



Effect of different levels of bell pepper (*Capsicum annuum*) alcoholic extract derived ointment on wound healing histopathological evaluation of male Wistar rats

Mones Moloody Tapeh¹, Damoun Razmjoue^{2,3}, Masumeh Jalalvand^{4*}

Abstract: The current study was aimed to evaluate effect of bell pepper alcoholic extract ointment in wound healing of male wistar rats. Twenty for male Wistar rats were randomly divided into four experimental groups. Experimental wound was induced on the anterior dorsal side of each Wistar rat. Group A served as control and received basal formulation ointment. Groups B, C and D received 2 and 4 and 6 percentage of bell pepper alcoholic extract. Wound healing activity as a closure percentage was determined on days five, 15 and 25 post operations. The number of fibroblast and macrophages, collagen deposition and neovascularization were assessed on 4, 8, 15 and 25 days after wound incision. Data showed that the wound area significantly decreased in bell pepper alcoholic extract treated groups compared to control group on days five, 15 and 25 post operations, respectively ($P \leq 0.05$). Application of bell pepper alcoholic extract, increased fibroblast and macrophages distribution and up regulated the new vessels formation and collagen deposition on days 4, 8, 15 and 25 and 30 after wound induction significantly ($P \leq 0.05$). In conclusion we suggested that the bell pepper alcoholic extract application at 2, 4, and 6 percentages was useful for wound healing in male Wistar rats.

Keywords: Bell pepper, Alcoholic extract, Ointment, Wound healing, Wistar rats.

<http://dx.doi.org/10.5935/1981-2965.20230028>

Recebido em 21.1.2020 Aceito em 30.09.2023

*Autor Correspondente:

¹*Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran*

²*Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran*

³*Traditional Pharmacy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

⁴*Department of Medical Biotechnology, Faculty of Medicine, Lorestan University of Medical Science, Khorramabad, Iran*

Corresponding author: Masumeh Jalalvand; Department of Medical Biotechnology, Faculty of Medicine, Lorestan University of Medical Science, Khorramabad, Iran

Introduction

Wound healing is a complex biological process involving cytokines with four phases: Hemostasis, inflammation, proliferation and remodeling. Understanding the molecular mechanism of the inflammation phase could improve wound healing in the clinic as excess inflammation is a critical point for dysregulation of normal wound healing. A variety of medicinal plants have been a source of healing in local communities around the world for thousands of years. It is still used as a primary health care method for 85% of the world's population (OKHOVATFARD AND REZAZADEH, 2023; BANIESMAEILI et al., 2023; KAZEMPOUR et al., 2023; BAZARI MOGHADDAM et al., 2023; RAMÍREZ et al., 2023).

Medicines made from various medicinal plants have many advantages such as less toxicity, economic value and cheapness, and sometimes they are more effective than chemical medicines (ASKARI et al., 2021; FADAEI RAIENI et al., 2020; ZANGENEH et al., 2023; SHAHMORADI et al., 2023; DARVISHI et al., 2023; DASTYAR and LYSIUK, 2023). Perhaps one of the reasons for the popularity of these drugs these days is these advantages. A bell pepper is the name for a plant species

(*Capsicum annuum*) cultivated for the bell-shaped fruits that come in red, yellow, green, and orange (ORTEGA et al., 2012).

They are excellent sources of phytochemicals, such as anthocyanins, vitamins, phenolic acids, flavonoids, carotenoids, and capsaicinoids (Oulai et al., 2018). Numerous studies have demonstrated the antibacterial activity of capsaicin and dihydrocapsaicin bioactive compounds isolated from bell pepper (EKOM et al., 2021). Bell pepper fruits are traditionally used in wound healing, but no scientific evidence is found in the literature that supports its activity (KOFFI et al., 2012). Some of study observation further suggests the contribution of the bell pepper fruit extract to the alteration of the microbial membrane, and the resulting leakage of intracellular materials may lead to microbial death, justifying its bactericidal effect.

The result of (Ekom et al., 2021) study demonstrates the antibacterial and therapeutic properties of the bell pepper fruit extract in an infected wound in a rat model. They also demonstrated that the gel based on bell pepper fruit extract showed a significant increase in the percentage of wound closure and caused a significant reduction of the number of colonies forming units of *S. aureus* at the infection site (SAMROT et al., 2018).

Considering the high availability and lower side effects of plants and plant-derived phytochemicals, interests are arising for herbal therapies (Sharifi Hosseini et al., 2018) Properties of different plants, many herbal extracts and their constituents have been tested for their promoting impact on wound healing. In spite of all the information available in literature, effect of bell pepper alcoholic extract on wound healing is not completely studied. Hence, this study was aimed to investigate the provoking effects of bell pepper alcoholic extract ointment on wound healing processing in male Wistar rats. To do that, the tissue fibroblasts, fibrocytes, macrophages distribution, vessel formation, and collagen and epidermis regeneration in rat model were analyzed.

Material and Methods

Fresh bell pepper was collected in Isfahan province, Iran. The bell peppers were identified in department of botany sciences faculty, Isfahan, Iran. Fruit parts were chopped and were dried and cleaned naturally on laboratory benches at room temperature (23-24°C) for 10 days until they were crisped and powdered by using an electric blender. The hydro distillation via cleverger type apparatus was performed to obtain essential oil from bell pepper (PIETRELLA et al., 2011). To assume wound healing property of bell pepper ointment, twenty-four male Wistar rats at 10 weeks old of ages, 220-240g of weight were used in this study. To adapt experimental rats in new experiment condition, they were kept one weeks prior the

study in a standard laboratory condition. Experimental rats kept in temperature of $22 \pm 3^{\circ}\text{C}$, $55 \pm 10\%$ of humidity and a 12h light and dark cycle.

During the study period experimental rats had ad libitum chew pellets and fresh water. All experiments were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of pain (Zimmermann, 1983) and current Iranian law for laboratory animal care. All experiments approved by ethic research committee of Islamic Azad University. To do the study plan four topical ointments were prepared. The control ointment was base formulation comprising Eucerin (25%) and Vaseline (75%) and three various bell pepper topical ointments were prepared. Ointment A was contained 2 g of bell pepper alcoholic extract in base formulation, Ointment B was contained 4 g of bell pepper alcoholic extract in base formulation and Ointment c was contained 6 g of bell pepper alcoholic extract in base formulation (SASIDHARAN et al., 2010).

The ointment was evaluated for primary skin irritation test on shaved back of the experimental rats for checking any abnormality of ointment (Laila et al., 2011). The experimental Wistar rats were starved for 12 hours prior to wounding. All of them anesthetized by intraperitoneal injection of ketamine 5%, 90mg per each kg by Ketaset 5% from Alfasan, Woerden, Netherlands Company and xylazine hydrochloride 2%, 5mg per

each kg by Rompun 2% from Bayer, Leverkusen, Germany, Company. The fur was aseptically and predetermined area marked on the back of experimental rats and they were fixed on the surgery table in ventral posture, then two circular wounds each about 200 mm² were made on the back of each experimental Wistar rat (MORTON AND MALONE, 1972).

Prepared ointments applied for evaluating its impact on wound healing (Shanbhag et al., 2006). Rats were kept individually under hygienic and controlled conditions in polypropylene cages with stainless steel top. The ointments were topically applied once a day, starting 24 hours post operation, on the wound area until wound completely healed. All of treated rats were monitored for any wound fluid or other abnormalities (SASIDHARAN et al., 2010).

Wound contraction percentage and wound closure time were used to assess wound healing property. A graph paper and permanent marker were used to measure size and the wound size was computed on days 5, 15 and 25 post operations (FARAHPOUR and HABIBI, 2012). Percent wound contraction was calculated from the days of measurement of wound areas. The wound healing percentage was calculated by the Walker formula (WALKER and MASON, 1968). The specimens from skin were taken on days 4,

8, 15, 25 and 30 after wound infection. Tissue samples were excised and fixed in neutral buffered formalin 10 percentage.

After that the sample tissues were processed routinely and the sections were microscopically evaluated to assess the predominant stage of wound healing include fibroblast, neovascularization, immune cells distribution and macrophages (Sasidharan et al., 2010). Data were assayed and analyzed by two-way analysis of variance (ANOVA process) using SPSS version 22.

Statically analysis

Obtained data result presented as mean \pm SEM and for treatment showing a main effect by ANOVA, means have compared by Dunnett's test and the ($P \leq 0.05$) were considered as significant differences between treatments.

Result

The results of bell pepper alcoholic extract ointment application on wound excision and contraction area are presented in table 1. As result shown, wound area significantly decreased in treated animals by 2,4 and 6 percentage of bell pepper alcoholic extract ointment compared to control groups on days 5, 15 and 25 post operation ($P < 0.05$). The wound closure was nearly completed in bell pepper alcoholic extract treated groups compared to control animals on day 25 and at the days 30.

Table 1: Effect of the bell pepper alcoholic extract ointment on wound excision area (mm²)

Day	Control	Bell pepper 2%	Bell pepper 4%	Bell pepper 6%
5	142±4 ^a	132±4 ^b	125±2 ^c	112±3 ^d
15	87±3 ^a	65±2.5 ^b	48±2 ^c	36±2 ^d
25	21±2 ^a	5±1.5 ^b	4±1.2 ^b	1±0.5 ^c
30	2±0.2 ^a	1±0.3 ^b	0	0

Values are presented in mean ± SEM. There are significant differences. Between groups with different codes in a the same rows (superscript letters a and b; P<0.05).

Data already showed that the number of fibroblast and macrophages was increased as well as vessel formation compared to control group on days 4, 8, 15 and 25 after incision (P<0.05). Also, there was significant difference for fibroblasts and macrophages distribution between 2, 4 and 6 percentage of bell pepper extract treated groups (P<0.05). The collagen bundles were well organized between vessels compared to control group on day 8 (P<0.05). Additionally, the mature collagen bonds formation and angiogenesis significantly increased in 3% treated group on day 15 post wound induction (P<0.05).

Discussion

The present investigation illustrated the wound healing properties of bell pepper alcoholic extract ointment in Wistar rats. Our data showed that, the bell pepper alcoholic extract improved wound contraction and is useful for wound healing in male rats. Wound healing is a dynamic cellular and physiological process which is mainly depends on rapid reformation of damaged tissue into normal

condition. In this regard, down-regulation of inflammation associated with provoked angiogenesis, even in early stages, promotes proliferative machinery such as fibroblasts, fibrocytes physiologic and compensatory functions (KUMAR et al., 2006).

Histological studies showed that, application of bell pepper alcoholic extract significantly increased fibroblast and fibrocytes distribution in wound area. Our data showed that mean distribution of mast cells increased in bell pepper treated rats were indicating the bell pepper alcoholic extract role in elevating mast cells proliferation. Bell pepper alcoholic extract have antioxidant, antimicrobial and antifungal agent's roles. Based on findings of current study we think wound healing properties of bell pepper alcoholic extract was not related only to the antioxidant activity and impress its effect by all mechanisms.

Conclusion

In conclusion we conclude that bell pepper alcoholic extract ointment application increased fibroblast proliferation and collagen

synthesis which finally shortened wound area. In According to the current study results, topical administration of bell pepper alcoholic extract ointment decreased inflammatory cells and afterward increased in fibroblast migration and wound contraction. However, delayed inflammatory phase during healing process increases abnormal cellularity in proliferation phase.

The bell pepper alcoholic extract ointment works as antioxidant, antimicrobial and antifungal agents. The wound healing properties of bell pepper alcoholic extract ointment was not related only to the antioxidant activity and impress its effect by all mechanisms. Further studies are needed to clear pharmacological properties and physiological mechanisms by those phenolic compounds in bell pepper alcoholic extract act and distinguish its potential for clinical use in clinical trials.

References

ADAM J, SINGER MD, RICHARD AF AND CKARK MD.1999. Cutaneous Wound Healing. The New England Journal of medicine 341:738-746.

ASKARI N, RANJBAR M, SHAFIEIPOUR A. 2021, Effect of oral administration of Blepharis.Persica extracts on CATSPER gene expression and sperm parameters in rats with spinal cord injury. *Agricultural Biotechnology Journal*, 13(2): 1-20. doi: **10.22103/jab.2021.17133.1290.**

BANIESMAEILI SY, AKBARZADEH A, RIAZI G, ABDOLLAHI F, NIROMAND M. 2023. Effects of dietary Moringa oleifera leaf powder and ethanolic extract on expression function of immune genes of Litopenaeus

vannamei. *Aquatic Animals Nutrition*, 9(1): 27-41. doi: 10.22124/janb.2023.24268.120.

BAZARI MOGHADDAM S, BAGHERZADEH LAKANI F, JALILPOUR J, MASOUMZADEH M, SHENAVAR MASOULEH A. 2022. Effects of Echinacea purpurea and Allium sativum powdered extracts on growth indices, survival rate and liver enzymes in farmed Huso huso. *Aquatic Animals Nutrition*, 8(1): 25-39. doi: **10.22124/janb.2023.21942.1164.**

BELDON. 2010. Basic Science of wound healing. *Surgery* 28.409-492.

Brancato SK and Albina JE 2011. Wound macrophages as key regulators of repair: origin, phenotype, and function. *American Journal of Pathology* 178(1):19-25.

DARVISHI M, OMRANI NAVA A, KARIMI E, NOURI M, MEIGOONI SS, HEJRIPOOR SZ. 2023. Human and animal bites. *Caspian Journal of Environmental Sciences*, 21(2): 445-456. doi: 10.22124/cjes.2023.6539.

DASTYAR N, LYSIUK R. 2023. Identification of the most important medicinal plants used for wound healing: An ethnobotanical study of Sistan and Baluchestan province, Southeastern Iran. *Journal of Biochemicals and Phytomedicine*. 2(1): 16–19. doi: **10.34172/jbp.2023.4.**

EKOM SE, TAMOKOU JD, KUETE V.2021.Antibacterial and therapeutic potentials of the capsicum annum extract against infected wound in a rat model with its mechanisms of antibacterial action. *Biomed Res Int*.4:4303902.

FADAEI RAIENI R, ENAIAT GHOLAMPOR T, SEPAHI J. 2020, Effects of Lavandula angostifolia on growth performance, survival rate and immunity system in Grass carp (Ctenopharyngodon idella). *Aquatic Animals Nutrition*, 6(2): 1-12. doi: **10.22124/janb.2021.18059.1112**

FARAHPOUR MR AND HABIBI M .2012. Evaluation of the wound healing activity of an ethanolic extract of ceylon cinnamon in mice. *Veterinary Medicine* 57(1):53-57.

KAZEMPOUR M, SHAHANGIAN SS, SARIRI R. 2023. Dracocephalum kotschy: Inhibition of critical enzyme relevant to type-2 diabetes, essential oil composition, bactericidal and antioxidant activity. **Caspian Journal of Environmental Sciences**, 2023; 1-15. doi: 10.22124/cjes.2023.6256.

KOFFI-NEVRY R., KOUASSI K. C., NANGA Z. Y., KOUSSÉMON M., LOUKOU G. Y. 2012. Antibacterial activity of two bell pepper Extracts: Capsicum annum L. and Capsicum frutescens. **International Journal of Food Properties** .15(5):961–971.

KUMAR R, KATOCH SS AND SHARMA S .2006. Beta adrenoceptor agonist treatment reverses denervation atrophy with augmentation of collagen proliferation in denervated mice gastrocnemius muscle. **Indian Journal of Experimental Biology** 44(5): 371-376.

OKHOVATFARD M, REZAZADEH H. 2023. Effect of Curcuma longa and its derivatives, curcumin and curcuminoids on treatment of oral lichen planus: A systematic review of clinical evidence. **Caspian Journal of Environmental Sciences**, 1-12. doi: 10.22124/cjes.2023.6824.

ORTEGA M. H., MORENO A. O., NAVARR M. D. H., CEVALLOS G. C., ALVAREZ L. D., MONDRAGON H. N.2012. Antioxidant, antinociceptive, and anti-inflammatory effects of carotenoids extracted from dried pepper (Capsicum annum L.) **Journal of Biomedicine and Biotechnology**.524019:10.

OULAI A. C., DJE K. M., EBA K. P., ADIMA A. A., KOUADIO E. J. P.2018. Chemical composition, antioxidant and antimicrobial activities of Capsicum annum var. annum concentrated extract obtained by reverse osmosis. **GSC Biological and Pharmaceutical Sciences** .5(2):116–125.

RAMÍREZ J H, CURAY RR, OCHOA JS, VÉLEZ JM, CAZAR CHÁVEZ DJ. 2023. Determination of the antimicrobial effectiveness of the essential oil of Origanum vulgare against strains of Escherichia coli isolated from bovine mastitis. **Caspian Journal of Environmental**

Sciences, 2023; 1-9. doi: 10.22124/cjes.2023.7202.

SAMROT V. A., SHOBANA N., JENNA R.2018. Antibacterial and antioxidant activity of different staged ripened fruit of Capsicum annum and its green synthesized silver nanoparticles. **Bio Nanoscience** 8(2):632–646.

SASIDHARAN S, NILAWATYI R, XAVIER R, LATHA LY AND AMALA R .2010. Wound healing potential of Elaeis guineensis Jacq leaves in an infected albino rat model. **Molecules** 15(5) 3186-3199.

SHAHMORADI MK, AMINI NOGORANI M, MANSOURI F, ZAREI L. 2023. Combination of zinc nanoparticles with chitosan scaffolds increased cytokine genes on wound healing of infected rats with methicillin-resistant Staphylococcus aureus (MRSA). **Advancements in Life Sciences**.

SHANBHAG TV, SHARMA C, ADIGA S, BAIRY LK, SHENOY S AND SHENOY G .2006. Wound healing activity of alcoholic extract of Kaempferia galangal in winster rats. **Indian Journal of Physiology and Pharmacology**.50 (4): 384-390..

SHARIFI HOSSEINI MM, TORBATINEJAD N, TEIMOURI YANSARI A, HASSANI S, GHOORCHI T, TAHMASBI R 2018, The effects of corn silage particles size and fat supplement on feed intake, digestibility, ruminal function, chewing activity, and performance in mid-lactating Holstein dairy cows. **Journal of Livestock Science and Technologies**, 6(2): 21-32. doi: 10.22103/jlst.2019.12908.1250.

ZANGENEH M M, ZANGENEH A, ETEMADI N, TABATABAEI AGHDA S J, POURKAMALZADEH M, ZAND S, et al. 2023. The Effect of Nano Silver Particles of the Aqueous Extract of Artemisia aucheri on the Healing Process of Skin Wounds in Rats. **Plant Biotechnol Persa** 5 (1) :1-7. doi: .

ZIMMERMANN M.1983. Ethical guidelines for investigations of experimental pain in conscious animals. **Pain** 16(2):109-10