applied up to 10 days. Sutures were removed on the 7th post wounding day. Wound breaking strength (WBS) was measured on the 10th post wounding day in anaesthetized rats. The wound breaking strength is expressed as the minimum weight (in grams) of water necessary to dragging apart the wound edges.

2.4.2. Excision wound model

To study the excision wound model twenty four (24) animals were evenly distributed into four groups of six animals each in the following order: Control (untreated), povidone-iodine, LH and FW extracts (5% w/w ointment). Rats were anesthetized with ketamine (30 mg/kg, i.p.) followed by a circular wound on the dorsal thoracic region of about 500 mm^2 was made. Wounds were traced on graph paper on the day of wounding and consequently on the alternate days until wound restoration was complete. The rate of wound contraction was calculated by the formula.[24]

$$\%\ \text{wound contraction} = \frac{\text{Healed area}}{\text{Initial wound area}} \times 100$$

Where, Healed area = Initial wound area − Remaining wound area.

2.4.3. Dead space wound model

In dead space wound model study the animals were divided into four groups of six animals each in the following fashion: Control (1 ml/kg of 1% gum acacia p.o.), vitamin E (VTE 200 mg/kg b. w. p.o.), LH and FW extracts (200 mg/kg b. w. p.o.) respectively up to 10 days. Rats were anesthetized with ketamine (30 mg/kg, i.p.) incision of about 1 cm was made on both the dorsal paravertebral sides followed by grafting of sterilized cylindrical grass piths (2.5 cm × 0.3 cm) wounds were closed with sutures.[11] After 6 h of interval of the last dose on the 10th post wounding day, the animals were sacrificed and granulation tissue formed around the implanted piths were carefully removed out, weighed, and processed for the estimation of free radicals, antioxidants, and collagen tissue parameters.[24]

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