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A NOVEL THERAPEUTIC EFFECT OF THE WOUND HEALING ACTIVITY OF *BOSWELLIA SERRATA* EXTRACT (BOSWEGEX®) BY USING THE HUMAN DERMAL FIBROBLAST (HDF) CELL LINE: AN *IN-VITRO* STUDY

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Keywords:

Boswellia serrata (Boswegex®), Wound scratch assay, Cytotoxicity, Wound healing activity

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ABSTRACT: The Indian medicinal plant *Boswellia serrata*, sometimes known as frankincense in the West, has been used in traditional medicine to heal burns and wounds. The objective of this study was to investigate the capability of *B. serrata* (Boswegex®) to enhance wound healing by carrying out an *in-vitro* scratch experiment on human dermal fibroblast cells (HDF). Povidone-Iodine 5% cream was used as a positive control to determine the efficacy of Boswegex®'s wound healing activity. The cytotoxicity, along with proliferation and migration activities, were evaluated against the human dermal fibroblast cell line HDF using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium] assay. Interestingly, Boswegex® was found to stimulate proliferation and migration of HDF cells at given concentrations and enhance the closure rate of the wound area within 24 hours after treatment. Boswegex® was evaluated for its cytotoxicity at different concentrations ranging from 1000 µg/ml to 0.975 µg/ml, which resulted in 7.78±0.06 µg/ml and hence the concentrations of 2µg/ml and 1µg/ml. In the scratch assay, 349.62µM and 234.8 µM migrations of cells were exhibited at 2µg/ml and 1µg/ml respectively. These findings suggest that Boswegex® promotes wound healing by enhancing fibroblast and endothelial cell proliferation and migration and has potential for the treatment of wounds.

INTRODUCTION: The largest organ of the human body, the skin, shields the visceral organs from damage and microbial infection. To replace damaged tissue and maintain tissue homeostasis, the wound healing process is necessary. It takes a variety of processes to generate new tissue, including inflammation, angiogenesis, and the development of granulation tissue, re-epithelialization, and the replacement of the extracellular matrix [1].

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Cells, which include fibroblasts, keratinocytes, macrophages, and other immune cells, rapidly proliferate and travel to the site of the skin injury, beginning the intricate healing process. Consequently, one of the crucial stages of the healing process for wounds is cell migration towards the wound, which is generally controlled by a variety of stimuli in the tissue microenvironment [2].

The most prevalent cells in skin tissue are called fibroblasts, and they play a key role in the breakdown of fibrin clots, the production of extracellular matrix (ECM) components, and the development of collagen structures that promote tissue homeostasis [3, 4]. The production of collagen and the development of granulation tissue are essential for wound contraction.

Due to this, current research on wound healing is concentrated on finding novel therapeutics that can stimulate the activation and modification of fibroblasts that produce collagen [5]. There is a pressing demand for new topical wound-healing regimens. Inflammation, wound malodor, and purulent discharges can all be controlled more effectively using a topical therapy that has little adverse impact on fibroblast proliferation and wound healing.

Even though the pharmaceutical drug organization has experienced tremendous growth, wound healing medications still fall short due to a lack of availability, a high price, and many adverse reactions [6]. Due to the widespread perception that natural product-based medications are secure, dependable, clinically effective, affordable, globally competitive, and better tolerated by patients, they are in high demand in many developing nations with strong herbal traditions [7, 8]. Additionally beneficial as a therapeutic agent for preventing wound infections are natural products [9]. However, we intended to use *Boswellia serratra* extract to create a natural medication.

In India, Northern Africa, and the Middle East, there are trees with moderate to huge branches called *Boswellia serrata* Roxb (Bursaraceae). Peeling back strips of bark reveals a gummy oleo-resin that is filled with oils, terpenoids, and gum. Alpha thujene and p-cymene make up the majority of the essential oils, which can make up to 16 percent of the resin. There are four additional pentacyclic triterpene acids, with ß-Boswellic acid being the main one. Gummy exudates have long been used in the Ayurvedic medical system. The term "guggals" additionally refers to these gum resins. By inhibiting 5-lipoxygenase, a crucial enzyme involved in the formation of leukotrienes, which promote inflammation, terpenes in boswellic acid reduce the synthesis of leukotrienes in intact neutrophils [10, 11] and this mechanism also supports wound healing.

The process of repairing a wound that has been caused to the skin or other soft tissues is known as wound healing. After an injury, an inflammatory reaction takes place, and the cells beneath the dermis start to produce more collagen. The epithelial tissue eventually regenerates [12]. Numerous procedures are involved in wound care and management, including dressing the wound and administering painkillers, anti-inflammatory drugs, and medications that speed up the healing process. These will have a variety of negative repercussions. As a result, numerous studies have demonstrated the ability of *B. serrata* to cure wounds. Additionally, this will prevent processes like contraction, inflammation, granulation, fibroplasia, and epithelization [13]. Drugs that reduce inflammation are known to slow down the healing process since inflammation occurs before healing. By interfering with any of the various stages of wound healing, a treatment may affect how a wound heals. The standard course of treatment entails keeping the wound tidy, dry, and covered. The purpose of the current study was to determine whether the indigenous medicine *B. serrata* was beneficial by using an HDF cell line in an *in-vitro* wound healing scratch assay.

Therefore, this study was carried out to find out how effectively *B. serrata* extract works to promote wound healing in human dermal fibroblast cell lines.

MATERIALS AND METHODS:

Chemicals and Reagents: Fetal bovine serum (FBS) and Dulbecco's modified Eagle medium (DMEM) with high glucose were procured from Gibco. Trypsin and Dulbecco's phosphate-buffered saline (D-PBS), which are both used in cell culture, were purchased from HI Media in India. By using the 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay, the cytotoxicity was evaluated. MTT Reagent and dimethyl sulfoxide (DMSO) were bought from Sigma in the United States and HI Media in India, respectively.

Preparation of the Plant Extract Boswegex®: Boswegex® is a mixture of fractions derived from an aqueous ethanolic extract of the gum resin from *B. serrata*. Boswegex® is standardized with 40 to 85% total boswellic acids (BAs) each in order to maintain quality and batch-to-batch consistency.

Standardization of Boswegex®: Processes for extraction and purification of Boswegex® were standardized. Accurately weighed 1.5 g of the sample and then dissolved it in 100 ml of alcohol. 8–10 drops of phenolphthalein were added as an

indicator. This was titrated against 0.1 N NaOH. The 100 ml of alcohol were given as blank. The End points were observed as pinkish-brown to colourless. The following formula was used to calculate the values:

Titter value x 45.67 x Normality of 0.1 N NaOH x 100 / Wt. of sample in mg x 0.1

Cell Line and Culture Medium: The human dermal fibroblast cell line HDF was grown in Ham's F-12 medium with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 g/ml) and amphotericin B (5 g/ml) at 37°C until confluent. TPVG solution was used to separate the cells (0.2% trypsin, 0.02% EDTA, and 0.05% glucose in PBS). The 96-well microtiter plates used for all studies were used to carry out experiments and produce the stock cultures, which were cultivated in 25 cm^2 culture flasks.

Cytotoxicity Assay: The MTT test was used to assess the cytotoxicity of Boswegex® on human dermal fibroblast cell lines (HDF). Ham's F12, containing 10% FBS, was used to trypsinize the monolayer cell culture and increase the cell density to 100,000 cells/ml. The diluted cell solution was diluted, and 0.1 ml was added to each well of the 96-well microtiter plate. Once a partial monolayer had developed after 24 hours, the supernatant was flicked off, the monolayer was washed with media once, and 100µl of various test drug doses were added. This was carried out on microtiter plates. The plates were subsequently incubated for 72 hours at 37°C in 5% CO_2 conditions, with microscopic examination and observations conducted every 24 hours. The drug solution in the wells was discarded after 72 hours and replaced with 50µl of MTT in PBS in each well. The plates were gently shaken and incubated for 3 hours at 37oC in a 5% CO2 condition. The generated formazan was solubilized by removing the supernatant, adding 100µl of propanol, and gently rotating the plates. A microplate reader operating at a wavelength of 540 nm was used to measure the absorbance. The data from the dose-response curves for the cell line were used to determine the dose of the test drug required to inhibit cell growth by 50% (CTC_{50}), and the percentage growth inhibition was then calculated.

% of viability = Mean absorbance of test sample × 100 / Mean absorbance of negative control

***In-vitro* Assessment of Wound Healing:** HDF cells were grown in a flask at 37°C and 5% CO_2 in 10% Ham's F-12 growth medium with FBS. At a density of 1.5 x 10^5 cells/ml, the cells were reseeding in three 60-mm petri dishes. Ham's F12 serum-free growth medium was added and cultured overnight once the cells reached confluency (greater than 70% cell density). After incubation, a sterile 1 ml tip was used to make a scratch that measured between 0.8mm and 1.0mm wide. The petri plates were cleaned twice with 1X PBS. Two safe doses of the drug were administered to the cells, with 1% DMSO providing the control, in three separate petri dishes. At 0, 24, and 48 hours, the plates were tested. The cells were incubated for 48 hours before being PBS washed, and then 10% formaldehyde was added to fix the cells. 2 ml of 0.05% crystal violet was added and incubated for 5–10 minutes after the formaldehyde solution was discarded. Under a microscope, the plates were examined, and five distinct spots for each interval were used to quantify distance using Motic microscopy software. By comparing intervals between 0 and 48 hours, the region of later cell migration was determined.

The percentage of wound healing that was inhibited by the scratch assay method was calculated using the formula as follows:

% of wound healing = scratch area at 0 h−scratch area at specific time × 100 / Scratch area at 0 h

Statistical Analysis: All experimental values are reported as the mean ± SD. Next an ANOVA evaluation of the data, the difference between the groups was determined using a paired t-test. Statistics were considered significant when P-values were less than 0.05.

RESULTS:

Cytotoxicity: The cytotoxic effect of Boswegex® on HDF cell lines was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The cells were exposed to different concentrations of the test compound for 72 hours, and the cytotoxic effect of the Boswegex® was evaluated. The percentage viability of HDF cells as CTC_{50} in Boswegex® was observed

to be 7.78±0.06. The concentrations of Boswegex® used for treatment and their corresponding percentage cell viability were tabulated in Table 1 and represented in **Fig. 1** and **2.**

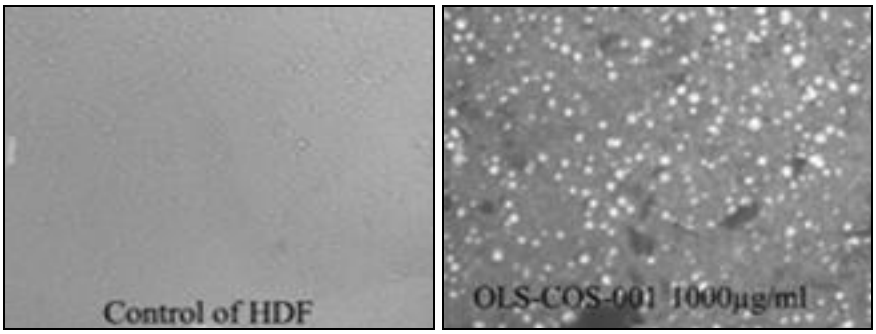
Effect of Boswegex®on *In-vitro* Scratch Assay: Table 2 and **Fig. 3** shows the images of scratch assays on HDF cells at 0, 24 and 48 h post injury time without treatment (control) and with treatment. All the images are shown progression of wound closure on scratch wounded HDF cells. Enhanced migration and wound closure were observed in Boswegex® treated cells as compared with standard drug Povidone-Iodine 5%. Rapid cell migration and wound closure rate of Boswegex® treated HDF cells were observed and these effects were comparable with positive control drug, Povidone-Iodine 5%. *In-vitro* wound healing assay of Boswegex® was evaluated on HDF and it exhibited significant efficacy by offering 349.62µM and 234.8 µM migrations of cells at 2µg/ml and 1µg/ml respectively.

TABLE 1: CYTOTOXICITY BOSWEGEX® AGAINST HDF CELL LINE. DATA ARE PRESENTED AS MEAN ± SD (N=3)

Name of Test Sample	Test Conc. (µg/ml)	% of Cytotoxicity	CTC_{50} (µg/ml)
Boswegex®	1000	87.07±0.3	7.78±0.06
	500	84.71±0.6	
	250	79.60±0.3	
	125	74.49±0.4	
	62.5	71.25±0.4	
	31.2	62.88±0.5	
	15.6	59.82±0.4	
	7.8	50.12±0.3	
	3.9	28.40±0.3	
	1.95	9.00±0.8	
	0.97	2.19±0.5	

TABLE 2: DISTANCE OF THE SCRATCH ON HDF CELL LINE

Sl. no.	Name of Test Sample	Time (Hour)	Test Conc. (µg/ml)	Distance At Five Different Locations (µM)	Average Distance (µM)	Distance Covered (0hr-48hr)
1.	Control Cells	O hr	--	550, 551, 543, 539.5, 549.9	546.6	--
2	Povidone-Iodine 5%		5 µg/ml	540.5, 536.9, 548.6, 535.5, 532.5	538.8	--
3	Boswegex®		2 µg/ml	504, 509, 508, 502, 500.9	504.78	--
4	Boswegex®		1 µg/ml	525.8, 520.6, 522.8, 506.8, 521.5	519.5	--
1.	Control Cells	24hr	--	447.5, 445.5, 441.5, 439.5, 449.9	444.78	101.82
2	Povidone-Iodine 5%		5 µg/ml	190.5, 176.8, 188.3, 175.7, 172.5	180.76	358.1
3	Boswegex®		2 µg/ml	215.5, 224.5, 212.7, 214.5, 211.1	215.66	289.1
4	Boswegex®		1 µg/ml	380.8, 370.6, 385.8, 370.8, 371.5	375.9	143.6
1.	Control Cells	48	--	308.5, 315.6, 302.6, 339.5, 315.9	316.4	230.2
2	Povidone Iodine 5%		5 µg/ml	88.5, 89.6, 87.6, 91.6, 85.5	88.56	450.24
3	Boswegex®		2 µg/ml	150.8, 160.5, 155.8, 149.8, 158.5	155.08	349.62
4	Boswegex®		1 µg/ml	290.8, 279.6, 287.8, 279.8, 285.5	284.7	234.8



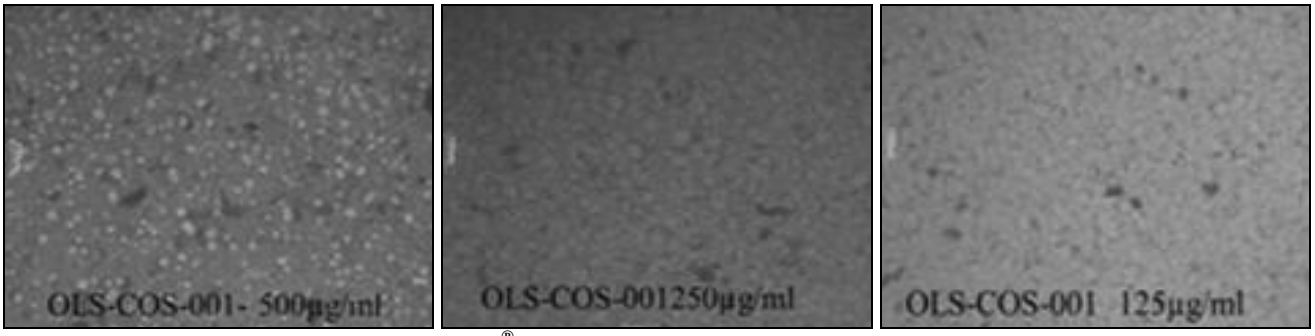


FIG. 1: CYTOTOXICITY OF BOSWEGEX® AGAINST HDF CELL LINE AT DIFFERENT CONCENTRATIONS

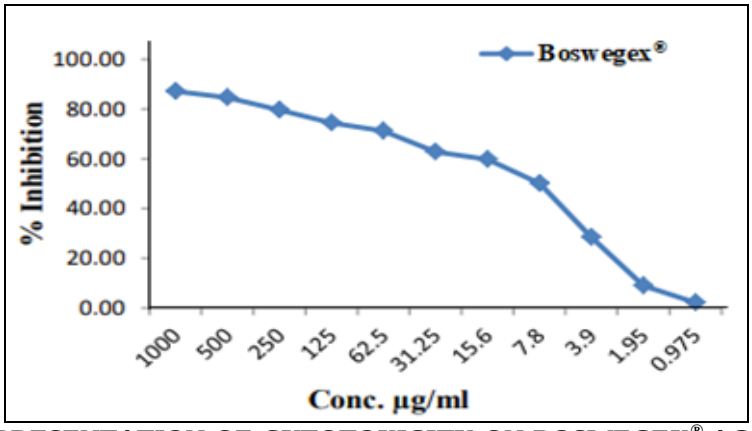


FIG. 2: GRAPHICAL REPRESENTATION OF CYTOTOXICITY ON BOSWEGEX® AGAINST HDF CELL LINE. Data are presented as mean ± sd (n=3).

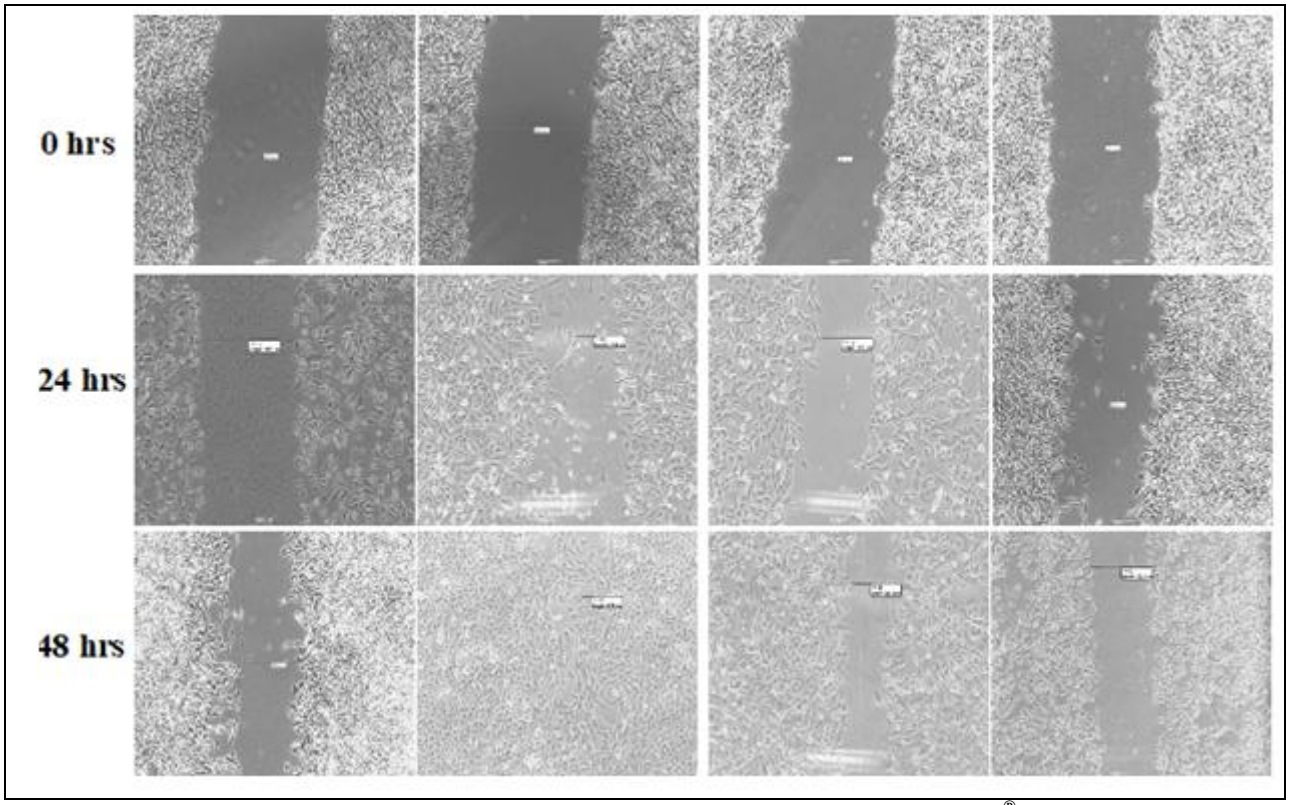


FIG. 3: REPRESENTATIVE IMAGE SHOWING THE EFFECT OF BOSWEGEX® ON HUMAN DERMAL FIBROBLAST (HDF) MIGRATION IN A WOUND SCRATCH TEST ASSAY

DISCUSSION: The skin offers defence and serves as an exterior barrier for human tissues and cells against microbial infection from the outside environment. The process of wound healing must

be used to quickly and efficiently repair any skin barrier damage. The primary layer of protection in cutaneous wound healing is the dermal fibroblast, which migrates and proliferates into the wound site in reaction to injury. It responds to cytokines produced by macrophages, such as TGF-1, by differentiating into myofibroblasts, which is dependent on fibronectin interaction [14].

After 24 hours, the viability of the fibroblast at various concentrations was assessed since *B. serrata* therapy on HDF proliferation and migration was not hindered by any toxicity. Based on a cell viability assay, our findings showed that after 24 hours of treatment, *B. serrata* extract had no harmful effects on HDF cells. According to these results, *B. serrata* was employed for additional therapy at non-toxic dosages. The scratch test is a viable and affordable screening technique to identify and confirm the *in-vitro* wound healing activities of *B. serrata*. This assay has a strong connection to the second stage of the healing process for wounds, which is marked by keratinocyte and fibroblast migration and proliferation [15, 16]. The *B. serrata* was used in this study as a treatment during the scratch assay, and the aqueous ethanolic extract increased the population of HDF cells in the 'wounded' or scratched area by causing cell migration as well as the proliferation of the migrated cells.

Physical harm that causes the skin to split or open up is called a wound. In order to restore broken anatomical continuity and the compromised functional state of the skin, wounds must be properly healed. It results from the coordinated response of several cell types to damage. An orderly and definite series of biological activities are present during cutaneous wound healing, beginning with wound closure and continuing through tissue remodeling and repair [17]. Results from the current study imply that Boswegex®'s *in-vitro* scratch assay method has sped up the healing of wounds. When compared to the control group, the treated excision wounds had a faster rate of wound contraction, which resulted in a larger amount of repaired tissue. To demonstrate Boswegex®'s effectiveness at promoting wound healing, tensile strength was tested. Increased collagen concentration and fibre stabilization may be to blame for the increased tensile strength of treated wounds [18]. The findings imply that Boswegex® treatment may be good for the various stages of wound healing, including fibroplasias, collagen synthesis, and wound contraction, leading to quicker recovery.

CONCLUSION: This study has shown that Boswegex® has the ability to increase the vitality and proliferation of human dermal fibroblast cells in an *in vitro* wound closure experiment. The boswellic acids in Boswegex® may stimulate fibroblast cell growth and proliferation to improve the activity of wound closure. Cell migrations at 2µg/ml and 1 µg/ml of the maximum Boswegex® concentration were 349.62 M and 234.8 M, respectively. The data from this study's observations and findings showed that Boswegex® significantly induced wound contraction. Comparing the treated groups to the control and standard groups, the breaking strength of the treated excision wounds increased. These findings may support Boswegex®s use in the management of wound healing.

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