RESEARCH PAPER

Evaluation of wound healing effect of *Solanum nigrum L.* leaf extract-loaded sodium alginate nanoparticles embedded in chitosan hydrogel, *In vivo* study

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ABSTRACT

Objective(s): Wound healing is one of the most fundamental issues in medical science. *Solanum nigrum L.* has been attracted great attention for its antioxidant, antimicrobial and anti-inflammatory activities. The aim of this study was to evaluate the effect of leaf extract of *S. nigrum L*-loaded sodium alginate nanoparticles (NPs) embedded in chitosan hydrogel on wound healing.

Materials and Methods: Ethanolic extract of *S. nigrum L.* leaves (5% v/v) were loaded into sodium alginate NPs using the ionic gelation technique and characterized (Ext-AG NPs). Then, NPs were incorporated into chitosan hydrogel (Ext-AG-CS hydrogel) and the properties of this formulation such as viscosity and release profile were evaluated. The antimicrobial activity of the extract alone and loaded into the hydrogel (Ext-CS hydrogel) was measured on the *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilius* by MIC assay. Finally, their wound healing effects were studied on full-thickness wound in rat animal model in 3, 7 and 14 days.

Results: The particle size of Ext-AG NPs was obtained 437±15 nm. The encapsulation efficiency of extract was about 91.6%. The in vitro release profile from NPs showed that the maximum released extract was 30% during 6 days. However, by embedding of NPs into hydrogel, the release of extract was about 12% after 6 days. The results showed that the extract could be release from hydrogel about 30% in the first 4h followed by about 70% release on the fifth day. Therefore, this formulation was used in subsequent studies. Ext-CS hydrogel 5% exhibited lower MICs on all tested microorganisms in compared with aqueous extract alone. Finally, the results of in vivo wound healing analysis revealed that on day 3, the extract solution and Ext-CS hydrogel were more effective in reducing inflammation than chitosan gel and positive control. The process of epithelial tissue formation on day 14, in all treated groups, seemed to be better than negative control, which shows the positive effect of these compounds on faster epithelial tissue formation.

Conclusion: In general, it seems that *S. nigrum L.* leaf extract 5% and Ext-CS hydrogel 5% were more effective in wound healing process than other treated groups. However, the chitosan hydrogel-extract formulation showed better antimicrobial activity.

Keywords: *Chemical synthesis, Characterization, Chloroquine diphosphate, Encapsulation, Gold nanoparticles*

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INTRODUCTION

The crucial role of the skin like hydration, thermal regulation, and protection from pathogens and chemicals, shows that how severe skin damage could be life-threatening. Wound healing is a complex process that basically involves inflammation, proliferation, and regeneration stages [1, 2]. Conventional wound therapy is usually associated with the formation of scars, functional alterations and aesthetic problems [3]. Recently, regenerative wound therapy, that aims to reestablish skin to its original function by regenerating injured cells and skin tissue without scar formation, has attracted a lot of attention [4]. For this reason, numerous studies have been focused on developing new drugs and technologies to achieve more effective and lower cost wound healing [1]. The *Solanum nigrum* complex also known as *Solanum L.* (black nightshade) is a member of the Solanaceae family [5]. *S. nigrum* has been traditionally used to treat numerous diseases such as pain, fever, inflammation, diuretic, hepatoprotective, and antipyretic agent [6-8]. Also, it has been reported that *S. nigrum L.* exhibits anticancer activity for hepatocellular carcinoma [9], human ovarian cancer [9], human colorectal cancer [10] and human endometrial cancer [10]. The leaf paste of *S. nigrum L.* was also applied directly for wound healing [5]. The polyphenolic compounds of this plant have several properties including antioxidant, antimicrobial, skin protection from UV rays, and skin repair [11, 12]. Recently, nanotechnology offers innovative approaches for the wound healing with its multiple benefits compared with traditional treatment approaches. Different nano-delivery systems designed for wound treatment such as nanospheres, nanocolloids, nanocarriers, nanofibers, nanoemulsions, nanoparticles (NPs), and nanocapsules. These systems can improve the drug penetration to damage tissue and also protect them from degradation [13]. Alginate with wound healing potential has extensively investigated to develop as a nanocarrier system because of its inherent properties including excellent biodegradability, antimicrobial activity, non-toxicity, non-immunogenic, cheap to buy, ability to absorb water, and high absorption profile [14]. Hydrogels are cross-linked networks containing different types of polymers with high capacity to absorb water. Hydrogel-forming polymers have hydrophobic or hydrophilic functional groups in their structure, which include amine (NH2), hydroxyl (-OH), amide (-CONH-, -CONH2) and sulfate (-SO3H). These groups enable hydrogels to absorb aqueous fluids, which leads to the expansion of the hydrogel and the occupation of more volume, a process known as swelling [15]. Chitosan (CS) is a natural cationic copolymer that shows good benefits for forming hydrogel structures [10]. The benefits of CS hydrogel in the treatment of wounds and burns are providing a moist wound environment, protection from secondary infections, removing of wound exudate, promotion of re-epithelialization and acceleration of angiogenesis and collagen maturity [16]. In this study, for the first time, the antimicrobial and wound healing effects of *S. nigrum L*. extract-loaded sodium alginate NPs embedded in CS hydrogel were evaluated in animal model.

MATERIALS and METHODS

Material

Sodium alginate, and chitosan (medium molecular weight) were obtained from Sigma Aldrich (Germany). Hematoxylin and eosin staining were purchased from Merck (Germany). ketamine, Diethyl ether and xylazine 2%were bought from Trittau (Germany). Other solvent bought from Merck (Germany).

Methods

Preparation of ethanolic extract of S. nigrum L. leaves

The maceretaion method has been used to prepare the alcoholic extract of *S. nigrum L.* leaves. Firstly, 100 g of *S. nigrum L.* leaves were collected from the areas around Mashhad, Iran. The leaves were well dried, powdered and soaked in ethanol 70% for 48 h, then this mixture was passed through paper filter. After removing the ethanol, the extract was placed in a freeze dryer and 49 g of dried extract leaves were obtained.

Determination of total polyphenols in S. nigrum L. extract

The total phenolic content of *S. nigrum L.* extract was determined by Folin-Ciocalteu method. A standard gallic acid curve was prepared at concentration 20-160 mg/ml in water. These dilutions and *S. nigrum L.* extract were mixed with of Folin-Ciocalteu reagent (at ratio 1:1) for 2h at 30°C. Then, the absorbance of all samples was read at 765 nm by UV spectrophotometry (Shimadzu, Japan). The amount of polyphenol percentage was calculated as following:

$$(\%\ \mathrm{w/w}) = \frac{A-b}{m} \times \frac{V \times D}{W \times 1000} \times 100$$

A: Sample absorption, b: Standard curve width, m: Standard curve slope, W: Sample weight, V: Sample volume, D: Dilution coefficient.

Preparation of Ext-AG NPs

in order to synthesize alginate NPs, the ionic gelation technique was performed as described previously [17]. At first, the solution of sodium alginate (3.3 mg/ml, pH=5.5) prepared. Then, *S. nigrum L.* extract solution (16.6 mg/ml) and calcium chloride (0.3% w/v, 6 ml) were added to the sodium alginate solution while shaking. After 30–60 min, the solution was sonicated with 90% power (4 min). Next, CS solution (2.25%) (PH 4-4.6) was added slowly the above solution. Finally, the synthesized alginate NPs were washed with deionized.

Characterization of synthesized NPs

Particle size, Poly Dispersity Index (PDI) and zeta potential of prepared Ext-AG NPs was performed by a Dynamic Light Scattering device (DLS) (Nanozszen3600, Malvern). To obtain morphological information of synthesized NPs, samples were investigated by Scanning Electron Microscope (SEM) (VP 1450 , Leo, Germany) [18].

Encapsulation efficiency

The encapsulation efficiency of *S. nigrum L.* extract in the AG NPs was calculated using the indirect method. After centrifuging, the supernatant containing unloaded *S. nigrum L.* extract was collected and the quantity of extract was measured at 268 nm. The encapsulation efficiency of *S. nigrum L.* extract was calculated by the following equation:

Drug encapsulation efficiency (%) = Weight of drug in particles/ Initial weight of drug added×100 [19].

In vitro release study

In vitro release profile of encapsulated extract from AG NPs was evaluated in simulated neutral environment (PBS 0.1 M, pH=7.4). 1 ml of Ext-AG NPs suspension containing 50 mg *S. nigrum L.* was placed into dialysis bags (cut off= 8000 Dalton) and immersed in PBS (5 ml) while shaking at 37 °C. At determined times (1, 2, 3, 4, 24, 48, 72, 96, hr), 300 µl of the released medium was removed and equally replaced with fresh PBS. The quantity of released *S. nigrum L.* extract was measured at 268 nm using the standard curve.

Preparation of CS hydrogel

The amount of 3 g of CS powder with medium molecular weight was gradually added to 100 ml of 1% acetic acid solution and stirred for one hour. It was then sonicated for 10 minutes to remove air bubbles inside the gel.

Loading of Ext-AG into the CS hydrogel

NPs containing 50 mg extract or 50 mg of the extract dissolved in a minimum amount of water was added to 1 ml of CS hydrogel 3% and stirred for 2 h (pH was set at 5.5). For sterilization, the products were exposed to UV light for 15 minutes for two times.

Evaluation of viscosity of the prepared hydrogel

The viscosities of CS hydrogel 3% and Ext-CS hydrogel 5% were measured separately by Brookfield R/S + rotational rheometer (Brookfield Co., USA) with SC4-25 spindle at a shear rate of 0–200 s^{-1}.

In vitro release study of Ext-AG-CS hydrogel

In vitro release profile of extract from CS hydrogel containing Ext-AG NPs was also carried out in PBS, pH 7.4. So that, 1 ml of CS hydrogel was transferred into the dialysis bag. The profile release assay was carried out as described in *in vitro* release study section.

Microbial limit tests

The quantitative and qualitative microbial limit tests were done to control the quality of tested formulations (CS hydrogel, aqueous solution of extract 5%, and Ext-CS hydrogel 5%). Although, all tested products themselves have antimicrobial effects, but the exact range of their effects was not known, and therefore the microbial limitation test was performed to identify their possible microbial contamination.

Qualitative assay

For the qualitative microbial limit test, all formulations were cultured in two media: SCDB (Soybean casein digest broth) and lactose broth. In this way, 1 mL of each sample was added to 9 mL of culture medium and incubated overnight at 37 °C. Then the growth of microorganisms was visually evaluated.

Quantitative test

The quantitative microbial limit test was performed by plate counting method. At first, a 10% dilution of the three tested formulations was prepared in sterilized SCDB culture medium. Then, 1 ml of different dilutions of each formulation was poured into the plates, and the sterile melted SCDA (Soybean casein digest agar) culture medium was added until the plate surface was completely covered. The plates were incubated for 3-5 days at 37 °C and then any microbial growth was measured by counting the colony forming units.

Determination of minimum inhibitory concentration (MIC)

The antimicrobial activity of CS hydrogel, aqueous solution of extract 5%, and Ext-CS hydrogel was evaluated by determination of their minimum inhibitory concentration on the *Staphylococcus aureus* (ATCC 6538P/ PTCC 1112), *Escherichia coli* (ATCC 8739 / PTCC 1330), *Pseudomonas aeruginosa* (ATCC 9027 / PTCC 1074) and *Bacillus subtilis* (PTCC 1247). In the first stage, different concentrations of each formulation were prepared in SCDB culture medium. A concentration of 106 CFU / ml of each microorganisms was prepared in the test tube. Then, 180 µl of the different dilutions of the formulations was poured in each well of 96 well microplates and eeach well was inoculated with 20 µl of each microorganisms. The culture medium and the culture medium inoculated with microbial suspension were considered as negative and positive control group, respectively. The plates were incubated overnight at 37°C. The MIC was determined as the lowest concentration of the samples that inhibited the growth of bacteria [8].

In vivo assays

The wound healing effect of CS hydrogel, aqueous solution of extract 5%, and Ext-AG-CS hydrogel 5% was evaluated and compared on the mice full thickness skin wound model.

In vivo wound model

In this study, 45 male NMRI (Naval Medical Research Institute) mice aged 1.5 to 2 months with a weight range of 30-35 g were used. All ethical considerations were made based on the approvals of the Animal Care Committee of the Mashhad University of Medical Sciences, Iran (project Approval ID: IR.MUMS.PHARMACY.REC.1397.104).

The mice were anesthetized by intra peritoneal (IP) injection of a mixture of Xylazine and Ketamine (Xylazine 7.5 mg/Kg, Ketamine 60 mg/Kg). Then, the hairs on the back of the mice were shaved and the skin was disinfected with povidone-iodine and then with 70% ethanol. Then, a full-thickness skin wound (about 15 mm-diameter) was made using a sterile surgical knife on the back and between the two shoulders of each mouse. The five tested groups including CS hydrogel 3%, extract 5%, Ext-CS hydrogel 5%, negative control group (normal saline) and positive control group (phenytoin cream) were applied topically to the wound site daily, and all wounds was completely covered. Each animal was kept in solitary cages in the animal room. To prevent infection at the wound site, each rat was injected intramuscularly (IM) with 50 IU of penicillin 6.3.3 twice at the first 24-hours. The appearance of the wound was evaluated for healing once a day. Wound condition was also examined for infection using some criteria like odor, increased secretion, presence of necrosis, discoloration to black or dark brown, and edema at the edges of the wound. On days, 3, 7 and 14, three mice were selected and sacrificed randomly for further histological study.

Histological evaluation of wound healing

Prepared samples for histological evaluation included tissue formed at the wound site and some of the surrounding healthy areas. The samples were placed in sampling containers containing 10% formalin buffer for 24 h. The specimens were cut and exposed to liquid paraffin for 8 h. Then, they were extracted from the liquid paraffin, fixed on the slide and stained by the Hematoxylin and Eosin (H&E). Some criteria for each slides were evaluated for measuring wound healing, including necrosis, inflammatory cell density, angiogenesis, fibroblast proliferation, collagen density, and epithelial regeneration.

Statistical analysis

GraphPad Prism 7 statistical software was used to statistically analyze the findings. One-way ANOVA and T Test were used to compare between different groups. In this study, the $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Total polyphenols in S. nigrum L. extract

Phenolic compounds are considered as the most important antioxidants agents in plant materials. The amount of total polyphenols in the alcoholic extract of *S. nigrum L.* leaves was

calculated as 37.42% using the standard gallic acid curve. In a study by Prasath et al, the total polyphenol content in the ethanol extract of S. nigrum was also obtained to be 322±2.66 mg/g equivalents of gallic acid [20].

Characterization of NPs

The particle sizes of Ext-AG NPs and NPs were obtained about 473±21 and 627±32 (PDI≤0.5), respectively. Zeta potentials were -31.5±2.4 for NPs containing *S. nigrum L*. extract and 29±1.5 for NPs alone. SEM image also approved the formation of NPs, which were spherical or ellipsoidal (Fig. 1).

According to the Fig. 1. Ext-AG NPs have a dense and lattice structure. The result showed that the encapsulation efficiency of extract into AG NPs was about 91.6%.

In this study, the particle size of synthesized AG NPs was about 627 nm. Interestingly, the size of Ext-AG NPs reduced to 437 nm, which could be due to electrostatic interactions between the negative charge of the extract and the positive charge of nanoparticles. Studies have shown that positively or negatively charged nanoparticles accumulate in damaged skin more than neutrally charged particles. Since the net charge of the surface skin is negative (due to the presence of sulfated proteoglycans), positively charged NPs have a greater ability to penetrate the skin. On the other hand, it has been observed that the high cell adsorption of negatively charged NPs is firstly related to the non-specific process of NPs adsorption on the cell membrane and secondly is related to the formation of NPs clusters. Therefore, the NPs with different surface properties can affect their cell uptake and intracellular distribution, and by modifying their surface charge, NPs can be delivered to specific intracellular targets (lysosomes, mitochondria, cytoplasm, etc) [21, 22].

In vitro release study

The in vitro release profile of extract from Ext-AG NPs was evaluated in PBS (pH 7.4) (Fig. 2). The results indicated that the highest release of extract was occurred during the first 24 hr, especially in the first 4 hr (30%). As shown in Fig. 2, a burst release of extract from hydrogel to about 30% has been occurred during the first 24 h, especially in the first 4 h. Then a controlled and slow release of extract to about 70% has been observed till the end of release study. The initial burst release may be due to rapid gel swelling resulted in adsorbed extract diffusion in the direction of the surface of the gel matrix. The slow release of extract may be attributed to the viscosity of the hydrogel which effects on drug penetration from gels through the skin [23]. By embedding of NPs into hydrogel, the release of extract was about 12% after 6 days. On the other hand, the results showed that the extract could be release from hydrogel about 30% in the first 4h followed by about 70% release on the fifth day. Therefore, this formulation was used in subsequent studies. According to these results, it can be concluded that the placement of NPs containing the extract in the chitosan gel, traps the extract and prevents its release. This is due to the electrostatic interaction between the negative charge of the extract and the positive charge of chitosan, and the production of high amounts of nanoparticles for wound healing was not cost-effective in the animal model. Therefore, in the next step of the study, we used chitosan gel containing the extract, which was released in two stages.

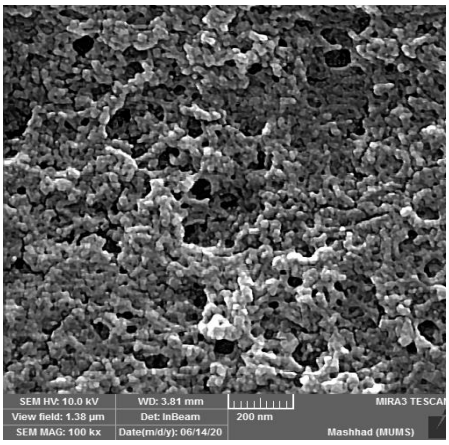

Fig 1. Morphological investigation of the Ext-AG NPs using SEM

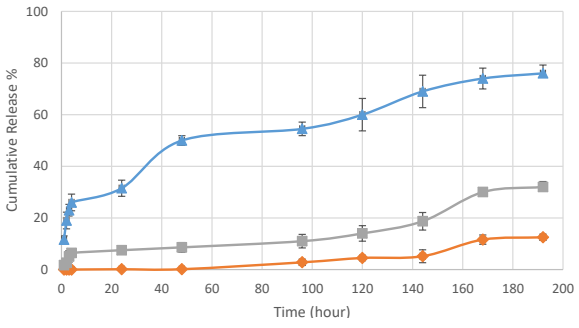

Fig 2. Release profile of Ext-AG NPs (gray), Ext-AG-CS hydrogel (red) and Ext-CS hydrogel (blue) in PBS (pH7.4) at predetermined time

Table 1. MIC (µg /ml) of prepared formulations against four types of microorganism

Microorganism / Formulation	*Staphylococcus Aureus*	*Bacillus Subtilis*	*Pseudomonas Aeruginosa*	*Escherichia Coli*
Chitosan hydrogel	<230	<230	<230	468
Extract 5%	12500	3125	1560	12500
Chitosan hydrogel-Extract 5%	<230/195	<230/195	<230/195	460/390

The viscosity of synthesized formulations

The results of the viscosity studies of the prepared formulations are shown in Fig. 3. According to these diagrams, it can be concluded that these gels have a pseudoplastic behavior. With increasing shear stress in all formulations, the viscosity of the solution were decreased. The gel containing the alcoholic extract of *S. nigrum L.* showed more decrease in viscosity than CS hydrogel.

The results of microbial limit tests

In different studies, the antimicrobial activity of *S. nigrum L.* has been investigated. Nabi Shariatifar et al. evaluated the antibacterial effect of aqueous extract of *S. nigrum L.*, *olive, artichoke* and *licorice* on some foodborne pathogens. The results showed that the inhibitory and lethal effect of the aqueous extract of black *S. nigrum L.* fruit was more than other aqueous extracts (in *Staphylococcus aureus* (MIC = 10 mg / ml). On the other hand, *Staphylococcus aureus* was the most susceptible bacterium and *Salmonella typhi Morium* was the most resistant to the other aqueous extracts [24]. After the time of incubation, which is mentioned for each of the quantitative and qualitative microbial limit tests, the culture tubes and plates were examined for microbial growth. For CS hydrogel, microbial growth was observed only for Gram-negative *P. aeruginosa*, but in the case of 5% extract and Ext-CS hydrogel 5%, no microbial growth was observed in the qualitative test. All three samples showed approximately less than 10 colony per milliliter of aerobic microorganisms, molds and yeasts. According to the European Pharmacopoeia, this number is acceptable in herbal skin products.

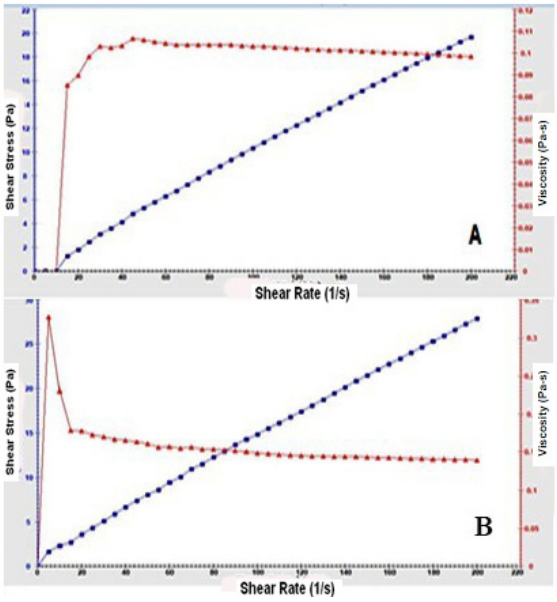

Fig. 3. Viscosity of A) CS hydrogel, B) Ext-CS hydrogel 5%. The red line shows the viscosity versus the share rate and the blue line represents the share stress/share rate

MIC determination

In this study, the MIC of Ext-CS hydrogel 5% on *S. aureus, P. aeruginosa and B. subtilius* was obtained at concentrations less than MIC of aqueous solution of *S. nigrum L.* leaf extract. Therefore, by loading the extract in CS hydrogel, more antimicrobial effects can be obtained. The results of obtained MIC are presented in Table 1. The Ext-CS hydrogel 5% formulation showed lower MIC compared to other groups maybe due to synergistic antimicrobial effects of chitosan and extract.

In vivo studies

In Fig. 4, images of the wound area is shown in different groups after 1, 3,7 and 14 days of treatments. Based on these results, treated groups with aqueous solution of 5% extract and Ext-CS hydrogel 5% showed the most wound healing effects. The difference in the amount of their effect with all other groups was visible from the third day and in the following days this difference became significant. So that, on the fourteenth day of the wound healing assay, new hair growth was seen at in the treated groups with aqueous the wound site solution of 5% extract and Ext-CS hydrogel 5%. In different studies, the wound healing effect of *S. nigrum L.* leaf extract has been evaluated.

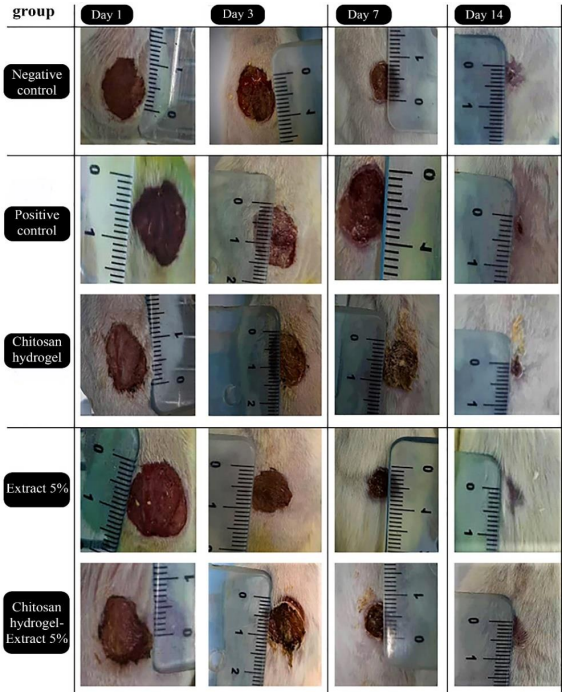

Fig 4. Evaluation the wound healing at the 1, 3, 7, and 14th days for different groups. The negative and positive control groups included the wound tissue treated with normal saline or phenytoin cream, respectively

Shirin Fahimi et al. used PHC (poly herbal cream) containing aqueous extract of Malva sylvestris and *S. nigrum L.* leaves and oil extract of Rosa damascene petals to repair burns [25].

At the end of the treatment period, there was a significant improvement in PHC-treated mice compared to the other groups. (For PHC 87.0% ± 2.1% compared to 32.2% ±1.6%; 57.0% ±5.3% and 70.8% ± 3.5% for control groups, basal cream and silver sulfadiazine cream). In addition, healed wounds in PHC-treated animals contained fewer inflammatory cells and had favorable re-epithelialization with significant neovascularization. In addition to its antioxidant activity, PHC exhibited antibacterial activity against *Staphylococcus aureus* [25]. In one study, Anam Javed et al. examined the effect of a 5% ethanolic extract of a combination of leaves and stems of neem (*Azadirachta indica*) and *S. nigrum L.* on wound healing for 4 weeks, in which adult male mice were grouped; A (*A. indica* + glycerin), B (*S. nigrum* + glycerin), C (*indica + S*. nigrum) and D (glycerin). Histopathological results showed controlled microbial attacks in groups A and B compared to groups C and D and re-epithelialization in the first week was significantly improved for group B. The results of the present study suggest that these herbal compounds have better regeneration rate and less side effect for skin wound healing and should be preferred over harmful industrial products [26]. Also, the antimicrobial and wound healing effects of chitosan have been extensively studied [16, 27].

Histopathological studies

The results of the criteria of histological examination for measuring wound healing, including inflammatory cell density, angiogenesis, fibroblast proliferation, collagen density, and epithelial tissue formation are shown in Fig. 4. On day 3, epiderm and dermis damage was observed with the presence of fibrin clots and inflammatory cells at the wound site. On this day, no tissue regeneration was observed in the negative control group (normal salin). In the positive control, Ext-CS hydrogel 5%, and the CS groups, thickening of the edges of the epithelial tissue was observed. However, the process of covering tissue regeneration in the extract solution treated group seemed better than other slides, where progressive covering tissue was observed. Granulation tissue, including forming blood vessels (angiogenesis) and fibroblast cell proliferation (fibroplasia) with collagen production, was observed in the groups treated with CS, Ext-CS hydrogel 5% and extract solution. On this day, the density of inflammatory cells in the extract and Ext-CS hydrogel groups was lower than the other groups. On day 7, the density of inflammatory cells in all slides was decreased. On this day, progressive epithelial tissue was observed in all groups. Granulation tissue including angiogenesis and fibroplasia were observed along with collagen production in all groups. The density of inflammatory cells in the negative control group was higher than the other groups. Remnants of necrotic tissue (scab) at the wound site were present in all groups. On the 14th day, in the extract solution, positive control and CS treated groups, epithelial regeneration progressed well and the epithelial tissue completely covered the wound surface. Progressive epithelial regeneration was observed in Ext-CS hydrogel 5% and negative control groups. Granulation tissue in the negative control group appeared fuller and younger than the other groups. The cell density of granulation tissue decreased in the extract, Ext-CS hydrogel

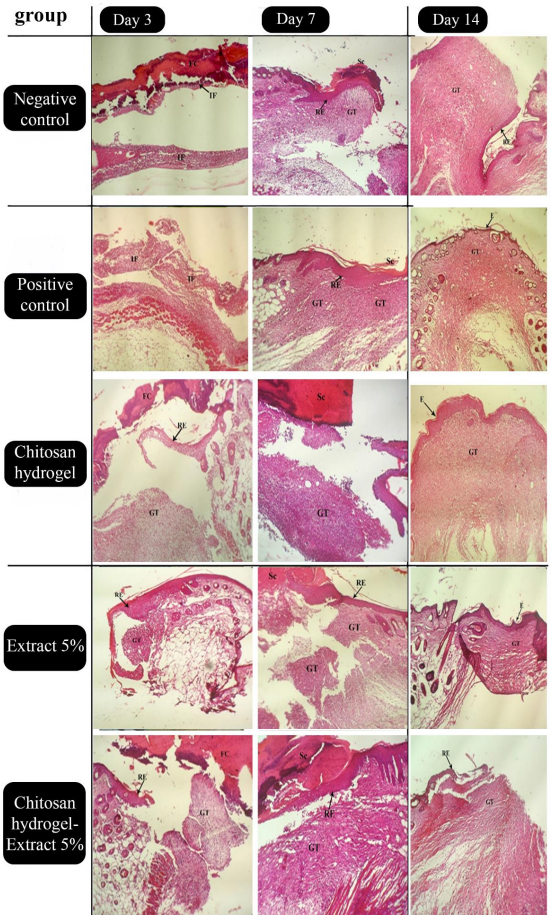

Fig 5. Histopathological assessment of wound healing by H&E staining on days 3,7, and 14. IF: Inflammatory cells, V: vessels, Sc: Scab, GT: Granulation Tissue FC: Fibrin Clot, ER: Epithelial Regeneration

5% and positive control groups, and thickened and regular collagen fibers were observed, and the granulation tissue appeared to be more mature than the CS and negative control groups. On this day, no remnants of necrotic tissue (scab) at the wound site were observed in all groups (Fig. 5).

The aim of this study was to load *S. nigrum L.* extract with antimicrobial and wound healing properties into CS hydrogel. Although extract alone and Ext-CS hydrogel 5%both showed good wound healing effects, but according to the microbial results, the amount of MIC of Ext-CS hydrogel 5% was lower than aqueous solution of leaf extract. Therefore, by loading the extract in CS, more antimicrobial effects can be obtained. In conclusion, it proposed that CS hydrogel containing *S. nigrum L*. leaf extract can effectively promote the wound healing process while simultaneously inhibit the inhibits infection at the wound site.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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