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The Use of Methanolic extract of *Boswellia serrata* in Combination with Dextrin and Glycerin for Treatment of Experimentally Induced Thermal Injuries in Rabbits

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Abstract

Background Thermal injuries and burns are considered as the major health care problem worldwide. Burns in general are life-threatening condition with high morbidity and mortality. Wound infection and delayed wound healing are the main essential problems in burned patients and represent the major goals for new therapeutic strategies. Frankincense, the gum resin of the *Boswellia serrata* tree have traditionally been used in folk medicine for centuries to manage different chronic inflammatory diseases, the anti-inflammatory potential of lipophilic *Boswellia serrata* extracts were confirmed by experimental data from animal models and clinical studies on humans.

Objective Comparing the wound healing effect of methanol extract of *Boswellia serrata*-dextrin-glycerin combination to that of silver sulfadiazine.

Methods The dried powder of the oleo gum resin was extracted by cold method (maceration) using methanol and then the dried extract was mixed with dextrin and glycerin (30 gm, 40 ml, 60 gm) respectively. Thirty-two domestic male rabbits were divided into four groups, eight animals for each group, the groups were; intact healthy (H), burned without treatment (Gr1), burned and treated with silver sulfadiazine (Gr2) and burned and treated with the methanolic extract of *Boswellia serrata*–dextrin-glycerin combination (Gr3). Thermal injury was induced in all groups (except the intact healthy group) and treated topically on burned area once daily for 28 days. Tissue levels of vascular endothelial cell growth factor (VEGF) and tumor necrosis factor alpha (TNF-α) were assessed in addition to skin histological examination at the end of the study (day 29).

Results Histopathological evaluation showed enhanced inflammatory response, granulation tissue formation, and collagen deposition due to the appropriate regulation of TNF-α and VEGF.

Conclusion Topical use of the methanolic extract of *Boswellia serrata*-dextrin-glycerin combination significantly enhanced wound healing activities.

Keywords *Boswellia serrata*, VEGF, TNF, thermal injury

List of abbreviation: *B. serrata* = *Boswellia serrata*. VEGF = Vascular endothelial cell growth factor, TNF-α = tumor necrosis factor alpha

Introduction

One of the most common universal problems worldwide is burns, which is the leading cause to ugly skin scarring and serious handicapping, the effect of burns extends to the entire body besides the skin [(1)]. The function of the skin is to protect the internal body organs from any hostile external environment of different pollution, temperature, humidity and radiation. Also, skin has important function, such as preserving water and heat regulation [(2)]. A burn is a kind of skin injury that is caused by heat, electricity, chemicals, light radiation, extreme cold or friction [(3)]. The world health organization

reports that more than 90% of burn injuries occur in the developing countries or underdeveloped ones where the death from large burns (more than 40% of total body surface area) reach 100% [4].

Frankincense, the gum resin of the *Boswellia serrata* (*B. serrata*) tree, was well known to ancient civilizations and is still considered as for ritual purposes in the Catholic Church and traditional ceremonies in Northern Africa [5]. High performance liquid chromatography (HPLC) analysis of Indian and African samples of B. serrata gum resin yielded 12 different pentacyclic triterpene acids, the most important is alpha boswellic acid and acetyl boswellic acid. This method provides differentiation and standardization of the resin of different origin and gum resin phytopharmaceuticals [6].

Dextrin is mainly produced by an enzyme called amylase in human that is usually present in saliva mixes with the food in the mouth, and then acts on the starch in a slightly alkaline medium to convert it to dextrin [7].

Glycerin (CAS No. 56-81-5) is a polyhydric alcohol which its molecular formula is $C_3H_8O_3$. Glycerin (also referred to as glycerol) is a simple polyol compound that has three hydroxyl (OH) groups [8].

The objectives of this study was to compare the wound healing effect of methanol extract of Boswellia serrata-dextrin-glycerin combination to that of silver sulfadiazine.

Methods

Thirty-two domestic male rabbits, weighing 1250-1750 grams were divided into four groups each of eight animals, they were housed in animal house of Collage of Medicine, Al-Naharain University. Before starting the study, the animals were left for 48 hours to acclimatize to the animal room conditions of controlled temperature, allowed free access to water and food. Thermal injury was initiated by a metal bar (20*20*100) mm, heated in boiling water and preserved in equilibrium for about 15 min. with the present of thermometer and the animals were anesthetized using ketamine: xylazine (22-50 mg/kg: 2.5-10 mg/kg IM) and the bar was applied for about 45 seconds on their shaved back [9]. The experimental protocols are:

H: intact healthy group

Gr1: induced burn without treatment

Gr2: induced burn and treated with silver sulfadiazine

Gr3: induced burn and treated with methanolic extract of *B. serrata*-dexrin-glycerin combination.

The extraction process was first done on the Oleo gum resin using methanol by maceration (cold method), then the methanolic extract was mixed with dextrin and glycerin with continuous heating and stirring using magnetic stirrer and electric heater, the final combination was left to cool down [10].

At the end of the experiment the animals have been sacrificed by ether on day 29. The skin tissue were cut in two parts; one for tissue homogenization to determine tissue levels of vascular endothelial cell growth factor (VEGF), tumor necrosis factor alpha (TNF-α) and the other part for histological examination to give scoring level of inflammatory response of the wound.

Principle of the assay of TNF-α and VEGF a quantitative sandwich enzyme immunoassay technique (ELISA); where antibodies specific for TNF-α and VEGF have been pre-coated onto a microplate. Samples and standards are pushed into the wells and the TNF-α contents are bound by the immobilized antibody; then removing the unbound substances, adding a biotin conjugated antibody to the wells, washing, adding avidin conjugated Horseradish Peroxidase to the wells, washing again, adding a substrate solution to the wells and color would appear in proportion to the amount of TNF-α and VEGF bound in the first step. The color must be stopped and the intensity of it is measured at 450 nm (This assay was done as directed by the assay layout sheet of the manufacturer company: Cusabio).

Preparation of skin tissue for histological examination by fixation in 10% formalin and processed according to Bancroft and Stevens [11].

Statistical analysis was performed using SPSS-21 and descriptive statistics were formulated as mean and standard error mean (mean ±SEM). One Way Analysis of Variance (ANOVA) and t-test was used to assess and the difference was considered significant when p value was equal to or below 0.0512.

Results

Gr3 group showed a significant reduction in the levels of TNF-α in skin tissue homogenate; in addition to significant reduction of VEGF in skin tissue homogenate compared to other groups ($P < 0.05$) as shown in figure 1 and 2 respectively, while Gr1 and Gr2 animal groups showed non-significant difference on TNF-α and VEGF level in skin tissue homogenate ($P > 0.05$) but significantly different compared with H group, and according to histopathological examination (figure 3,4,5 and 6) of skin, Gr3 showed better inflammatory response, granulation tissue and fibrosis.

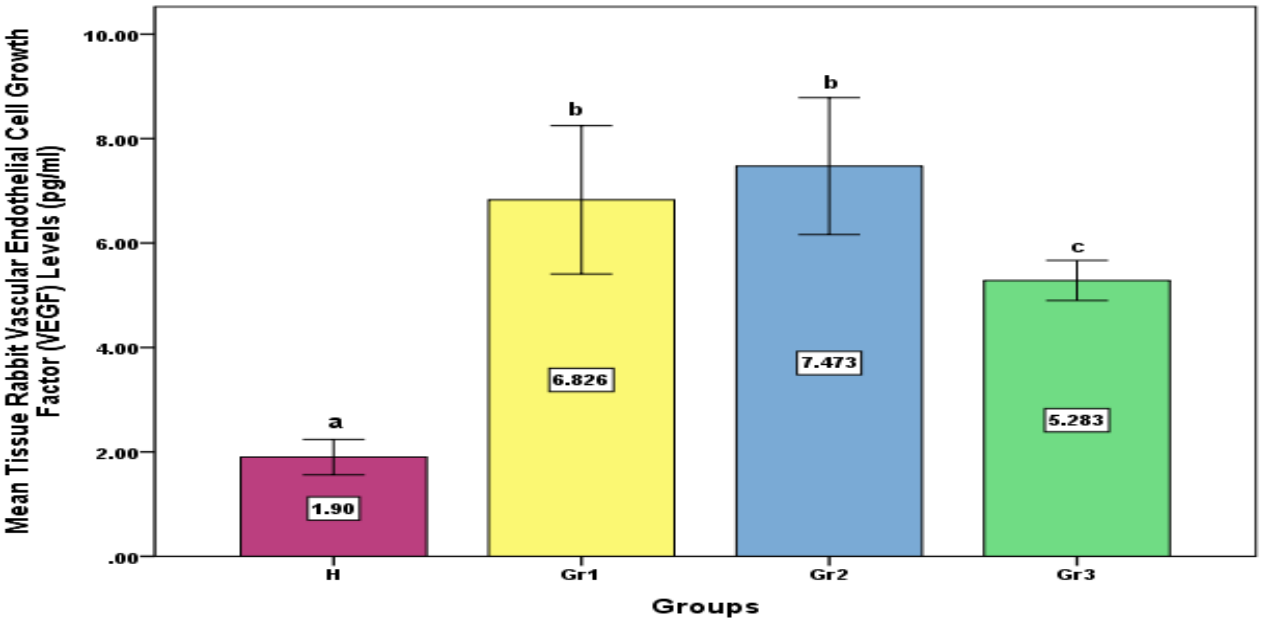

Fig. 1. Tissue VEGF level in the study groups.
H= intact healthy, Gr1= burned without treatment, Gr2= burned and treated with silver sulfadiazine, Gr3= burned and treated with ME of B. serrata-dextrin-glycerin compound

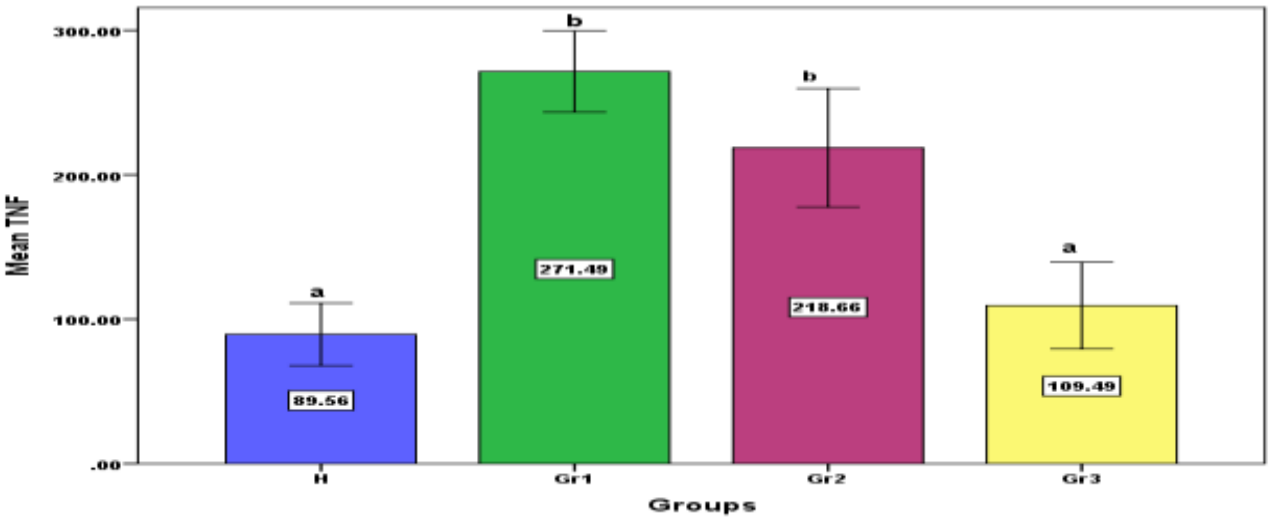

Fig. 2. Tissue TNF-α level in the study groups. H= intact healthy, Gr1= burned without treatment, Gr2= burned and treated with silver sulfadiazine, Gr3= burned and treated with ME of B. serrata-dextrin-glycerin compound

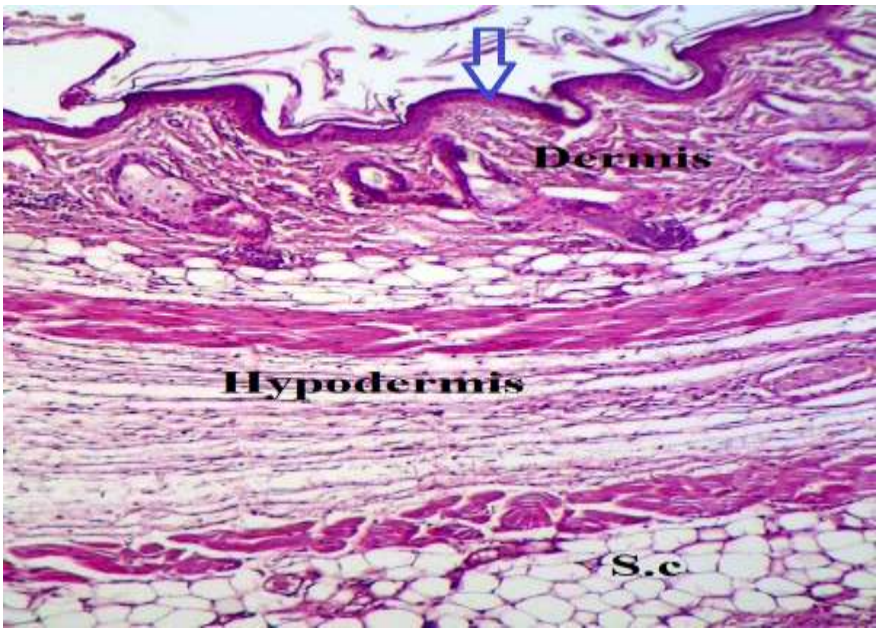

Fig. 3. Light microscopic section of rabbit skin tissue of H group (the intact healthy) showing normal skin tissue: epidermis (blue arrow), dermis, and hypodermis and subcutaneous. H&E (40X), (S.c) subcutaneous

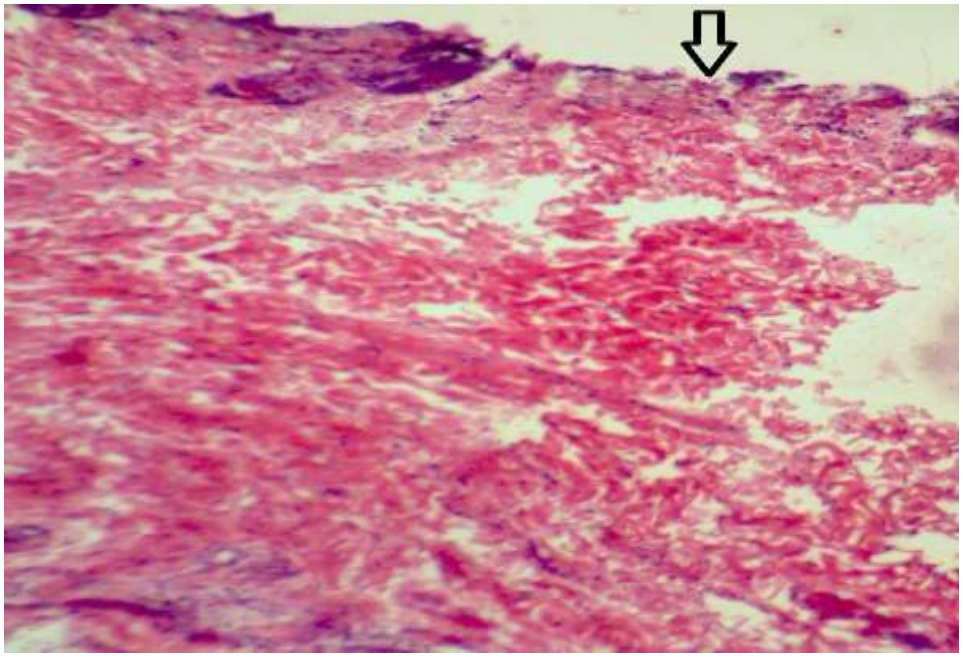

Fig. 4. Light microscopic section of rabbit skin tissue of Gr 1 (burned without treatment) showing burning skin layers and ulceration (black arrow). H&E (40X)

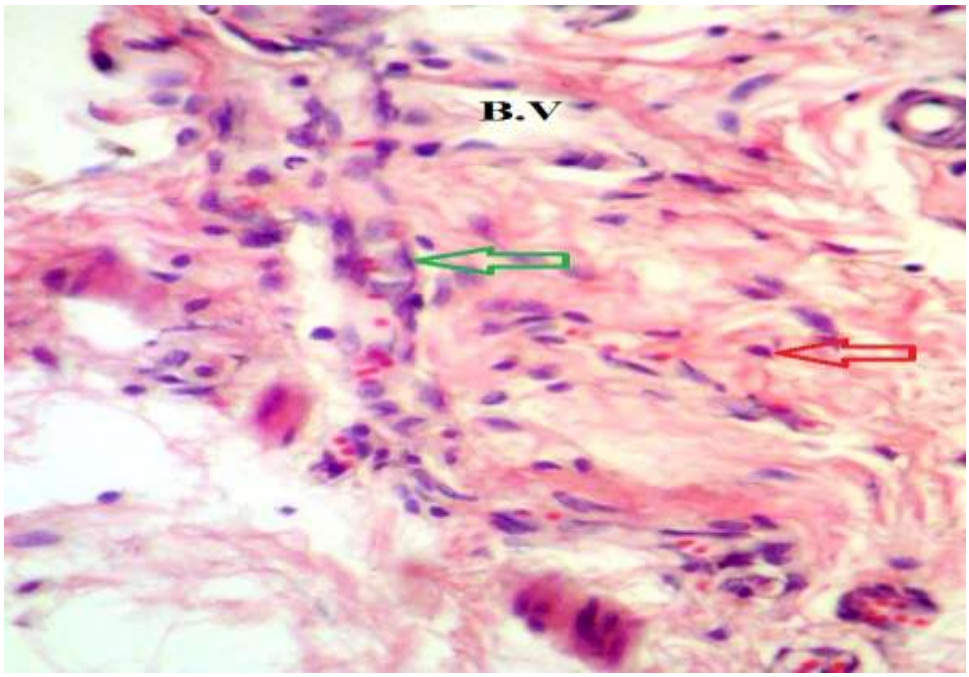

Fig. 5. Light microscopic section of rabbit skin tissue of Gr 2 (Silver sulfadiazine treatment) showing inflammatory (red arrow) with mild reactive fibroblast (green arrow) and collagen fiber. H&E (40X). (B.V) blood vessel

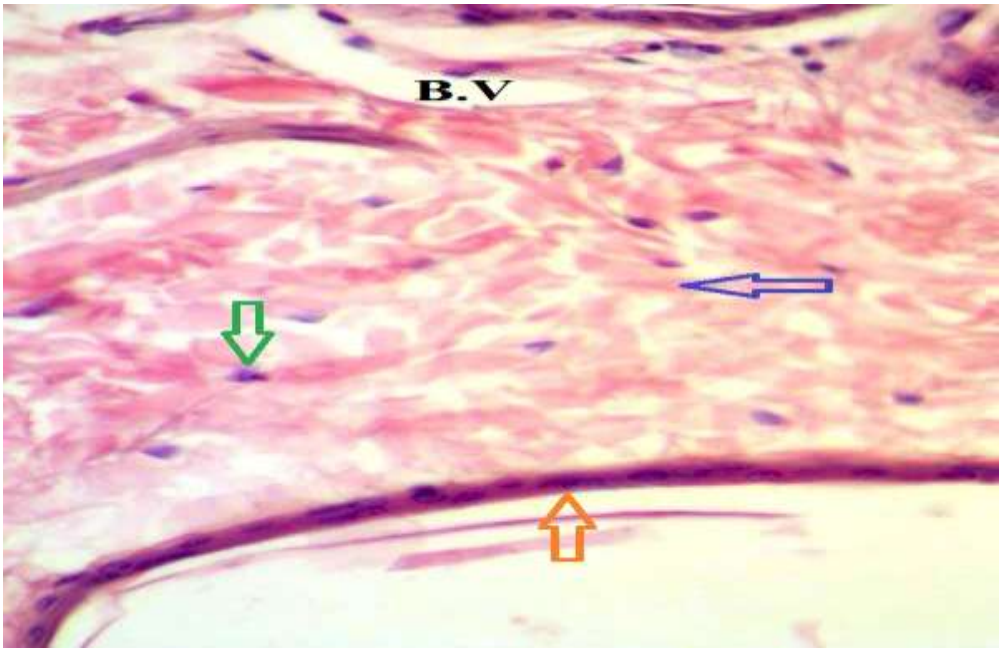

Fig. 6. Light microscopic section of rabbit skin tissue of group3 (Methanolic extract of *Boswellia serrata* -dextrin-glycerin combination) showing reactive fibrous tissue formation with present of fibroblast cells (green arrow), epidermis (orange arrow) and abundant of collagen (blue arrow). H&E (40X). (B.v) blood vessel

Discussion

Burn is the most widespread injury where oxidation process takes place associated metabolic and biological changes. Animal studies is useful because the pathophysiology and histopathology of thermal injury in animals is very similar to in humans [13]. Oleo gum resins from Boswellia species are being used in traditional medicine for the management of a different disease [14], experimental data from animal models as well as clinical studies on humans confirmed the anti-inflammatory potential of lipophilic *B. serrata* extracts [5].

The anti-inflammatory effect of the Methanolic extract (ME) of *B. serrata* may be attributed to the presence of diversity of phytochemicals in the methanolic extract such as α & β pinenes, α, β and γ boswellic acids and other terpenoids [15]. These chemical constituents may exert the direct anti-inflammatory effect by the direct inhibition of the vascular endothelial growth factor by interfering with VEGFR2 activation [16,17].

In this study, the extraction process was done sequentially and using the cold method "maceration", cold method for extraction were used because this method is more suitable for thermosensitive constituents so that it will ensure the essential phytochemical constituents was not subjected to degradation by higher temperatures [18]. It had been noticed that there are several factors seem to affect the variation in the yield and the composition of phytochemicals in the extract, these include: type of extraction method, duration of the extraction process, temperature of the water bath, agitation, solvent type used and its pH, concentration and polarity; particle size of the powdered plant part and solvent to sample ratio [19]. The vast majority of the hot aqueous extracts of the antibacterial active plants exhibited low activity when compared to the methanolic extract. The extraction yield of the current study was 14.2 % w/w, which come to agree with Lin and coworkers [20] in 2013 who showed that the typical yield of frankincense essential oil was 10% (w/w) of gum resins within a range of 8-13%.

Wound healing process is a physical building of molecules for tissue repair and secreted by

fibroblasts and others present at the site of the burned wound [20]. It's well known that wound healing process is affected by tissue levels of VEGF which in turn will affect angiogenesis and the direct and indirect activation of the fibroblast, and there is a link between angiogenesis and scarring and suggest a novel role for VEGF in mediating the quantity and quality of scar tissue generated during wound repair [21]. Johnson and Wilgus [22] in 2014 said that treatment of hypertrophic scar patients with interferon α2b has been linked to a reduction in angiogenesis and VEGF suggesting that reducing VEGF may improve scars, similarly, treatments used to induce keloid regression have been shown to reduce VEGF levels in keloid tissue.

Tumor necrosis factor-α is an important mediators of the acute and severe inflammatory reaction in thermal injury that affect wound healing process [23], it is involved in the early initiation of wound healing process and low levels can promote wound healing indirectly, however, high levels can delay wound healing [24]. Rapala [25] showed in 1996 that after daily applications of TNF-α for 4 days; an inhibitory effect on tissue repair was observed after 4 and 7 days. Collagen formation, indicated by the hydroxyproline content of the sponge, was significantly lower in the group treated with TNF-α than in the controls; this effect could be abolished with indomethacin and Indomethacin alone stimulated collagen production by 40%.

All these findings come to agree with our study results, which showed that VEGF and TNF-alpha tissue levels in the ME of *Boswellia serrata* treated group was significantly less than burn without treatment group (Gr1) and SSD treated group (Gr2).

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Author contribution

Abbas is a researcher who has done the technique of this work and conducted the writing of manuscript. Dr. Abdulkareem and Dr. Bahaa participated in supervision and in scientific review of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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