Journal of Ayurveda and Integrative Medicine 7 (2016) 14–22

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original research article (Experimental)

Preliminary investigation for wound healing and anti-inflammatory effects of *Bambusa vulgaris* leaves in rats

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ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form
6 July 2015
Accepted 31 July 2015
Available online 11 April 2016

Keywords:
Anti-inflammatory
Bambusa vulgaris
Wound healing

ABSTRACT

Background: *Bambusa vulgaris* (Family: Poaceae) used in Ayurveda for paralytic complaints, inflammatory disorders and externally to skin disorders. It has various medicinal uses with good nutritional composition and a rich source of vitamins, proteins, amino acid, beta-carotene and phenolic compounds.
Objective: The present study was aimed to evaluate wound healing and anti-inflammatory potential of ethanol extract of *B. vulgaris* leaves in rats.
Materials and methods: The *B. vulgaris* leaves were evaluated for wound healing on incision and excision wound methods. Anti-inflammatory effect was evaluated by measurement of paw edema in carrageenan-induced inflammation in rats. Ethyl acetate (BVL-A) and aqueous (BVL-B) fractions from the ethanol extract of leaves were screened for wound healing effects by measuring tensile strength and biochemical parameters in incision wound method. The wound contraction area, antioxidant status and histopathological studies were done in excision wound method.
Results: Tensile strength and hydroxyproline level of 5% w/w ointment of BVL-A and BVL-B treated groups were found significantly ($P < 0.01$) higher and comparable to the reference group. The histopathological study showed the proliferation of collagen, fibrous tissue, and capillaries with epidermal covering at the margin of the wound. The percent inhibition of paw edema was significantly decrease by increasing concentration of BVL-A and BVL-B fractions. In addition, it was found that *B. vulgaris* possesses antioxidant properties, by its ability to increase antioxidants level.
Conclusions: The results obtained in the present study were indicated that ethyl acetate fraction of *B. vulgaris* leaves inhibits paw edema and accelerates cutaneous wound healing.

1. Introduction

Wound healing is a normal biological process in the human body that includes three overlapping phases: inflammation, tissue formation, and remodeling. It involves soluble mediators, blood cells, parenchymal cells, and extracellular matrix [1]. As the blood components fall into the injury site, the platelets move toward into contact with exposed collagen. This result, the platelets releases clotting factors and essential growth factors. In hemostasis, the neutrophils go in to the wound site and begin phagocytosis to remove bacteria, foreign materials, and damaged tissue. The macrophages appear in the inflammatory phase and continue the phagocytosis process. Once the wound site is cleaned out, fibroblasts migrate to start the tissue formation and deposit new extracellular matrix. The new collagen matrix organized by cross-linking during the final remodeling phase [2].

Herbal medicines are being used by about 80% of the world population for primary health care due to their efficacy, safety, cultural acceptability, and less side effects. The plant constituents are a part of the physiological function of living flora and hence they have better compatibility with the human body [3]. Scientists who are trying to develop newer drugs from natural resources are looking toward the Indian traditional system of medicine, Ayurveda. Numerous drugs from plant, animal and mineral origin are described in the Ayurveda for their wound healing properties under the term Vranaropaka. According to the Ayurveda, *Vrana*

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Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2015.07.001

(wounds) is the discontinuation of the lining membrane that leaves a scar for life after healing, closely similar to the modern definition. Similarly, inflammation is an early phase in the pathogenesis of wounds termed *Vranashotha*. Due to a defect in human functional units, different types of wounds are mentioned in Ayurveda, such as *Vata*, *Pitta*, and *Kapha* types or exogenous due to trauma, such as *Chinna* (cut wound), *Bhinna* (perforated wound), *Kshata* (lacerated wound), *Viddha* (punctured wound), *Picchita* (contusion), and *Ghrista* (abrasion wound). These steps have striking similarities with wounds described in modern medicine [4].

The current treatment for the wounds are the application of silver products, steroids, advanced dressings and skin substitutes, negative pressure wound devices, growth factor, and hyperbaric oxygen [5]. All these therapies are including issues of resistance and are costly. The plant constituents have a dual mechanism of action, not only they enhance wound quality and closure rates, but they can also act as an antimicrobial agent which is an important clinical property in wound healing.

Bambusa vulgaris, commonly known as bamboo is taxonomically a grass, but its habit is tree-like. The leaves of *B. vulgaris* revealed that it contains crude protein of 10.1%, phosphorus 86.0 mg/100 g, iron 13.4 mg/100 g, vitamin B1 0.1 mg/100 g, vitamin B2 2.54 mg/100 g, and carotene 12.32 mg/100 g [6]. Bamboo leaves have been claimed to be used as an astringent, ophthalmic solution, emmenagogue, vulnerary, and febrifuge to heal the wounds and to control diarrhea in cattle. In ayurvedic medicine, leaves are traditionally used in paralytic complaints and to treat various inflammatory conditions [7]. Evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy leads to the discovery of newer and cost-effective drugs for treating various ailments. *B. vulgaris* has an impressive range of medicinal uses with high nutritional value and serves as a good source of vitamins, proteins, amino acid, beta-carotene, and various phenolics [6,8]. It was reported to possess antimicrobial action [9,10], antidiabetic [11], antioxidant [12], and abortifacient properties [13]. It has also been reported to possess antidiabetic activity through antioxidant nature. Stem decoction of *B. vulgaris* is used to control menstrual pain [14]. Leaves are used in against fever and diabetes [15]. *B. vulgaris* is used for stomach problems, pain, and internal parasites. It is used for skin problems in Trinidad and Tobago [16]. In Nigerian folklore medicine, bamboo is used as an emmenagogue, abortifacient, appetizer, and for managing respiratory diseases as well as gonorrhea [17]. Leaves and stems are used by the tribes of Raisen, Madhya Pradesh, to treat healing of skin injuries topically. Though the plant and its extracts have been used in the folklore medicine extensively, there is no scientific evidence for wound healing activity of the plant. The present work, therefore, was aimed to evaluate wound healing activity of different fractions of *B. vulgaris* leaves in rats.

2. Materials and methods

2.1. Animals

Wistar albino rats (150–200 g) of either sex were selected for the experiment. Animals were procured from Defense Research and Development Establishment, Gwalior, MP, India. They were housed individually in well-ventilated, temperature controlled (26 ± 2 °C) animal room for 7 days of the period prior experiment. The animals were given the standard commercial pellet rodent diet (Hindustan Lever Pvt., Ltd., Bengaluru, India) and water ad libitum. The procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. No. 1471/P0/a/11/CPCSEA).

2.2. Chemicals and reagents

Petroleum ether (60–80 °C), ethyl acetate, glacial acetic acid, sodium tartrate, copper sulfate, sodium carbonate, hydrochloric acid, chloroform, and perchloric acid were purchased from Ranbaxy Fine Chemicals Ltd., Thane, India. All these chemicals were of analytical grade. Folin-Ciocalteu reagent, Ehlrich reagent, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were purchased from Sigma Chemical Co., Ltd., USA. Hydroxyproline, carrageenan, chloramine-T, and trichloracetic acid (TCA) were purchased from Hi Media Laboratories Pvt., Ltd., Mumbai, India.

2.3. Plant material

The leaves of *B. vulgaris* were collected from village Kukri kheda near to the college campus, during the month of January. Plant material was identified by Dr. M. K. Shrivastava, Department of Botany, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, M.P., India, and a voucher Specimen (No. BV/472/13) was submitted in the department. The plant materials were dried in the shade, powdered moderately, and pass through sieve No. 10.

2.4. Extraction and fractionation

The powdered leaves (2 kg) were defatted with petroleum ether (60–80 °C) and extracted with ethyl alcohol (95% w/w) for 24 h in soxhlet apparatus. The dried ethyl alcohol extract was suspended in distilled water and extracted with ethyl acetate in a separating funnel repeatedly. The removal of solvent from ethyl acetate fraction yielded a brown powdered product which gave positive Shinoda tests (magnesium hydrochloride reduction test) for the presence of flavonoids. The aqueous layer of the fraction was dried and stored for study. The dried ethyl acetate extract of leaves was referred as BVL-A, and aqueous fraction of leaves was referred as BVL-B, which were subjected to preliminary screening for wound healing and anti-inflammatory activity.

2.5. Phytochemical studies

The phytochemical screenings of ethanol extract were performed to detect the presence of different phytoconstituents by qualitative chemical tests. High-performance thin layer chromatography (HPTLC) fingerprinting of both fractions of *B. vulgaris* was carried out to study the presence of different phytoconstituents and detect the presence of ascorbic acid in the fractions.

2.6. High-performance thin layer chromatography fingerprinting and quantification of ascorbic acid

The HPTLC fingerprinting of ethyl acetate and aqueous fractions was carried out on a pre-coated silica gel plate (0.2 mm, Merck 60 F-254, Germany) as the stationary phase and chloroform: methanol:glacial acetic acid:water (8:6:1:2) as a mobile phase. The dried fractions were dissolved in methanol (10 mg/ml) and filtered the solutions. The samples (10 μL) of fractions and standard ascorbic acid (Sigma–Aldrich, USA) were spotted in the form of bands of width 6 mm with a 100 μL Hamilton syringe on pre-coated silica gel aluminum plate (10 cm × 10 cm) with the help of Linomat 5 applicator. The applicator was attached to HPTLC system CAMAG which was operated through winCATS software (CAMAG Scientific Inc., USA).

The linear ascending development was carried out in a 20 cm × 10 cm twin through glass chamber saturated with the mobile phase. The developed plate was dried by hot air to