

## EXPERIMENTAL PAPER

# Wound healing potential of *Capparis spinosa* against cutaneous wounds infected by *Escherichia coli* in a rat model

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## Summary

Wound infection has become a major medical problem in recent years. This study was conducted to evaluate the healing activity of *Capparis spinosa* against surgical wounds infected by *Escherichia coli*. Twenty male rats were divided into two groups. Excisions were created surgically on the animals' skin and then infected with *E. coli*. Group 1 was treated with *C. spinosa* while Group 2 was untreated. Wound biopsy specimens were collected on days 5, 10 and 16 and analyzed. Results showed that the hydroxyproline content in treatment group was significantly higher in various post wounding days. Protein content increased gradually in ten days. Results of histopathological studies showed moderate to intense granulation tissue formation in treated group on day 10. The histopathological studies showed, that the new epidermis in treated group was thicker than in control group on day 16 post wounding. The present study has demonstrated that ethanol extract of *C. spinosa* includes properties that accelerate wound healing activities.

**Key words:** *Infected surgical wound, Capparis spinosa, Escherichia coli*

## INTRODUCTION

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. Wound healing is a programmed biological process that restores tissue continuity after injury and is a combination of physical, chemical and cellular events that restore the wounded tissue or replace it with collagen. Wound healing can be divided into three stages, including inflammation, proliferation and remodeling and maturation phases which involve the interaction of various cells, cytokines and growth factors [1]. The normal healing response begins immediately after the injury. When blood spills into the site of injury, the blood platelets contact with collagen and other elements of the extracellular matrix. This triggers the release of clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF- $\beta$ ). The inflammatory phase begins after the migration of neutrophils to the wound site to clean the tissue. Then, fibroblasts migrate into the tissue to begin the proliferative phase and deposit new extracellular matrix. This new collagen matrix becomes cross linked and organized [2].

Due to poor hygienic conditions in undeveloped and developing countries, wound infection has become a common disease in recent years [3]. Several reports associating the enterobacterium *Escherichia coli* with skin and soft tissue infections have been published: *E. coli* was found to be the causative agent of neonatal omphalitis [4], cellulitis localized to lower or upper limbs [5], necrotizing fasciitis [6, 7], surgical site infections [8], infections after burn injuries [9]. A public tendency towards the use of herbal wound healers is increasing, possibly due to lower side effects and prices of herbals, compared to chemical drugs.

*Capparis spinosa* (caper) (family *Capparidaceae*) is one of the most common aromatic plants growing wild in dry regions around the West or Central Asia and the Mediterranean basin. The caper has been known for centuries in traditional phytomedicine, which exploited its properties for several purposes. The aqueous extract from total aerial parts of the plant has been used for its antifungal [10], anti-inflammatory [11], antidiabetic [12], and antihyperlipidemic [13] activities and is among the constituents of polyherbal formulations to treat liver ailments [14], diuretic, antihypertensive and poultice [15]. Various parts of caper plant which can be used as drugs, cosmetics and foods are also used in different areas for landscaping, control of erosion or animal feeding [16].

The aim of this study was to assess the healing activity of *C. spinosa* against surgical wounds infected by *E. coli* in a rat model. To the best knowledge of the authors, no data has been published on wound healing activity of *C. spinosa*.

## MATERIAL AND METHODS

### The plant and extract preparation

The roots of *C. spinosa* were collected from varamin, province of Tehran, Iran. The root of the plant were dried and grounded into fine powder using an electric blender. Extract was prepared by cold maceration with distilled water for 24 h. Briefly, 50 g of powder were suspended at 100 ml ethanol for 24 h at a room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1). The extract was concentrated using vacuum distillation.

### Bacterial dilution

*E. coli* (ATCC 8739) was used as standard for inoculation and characterization of antibacterial activity of *C. spinosa*. A total of  $1 \times 10^6$  CFU/ml of the bacteria was used. *E. coli* was inoculated onto blood agar plates. Colonies were isolated and suspended in sterile distilled water. Serial dilutions were prepared and McFarland scale was used using spectrophotometry to achieve 0.5 McFarland dilution (wave length of 630 nm). The bacterial concentration was calculated  $1 \times 10^6$  CFU/ml in the dilution.

### Minimum inhibitory concentration (MIC)

To assess the minimum inhibitory concentration (MIC) of the extract, a stock solution of *C. spinosa* (30,000 ppm) was made. Briefly, four drops of span 20 (Croda Europe Ltd, England), as an emulsifier, were added to 0.3 g of hydroalcohol extract of *C. spinosa* in a sterile custom. Then, 10 ml of sterile phosphate buffer saline (PBS) were added to the solution and homogenized. A two-fold dilution of the extract in nutrient broth was made in sterile tubes and then adjusted inoculum of *E. coli* ( $1 \times 10^6$  CFU/ml) was added to each tube. Tubes were mixed gently and incubated at 35°C for 24 h. Then, the bacterial turbidity was assessed by visual examination. The last dilution, at which the growth of the bacteria was inhibited, was selected as the MIC of the extract [17].

### Animals

30 male *Sprague-Dawley* rats weighing  $254 \pm 14$  g were selected for the study. The animals were housed in a standard animal house (Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch). Rats were left for seven days at room conditions for acclimatization. They were supplied with water and food *ad libitum* and maintained at  $25 \pm 2^\circ\text{C}$ , 45–57% RH and 10:14 hr L:D cycle during the study. The animals were periodically weighed before and after experiments. All experiments on the animals were carried out according to “Guide for the Care and Use of Laboratory

Animals” published by the National Institute of Health (NIH) – approval No. A10/GVS/IAEC/0128 by the IAEC, University College of Veterinary Medicine, Garmsar, Iran.

## Wound excision

Animals were anaesthetized with a combination of 10% ketamine hydrochloride (50 mg/kg) and 2% xylazine hydrochloride (5 mg/kg). Dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound was marked. The site of excision was sterilized with povidine iodine followed by 70% ethanol. A full thickness of the excision of 225 mm<sup>2</sup> was created and left open. One milliliter of bacterial dilution ( $1 \times 10^6$  CFU/ml) was inoculated topically at the site of wound. Animals were divided in two equal groups and three subgroups (days 5, 10 and 16). Group 1 was topically treated with hydroalcohol extract of *C. spinosa*, while Group 2 was not treated with any solution. *C. spinosa* extract was used topically 4 h after bacterial inoculation. Treatment was continued daily for sixteen consequent days (every 24 h).

## Biochemical analysis

Wound biopsy specimens were collected from each group on days 5, 10 and 16 post wound excision. At the end of the experiment, animals were scarified and maintained in  $-80^{\circ}\text{C}$  until analysis. Hydroxyproline concentration was assessed as described by Woessner [18]. Total protein was measured using Lowry method [19].

## Histopathological studies

Wound biopsy specimens from each group were collected on days 5, 10 and 16 post excision. Tissues were fixed in 10% buffered formalin and routinely processed by standard procedures and then stained with hematoxylin and eosin (H&E). Stained specimens were microscopically evaluated to assess the histopathological changes during wound healing.

## Statistical analysis

Statistical analyses were carried out using SPSS, v16.0 (SPSS, USA) and the data were expressed as mean  $\pm$  standard deviation (SD). These used independent *t* test and analysis of variance. Differences were considered significant when  $p < 0.05$ .

## RESULTS

Mean concentrations of hydroxyproline and total protein are shown in table 1. A common pattern of changes in hydroxyproline was seen in all groups. The collagen

content increased gradually by time and reached the maximum level on Day 16 of post wounding. The hydroxyproline content in treatment group was significantly higher in various post wounding days ( $p < 0.05$ ). Protein concentration increased gradually by time and reached the maximum level on Day 16. Total protein increased significantly in wound tissues of treatment group ( $p < 0.05$ ). Histopathological studies showed an intense inflammatory response on Day 5 post wounding in both groups (fig. 1). A mild inflammatory response was continued in control group until Day 10 post wounding, while no inflammatory response was seen in treatment group. Results of histopathological studies showed moderate to intense granulation tissue formation and neovascularization characterized with increased fibroblasts in treatment group on Day 10 post wounding (fig. 2). The histopathological studies revealed matrix formation and collagen fiber deposition in treatment group on Day 16 post wounding. Also, thickness of the new epidermis on the wound borders in treatment group was  $1 \mu$  while it was  $0.4 \mu$  in control group (fig. 3).

Table 1.

Effect of *C. spinosa* extract on wound healing biochemical parameters

Biochemical parameters	Days	Groups	
		<i>C. spinosa</i>	Control
Hydroxyproline [mg/g]	5	18.8±5.71	13.23±0.35
	10	31.63±1.25	23.66±2.44
	16	74.8±1.15	64.63±3.18
Total protein [mg/100 ml]	5	17.2±0.85	17.3±0.75
	10	37.9±1.90	31.96±3.86
	16	45.13±0.70	34.63±0.55

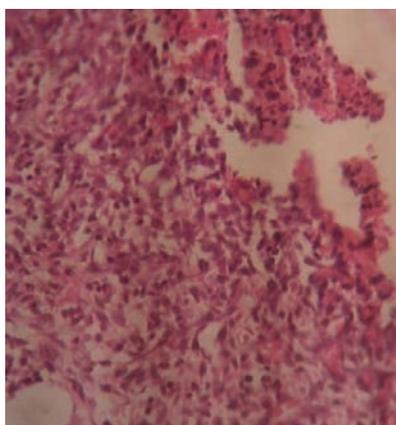
Values are expressed as mean ±SD; \* $p < 0.05$ 

Figure 1.

H&E sections of the wounds on Day 5 ( $\times 40$ ). An intense inflammatory response was seen in both groups

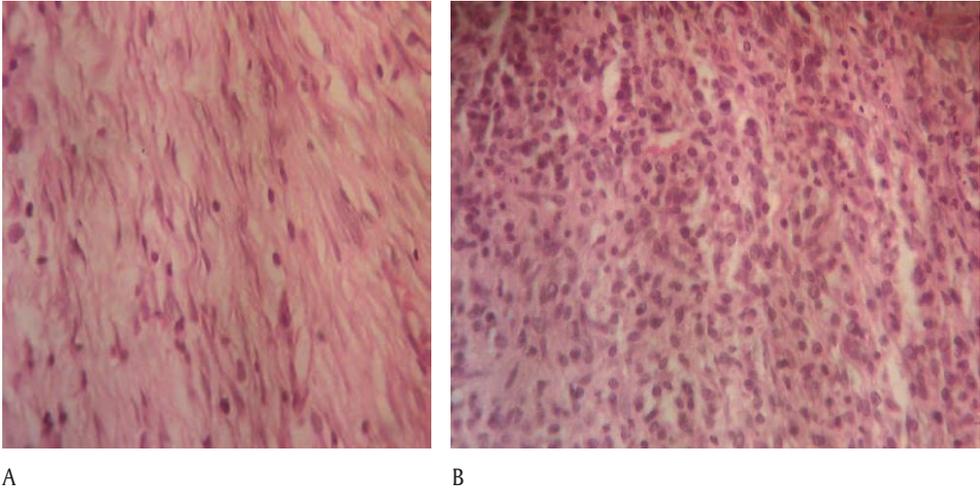


Figure 2.

H&E sections of the wounds on Day 10. A) treatment group: increased fibroblasts ( $\times 40$ ), B) control group: inflammatory response was seen in control group ( $\times 40$ )

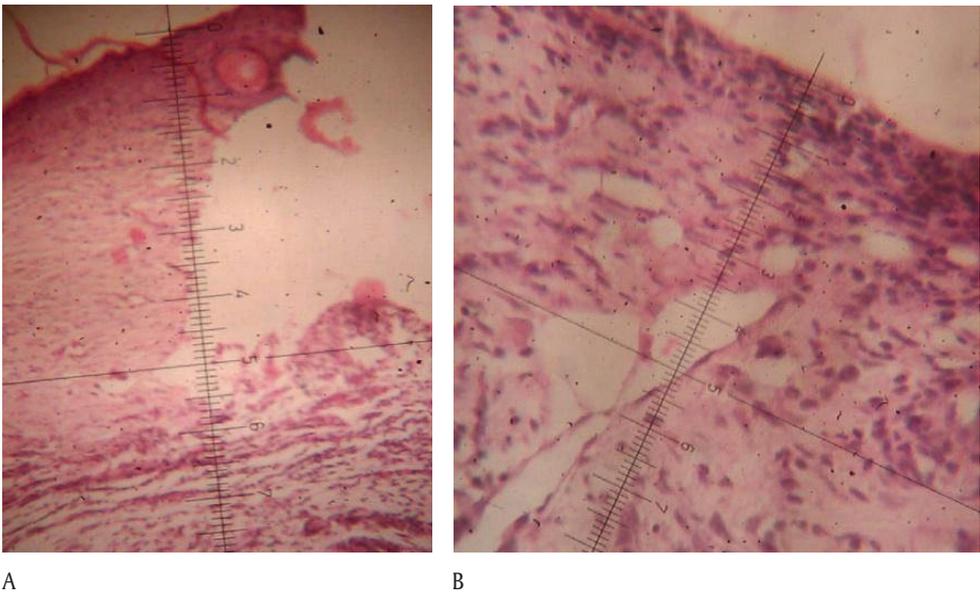


Figure 3.

H&E sections of the wounds on Day 16. A) thickness of the new epidermis on the wound borders in treatment group ( $\times 10$ ), B) and in control group ( $\times 10$ )

## DISCUSSION

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal [20]. In normal conditions, wound healing is considered as an accurately programmed insult that does not require considerable medical interventions. However, efficient and organized healing processes are absent in pathological conditions such as ulcers and infections. In such conditions, chronic inflammation is the predominant event characterized by abundant neutrophil infiltration, associated to reactive oxygen species and destructive enzymes [2]. Infection of wounds is one of frequent complications in patients undergoing surgeries. Nowadays, an increased interest is seen amongst researchers to use herbal agents in complicated infectious wounds due to increased antibiotic resistance in microorganisms. Plant products are potential agents for wound healing and are largely preferred because of their widespread availability, non-toxicity, lack of unwanted side effects and their effectiveness as crude preparations. A therapeutic agent selected for wound healing must improve at least one phase of healing process without producing any side effects [21]. Many plants are used as topical wound healers in Persian traditional medicine. One of these plants, *C. spinosa*, is used as an antiseptic agent in traditional medicine.

Hydroxyproline is the major constituent of collagen and found almost exclusively in collagen. The estimation of hydroxyproline is an accepted method of biochemical evaluation of total collagen content of a sample [22] and is also used as a marker of collagen synthesis [23]. An increase in the collagen content of the extracellular matrix is a characteristic change observed in the proliferative phase of wound healing [22]. Biochemical analysis of the excisional wound tissue of *C. spinosa* treated wounds demonstrated a significant increase in total collagen content compared to that of untreated wounds. The higher level of hydroxyproline in treatment group has indicated that more collagen has been produced. This is possibly seen due to increased collagen synthesis following fibroblast proliferation. In the current study, hydroxyproline concentration was increased gradually during study and reached the maximum on Day 16. Chithra et al. [24] and Pather et al. [25] showed that collagen content reached its peak on day 8 and then a mild continuous decline occurred until day 16 [24, 25]. In 2005, Gupta et al. [26] demonstrated that topical application of 1% seabuckthorn leaf extract significantly improved the healing process, as evidenced by increase in the content of hydroxyproline and protein as well as the reduction in wound area on day 8 post wounding. In a study by Nayak et al. [27] contents of protein and hydroxyproline were increased significantly in groups treated by *Cecropia peltata*, meaning that proliferative stage reached a peak on day 7 or 8 of post wounding [24, 25]. In the current study, biochemical assessment of collagen content (with maximal collagen content on day 16) showed different results, compared to other studies. This might be occurred due to moiety

of wound induced in the current study. Infection in wounds is considered as a complication and leads to delayed proliferation responses. Overall, higher hydroxyproline concentrations in various days revealed that the healing process proceeded in a correct manner in treatment group, as compared to control group. A relationship was seen between hydroxyproline concentration and histopathological findings. An intense inflammation was seen in wound tissues in both groups on day 4 post wounding; thus, hydroxyproline concentration was low in both groups on day 4 post wounding. Furthermore, an increased concentration of hydroxyproline was seen on Day 10 in treatment group, while control group had lower hydroxyproline concentration. This possibly occurred because of moderate granulation tissue formation and neovascularization in treatment group, while subsided inflammatory response was the prominent feature in control group.

Total protein content is an indicator for the protein level and cellular proliferation of the wound tissue [1]. Results of this study showed that total protein concentration of wound tissue in treatment group was higher than that in control group, which might be seen due to cellular infiltration or increased collagen synthesis rate. The present study has demonstrated that ethanol extract of *C. spinosa* includes properties that accelerate wound healing activities that may be due to its antimicrobial activity. The antimicrobial activity of each extract is also related to its chemical components. Flavonoids, as the major class of phenolic group, show antimicrobial activity by inhibition of nucleic acid synthesis, cytoplasm membrane function and energy metabolism [28]. According to literature reviews, this study is the first *in vivo* study that evaluates the healing activity of *C. spinosa* against wounds infected by *E. coli*. However, further and complementary studies are recommended to better understanding of the antimicrobial effects of *C. spinosa*.

## ACKNOWLEDGEMENTS

The authors wish to thank M. Gholibeigi, R. Nabi, H. Jourablo, K. Khosravi and M. Yazdani for excellent technical assistance.

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## MOŻLIWOŚĆ LECZENIA RAN CIĘTYCH ZAINFEKOWANYCH *ESCHERICHIA COLI* ZA POMOCĄ *CAPPARIS SPINOSA* NA MODELU SZCZURZYM

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### Streszczenie

Infekcje ran stały się ostatnio dużym problemem medycznym. W prezentowanym artykule przedstawiono badania przeprowadzone w celu określenia działania *Capparis spinosa* przeciwko infekcjom ran pooperacyjnych przez *Escherichia coli*. Dwadzieścia szczurów płci męskiej podzielono na dwie grupy. Skórę zwierząt nacięto operacyjnie, a następnie ranę zainfekowano *E. coli*. Grupę 1 leczono *C. spinosa*, grupa 2 nie była leczona. Biopsję ran przeprowadzono 5., 10. i 16. dnia, a następnie poddano analizie. Wyniki wykazały, że zawartość hydroksyproliny w grupie leczonej była wyraźnie wyższa w poszczególnych dniach następujących po powstaniu rany. Zawartość białka rosła stopniowo przez 10 dni. Obserwacje histopatologiczne wskazywały na umiarkowaną do intensywnej granulację tkanki w grupie leczonej 10. dnia. Na ich podstawie stwierdzono również, że nowa epiderma w grupie leczonej była grubsza niż w grupie kontrolnej po 16 dniach od zranienia. Prezentowane badania wskazują, że wyciąg etanolowy z *C. spinosa* ma właściwości przyspieszające gojenie się ran.

**Słowa kluczowe:** zainfekowana rana pooperacyjna, *Capparis spinosa*, *Escherichia coli*