Research Article

Wound closure activity of *Emblica officinalis* extract in comparison with methylsulfonylmethane

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ABSTRACT

Wounds are the result of injuries to the skin that causes disturbances or problems to other soft tissues. Healing of a wound is a complex and lengthy process of tissue repair and remodeling in response to injury. Various plant products have been used in wound healing since premodern era. It has been found that herbal extracts help in blood clotting, fight against infections, and help in accelerating wound healing process. Emblica officinalis, also known as Indian gooseberry or amla, is a deciduous tree of the family Phyllanthaceae. Here, in this study, the effect of oral and topical application of *E. officinalis* extract on excision wounds in human skin fibroblast cell lines was checked.

KEY WORDS: *Emblica officinalis*, Wound closure, Traditional Indian medicine

INTRODUCTION

Emblica officinalis, also known as *Phyllanthus emblica*, emblic,[1] emblic myrobalan, myrobalan,[1] Indian gooseberry,[1] Malacca tree,[1] or amla,[1] is a deciduous tree of the family Phyllanthaceae. It has edible fruit, which is usually referred as amla. It is found in the deciduous regions of tropical India and on the hill slopes up to 2000 m. It is commercially cultivated in the state of Uttar Pradesh in India and is also grown in Tamil Nadu, Rajasthan, and Madhya Pradesh.

In traditional Indian medicine, dried and fresh fruits of this plant were used. All parts of the plant are used in various Ayurvedic medicine and herbal preparations including the fruit, seed, leaves, root, bark, and flowers. In Ayurvedic polyherbal formulations, Indian gooseberry is a common element and the characteristic feature of this plant is that it is the principal ingredient in an ancient herbal dietary supplement called Chyawanprash. Amla fruit is sour and astringent in taste, with sweet, bitter, and pungent secondary tastes. Its post-digestive effects are cooling. The fruit is the richest source of Vitamin C and contains high amounts of ascorbic acid.[2] It has a bitter taste that may be derived from a high density of ellagitannins, such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%), and pedunculagin (14%).[3] It is believed to have a diuretic property as well.

Wound closure is the esthetic closure of traumatic or surgically induced wound and is based on the healing mechanisms, skin anatomy, as well as the selection of suture material and the closure technique. It is a critical and significant event, in general, as well as in oral surgery. Principally, the wound is defined as a lesion and rupture on skin surface that is caused by physical or thermal trauma, which needs medical therapy.[4] Wounds are the result of injuries to the skin that causes disturbances or problems to other soft tissues. Healing of a wound is a complex and lengthy process of tissue repair and remodeling in response to injury. Various plant products have been used in wound healing since premodern era. It has been found that herbal extracts help in blood clotting, fight against infections, and help in accelerating wound healing process. The skin protects the body's internal environment. Extensive damage to the skin may

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cause critical problems. Skin is made up of epidermis and dermis that is placed over the underlying connective tissue.[5] The epidermis consists of outermost layers and forms a protective barrier over the body's surface, responsible for maintaining water balance in the body and preventing pathogens from entering, thus forming a defense mechanism for the body. The epidermis is avascular and cells in the deepest layers are nourished by diffusion from blood capillaries extending to the upper layers of the dermis.[6] The dermis is the layer which is present beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils and elastic fibers, embedded in hyaluronan, and proteoglycans.[7] The epidermis and dermis are separated by a sheet called the basement membrane. The basement membrane not only controls the traffic of the cells and molecules between the dermis and epidermis but also serves, through the binding of a variety of cytokines and growth factors, as a reservoir for their controlled release during physiological remodeling or repair processes.[8]

The conventional method to screen the wound healing property of plant products is invasive procedures in animal models.[9] Many *in vitro* assays can be used to screen many plant products having antioxidant, cell mobilization, and angiogenic properties essential for wound healing. Moreover, plants are explored for their various pharmacological activities such as antihyperglycemic activity, anticancer, and antimicrobial activities.[9-12] Here, in this study, the effect of oral and topical application of *E. officinalis* extract on excision wounds in human skin fibroblast (HSF) cell lines was checked. *E. officinalis* extract is an herbal extract of the plant *E. officinalis* which has a good antimicrobial activity. In comparison, methylsulfonylmethane (MSM), an antimicrobial denture resin, is taken and the wound closure activity of these is compared with the standard drug, ascorbic acid (Vitamin C) which has been proved to have 100% wound closure property.

MATERIALS AND METHODS

The MSM powder and ascorbic acid were purchased from Sigma-Aldrich Company.

Preparation of MeOH Extract

E. officinalis (500 g) were refluxed with MeOH (3 × 3 L) for 3 h, and the filtrates were concentrated to dryness *in vacuo* at 40°C to render the MeOH extracts. These MeOH extracts were separately suspended in distilled H_2O.

Cell Culture

HSF cells were seeded in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum, 100 IU/ml penicillin, 2.5 µg/ml streptomycin, 2.5 µg/ml amphotericin B, and 50 µg/ml ascorbic acid, which was replaced twice a week and incubated in a 5% CO_2 humidified atmosphere at 37°C.

In Vitro Wound Closure Activity

HSF 1184 was used in this scratch wound closure assay to observe the migration capability of the cells wound closure. The cells were developed at a density of 3 × 105 cells/well in 6-well plate to 80% confluence. A straight line scratch was cut with a sterile 200 µL pipette tip (yellow tip; 2–200 µL, 53 mm) to create a 0.4 mm–0.5 mm width wound on the monolayer culture. Cell debris was washed with PBS twice and replaced with 3.0 mL extract of serum-free DMEM media (control), MSM (1–8 mg/ml), EOE (1–8 mg/mL), and ascorbic acid (5 mg/ml). All experiments were done in triplicate. The absorbance was measured at 570 nm. Wound closure at the same location was observed under inverted microscope at various intervals (0 h, 6 h, 12 h, and 24 h) and digitized images were taken.

RESULTS AND DISCUSSION

The wound closure was analyzed using Image J software. The percentage of wound closure was calculated using the equation:

$$\% \text{ Wound closure} = (W_{0h} - W_{xh})/W_{xh} \times 100\%$$

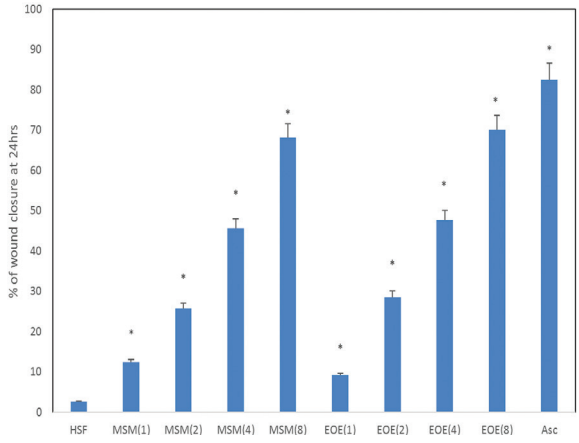
Where,
W_{0h} = Wound at 0 h
W_{xh} = Wound at "x" h ("x" = 0 h, 6 h, 12 h, and 24 h)

Statistical Analysis

Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance (ANOVA) and *post hoc* least significant difference test. $P < 0.05$ was considered statistically significant.

S. No	Treatment	Conc. (mg/ml)	% of wound closure at 24 h
1	HSF 1184 untreated cells	-	0±0
2	EOE	1.0	0.23±0.06*
3		2.0	0.99±0.04*
4		4.0	2.5±0.03*
5		8.0	6.9±0.02*
6	MSM	1.0	0.20±0.01*
7		2.0	0.89±0.07*
8		4.0	1.83±0.04*
9		8.0	6.1±0.02*
5	Ascorbic acid	5	8.5±0.05*

Values are expressed as Mean±SD (n=3); *P<0.05, as compared with HSF control. HSF: Human skin fibroblast, MSM: Methylsulfonylmethane



Results were expressed as mean ± SD ($n = 3$). *$P < 0.05$ significantly different as compared with HSF control.

Both the herb (*E. officinalis)* and MSM exhibited good wound closure property as compared to the standard drug, ascorbic acid. On comparison, it was observed that *E. officinalis* extract possessed a better wound closure activity than MSM.

CONCLUSION

From the above study, it is evident that both the herbal extract, i.e., *E. officinalis* extract and MSM possessed a good wound closure activity. Further, research can be done to analyze the use of *E. officinalis* or its extract as an *in vivo* drug. Since it is non-toxic than any other synthetic drug and it is edible, as a dental student, I would suggest to put forward a practice of prescribing patients to consume *E. officinalis* or its extract either once or twice a week so that it does natural wound healing inside the oral cavity, as having a wound is very common nowadays.

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