Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net

Effect of Chitosan and Dextrin Combination on Experimentally-Induced Thermal Injury in Rabbits

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Abstract

Background Burn is a major health problem, life threatening with a high mortality and morbidity; Chitosan and its formulations are used as a topical dressing in wounds and burns management due to its nontoxic, hemostatic, healing stimulant, antimicrobial, biocompatible and biodegradable properties as well as its vehicle use to deliver biopharmaceuticals, antimicrobials and growth factors into tissue.

Objective To evaluate the effects of chitosan-dextrin combination on induced burn in rabbits.

Method Forty domestic male rabbits, weighing 1250-1750 kg were divided into five groups, each of eight animals: AH group: apparently healthy rabbits, BWT group: left with no treatment, AR group: treated with *Aqua Rosea*, AG-S group: treated with silver sulfadiazine cream and CH-D group: treated with chitosan – dextrin combination; all animals (except AH group) wereinduced burn and treatedtopically on burned area once daily for 28days. Tissue levels ofvascular endothelial cell growth factor (VEGF), tumor necrosis factor alpha (TNF-α) and skin histological examination.

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

Conclusion Topical use of chitosan – dextrin combinationshowed effective and enhance wound healing activities.

Key words Thermal injury, burn, chitosan, dextrin.

List of abbreviations: AR = *Aqua Rosae*, Ag-S = Silver sulfadiazine, B.v = blood vessel, BWT = Burned animal with no treatment, CH-D = Chitosan – dextrin combination, ELISA = Enzyme linked immunosorbant assay, Pg = Pico gram, s.c = Subcutaneous, TNF-α = tumor necrosis factor alpha, VEGF = vascular endothelial growth factor.

Introduction

Burn is common universal problem that may lead to ugly scarring, serious handicapping. It can severely affect not only the skin but the whole body [(1)] because it is a coagulative necrosis of tissue and the damage depth is depending on the temperature and the duration of the exposure to the causative agent as heat, electricity, light, chemicals, friction or radiation. It affects the integrity of the skin because it is a barrier that protects the body from the external invasion (*e.g.*, microbial aggressions) [(2)].

Burns areserious problem in both the developed and developing world represented as physical and psychological assault on patients and considered a diverse and great challenge to medical staff [(3)].

Chitosan is modified natural carbohydrate polymer that produced commercially by deacetylation of chitin which found naturally in the skeleton of invertebrates, insects and some algae usually find in cell walls of squids, crabs, shrimps, crayfish and oysters as well as fungi cell wall [(4)]. Chitosan is a wound healing accelerator and it enhance every stage of healing because of improvement the functions of inflammatory cells like macrophages,

polymorphonuclear leukocytes and fibroblasts and increase the tensile strength of wound. These effects enhanced according to deacetylation degree, molecular weight and the state of chitosan [5].

Dextrin is produced by an enzyme called amylase in human and can be produced from a wide variety of starch, such as wheat, rice, corn, potato, and tapioca [6]. Dextrin act as formulation aidfor producing a desired texture in product, stabilizer and thickener forproducing viscous solutions or dispersions to enhance consistency and stabilize emulsions [7].

The current study was performed to investigate healing effect of chitosan – dextrin combination against burn injury.

Methods

Forty domestic male rabbits, weighing 1250-1750 kg were divided into five groups each of eight animals; they were housed in animal house of Al-Naharain Collage of Medicine. 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 done by a metal bar (20*20*100) mm, heated in boiling water and preserved in equilibrium for about 15 min. with the present ofthermometer and the animals were anesthetized using ketamine: xylazine (22-50 mg/kg: 2.5-10 mg/kg IM) and put the bar for about 45 seconds on their shaved back [8]. The experimental protocol is as follows:

AH group: apparently healthy rabbits, BWT group: induced burn animal with no treatment, AR group: induced burn animals with treatment with *Aqua Rosea*, AG-S group: induced burn animals with treatment of silver sulfadiazine, and CH-D group: induced burn animals with treatment of chitosan – dextrin combination; each treatment was given topically on burned area once daily for 28 days.

In preparation of Chitosan Dextrin combination, Chitosan solution was prepared by dissolving of chitosan in distilled water containing of glacial acetic acid with the present of a magnetic stirre [9]. Chitosan solution was prepared by dissolving 0.1 g of chitosan in 50 ml of distilled water containing 1 ml of glacial acetic acid with the aid of three hours stirring by magnetic stirrer for maximum solubility then the addition of 30 g dextrin with continuous stirring and finally mixing with 20 g *aqua rosea* to get cream constancy.

At the end the animals have been sacrificed by ether on day 29, also skin tissue were divided in two parts for homogenation and staining to determine tissue levels of vascular endothelial cell growth factor (VEGF), tumor necrosis factor alpha (TNF-α) by and histological examination.

Principle of the assay of TNF-α andVEGFa quantitative sandwich enzyme immunoassay technique ELISA; where antibodies specific for TNF-α and VEGF have been pre-coated onto a microplate. Samples and standards and 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 avid in 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.

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

Statistical analysis was performed using SPSS-21 andDescriptive statistics were formulated as mean and standard error of 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 0.05 [11].

Results

CH-D group showed a significant reduction in the levels of TNF-α in skin tissue homogenate (Figure 1), in addition to the significant elevation of VEGF in skin tissue homogenate

compared to other groups ($p < 0.05$), while BWT, AR and AG-S animal groups showed non-significant difference on TNF-α and VEGF level in skin tissue homogenate ($p > 0.05$) but significantly different comparing with AH group (Figure 2). In BWT and AR animal groups showed non-significant difference on all study parameters ($p > 0.05$), and according to histopathological examination of skin, CH-D group better inflammatory response, granulation tissue and fibrosis (Figure 7), when compared with other study groups: AH group (Figure 3), BWT group (Figure 4), AR group (Figure 5) and AG-S group (Figure 6).

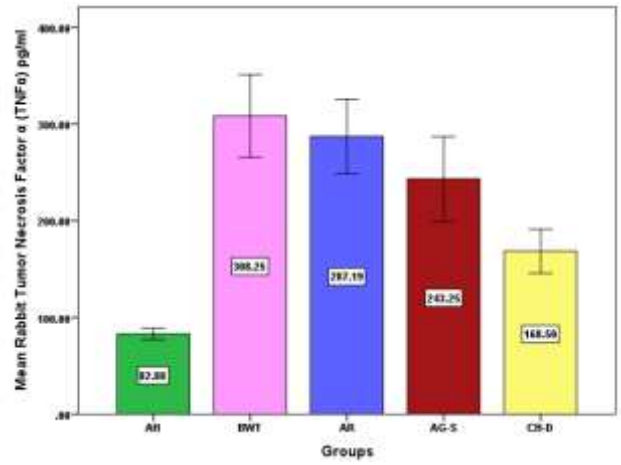

Fig. 1. Tissue TNF-α levels (pg/ml) of study groups.

AH = apparently healthy group, BWT = burned without treatment group, AR = treated with *Aqua rosea* group, AG-D = treated with Silver sulfadiazine group, CH-D = chitosan- dextrin treatment group.

Discussion

Burn is one of the most widespread injuries, where an oxidation process associated with biological and metabolic alterations; the pathophysiology and histopathology of thermal burn in animals is very similar to that in humans [12]. Many studies have demonstrated the therapeutic properties of chitosan because of its biodegradability that makes it dissolves with time when used in wound [5] and the addition of dextrin to aqueous solvents as formulation aid and thickening agent to get desired texture as well as enhance consistency [7].

Wound healing is a physical rebuilding process, molecules for wound repair are secreted by fibroblasts and others present at the wound site. VEGF is a critical cytokine that exhibits chemoattractant properties [13]. Angiogenesis is an important factor in proliferative phase of wound healing and forsupplying nutrients and oxygen needed for skin regrowth thus VEGF is one of the most potent proangiogenic growth factors in the skin [14], wound repair is a process that granulation tissue gradually replaces necrotic tissue and abnormal expressions of VEGF is involved in this process [15]. Reduced levels of endogenous growth factors and diminished angiogenesis are contributory factors for impaired wound healing. VEGF is the most potent angiogenic growth factor, which accelerates healing [16].

Chitosan stimulated inflammatory cells, endothelial cells, newly formed blood vessels, reticular – collagen fibers and VEGF in the wound healing area,chitosan accelerates granulation tissue formation and accelerate the wound healing through increasing VEGF secretion in all stages of wound healing process, activates fibroblasts in the granulation tissue, makes them proliferate and increase extracellular matrix production [17].

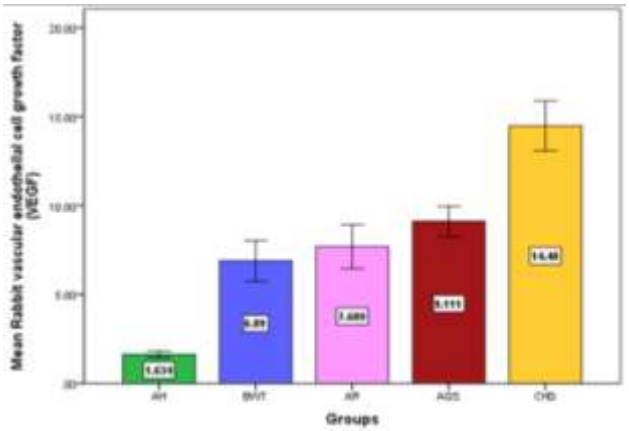

Fig. 2. Tissue VEGF levels (pg/ml) of study groups.

AH = apparently healthy group, BWT = burned without treatment group, AR = treated with *Aqua rosea* group, AG-D = treated with Silver sulfadiazine group, CH-D = chitosan- dextrin treatment group.

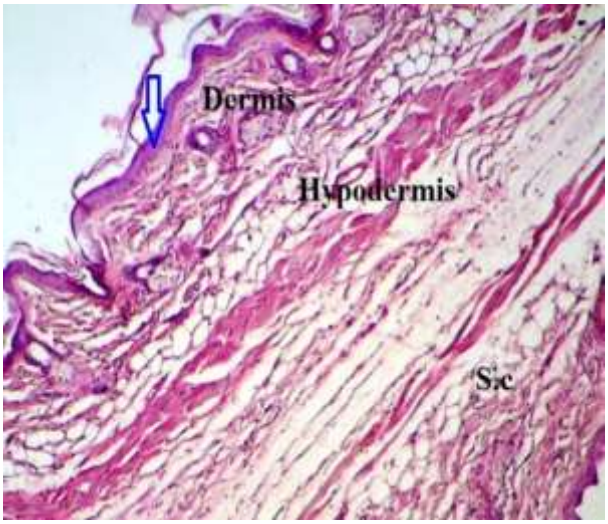

Fig. 3. Light microscopic section of rabbit skin tissue of AH group (the apparently healthy) showing normal skin tissue: epidermis (blue arrow), dermis, and hypodermis and subcutaneous. H&E (40X), (s.c) subcutaneous.

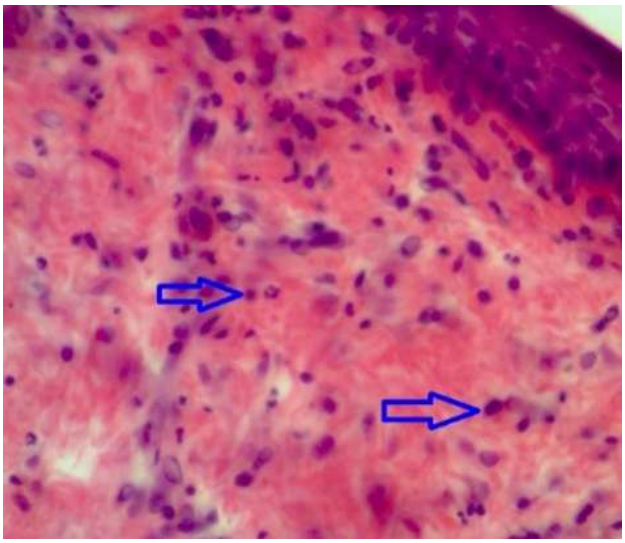

Fig. 5. Light microscopic section of rabbit skin tissue of AR group (*Aqua rosea* treatment) showing focal burning layer of epidermis with damage to dermis and hypodermis layers with certain inflammatory reaction (blue arrow). H&E (40X).

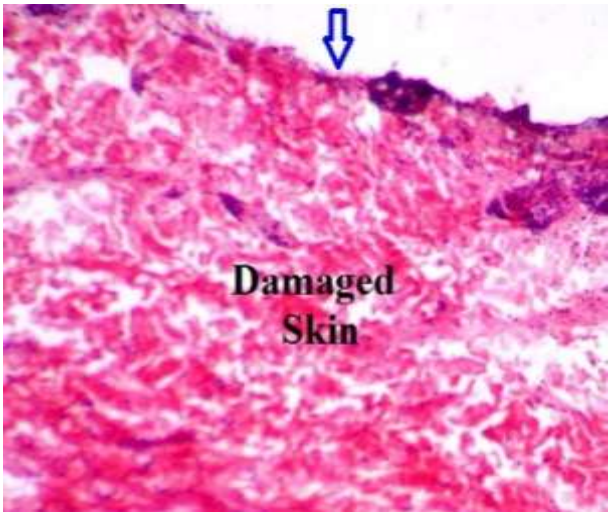

Fig. 4. Light microscopic section of rabbit skin tissue of BWT group (burned without treatment) showing burning skin layers with discontinuation of epidermis ,no skin appendages in the dermis layer and ulceration (blue arrow). H&E (40X).

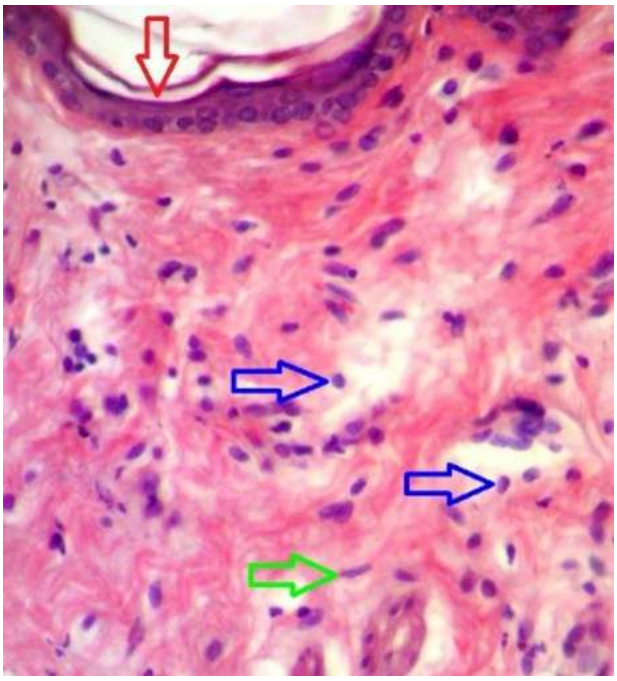

Fig. 6. Light microscopic section of rabbit skin tissue of AG-S group (Silver sulfadiazine treatment) showing more inflammatory (blue arrow) with mild reactive fibroblast (green arrow), re-epithelialization (red arrow) and collagen fiber. H&E (40X).

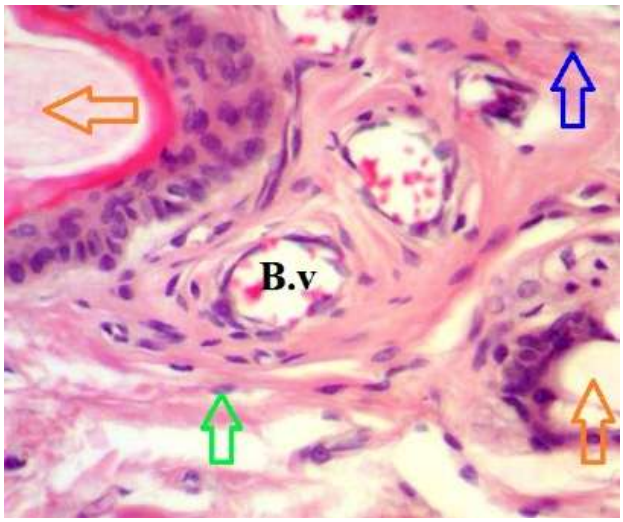

Fig. 7. Light microscopic section of rabbit skin tissue of CH-D group (combination of chitosan –dextrin treatment) showing more inflammatory (blue arrow) with reactive fibrous tissue formation with present of fibroblast cells (green arrow), hair follicles (orange arrow) and abundant granulation tissue (formation of new capillaries). H&E (40X). (B.v) blood vessel.

The physiopathological events following thermal injury related to acute inflammatory reactions in which hyperactive macrophages are primed to stimulate the down regulation or up regulation of certain inflammatory cytokines [18] and abnormal levels of tumor necrosis factor alpha, has been reported both systemically and locally in burn patients [19]. Low levels of TNF-α promote wound healing indirectly but high levels of TNF-α delay wound healing [20], up-regulated TNF-α during the inflammatory phase should decreased for the rapid wound healing response,moreover, a prolonged increase in levels of TNF-*α* may has a role in the development of multiple organ failure after thermal injury [21]. The current study found that chitosan-dextrin combination treatment animals groups had the lowest skin tissue TNF-α level when compared with other groups and these result suggested that chitosan promote wound healing via decreasing of up-regulated TNF-α levels as tableand increasing of up-regulated VEGF levels.

Acknowledgment

The authors thank all members in the Department of Pharmacology, College of Medicine, Al-Nahrain University for technical assistance and logical support.

Author contributions

Noor 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.

Funding

Self-funding.

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Received 20th Oct. 2015: Accepted 27th Mar. 2016