VETERİNER HEKİMLER DERNEĞİ DERGİSİ
JOURNAL OF THE TURKISH VETERINARY MEDICAL SOCIETY

ISSN: 0377-6395
e-ISSN: 2651-4214
Dergi ana sayfası:
Journal homepage:
http://dergipark.org.tr/vetheder

doi: 10.33188/vetheder.1183380

Araştırma Makalesi / Research Article

Wound healing effects of bee venom on adipose tissue derived mesenchymal stem cells

Özge ÖZGENÇ ÇINAR [1a*], Sedat SEVİN [2 b]

[1] Ankara University, Faculty of Veterinary Medicine, Department of Histology Embriyology, Ankara, Turkey
[2] Ankara University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ankara, Turkey
ORCID: 0000-0002-8776-4788 [a]; 0000-0003-0475-9092 [b]

MAKALE BİLGİSİ / ARTICLE INFORMATION:

Geliş / Received:
13 Ekim 22
13 October 22

Revizyon/Revised:
15 Aralık 22
15 December 22

Kabul / Accepted:
21 Aralık 22
21 December 22

Anahtar Sözcükler:
Bal arısı
Arı zehri
Mezenkimal Kök Hücre
Yara İyileşmesi

Keywords:
Honey bee
Bee venom
Mesenchymal Stem Cell
Wound Healing

ABSTRACT:

The objective of this study is to determine the effects of bee venom on the proliferation capacity of mesenchymal stem cells and wound healing. For this purpose, mesenchymal stem cells were isolated from canine adipose tissue and bee venom samples were collected from *Apis mellifera* anatoliaca in Muğla province of Türkiye. Cell viability test was performed on mesenchymal stem cells exposed to various concentrations (40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm) of bee venom. And wound healing test was performed on cells treated with the doses (5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm) and imaged every two hours for 16 hours. According to the results of our study's cell proliferation assay and wound healing test, bee venom had no proliferative effect on mesenchymal stem cells within the defined dose range. The study's outcomes may be enhanced by investigating the effect of bee venom on mesenchymal stem cells in combination with other substances or by improving the bee venom's purification process. Even while we have a better understanding of the mechanisms of action of bee venom components, there are still a lot of unanswered questions on the subject. It is believed that figuring out how bee venom affects wound healing may be useful for advancing wound care in both veterinary and human medicine.

Arı zehirinin yağ dokusu kaynaklı mezenkimal kök hücre üzerindeki yara iyileştirici etkileri

ÖZET:

Bu çalışmanın amacı, arı zehirinin mezenkimal kök hücrelerin çoğalma kapasitesi ve yara iyileşmesi üzerindeki etkilerini belirlemektir. Bu amaçla köpek yağ dokusundan mezenkimal kök hücreler izole edilmiş ve Muğla çevresinde bulunan *Apis mellifera* anatoliaca'dan arı zehiri örnekleri toplanmıştır. Farklı konsantrasyonlarda arı zehirine maruz bırakılan mezenkimal kök hücreler üzerinde hücre canlılığı testi (40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0,625 ppm ve 0.312 ppm) yapılmıştır. Hücreler üzerinde 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm arı zehri kullanılarak yara iyileştirme deneyi yapılmış ve 16 saat boyunca her iki saatte bir görüntülenmiştir. Çalışmanın hücre canlılığı ve yara iyileşme deneylerinin sonuçlarına göre, arı zehirinin köpek yağ dokusu kaynaklı mezenkimal kök hücreler üzerinde tanımlanan doz aralığında proliferatif bir etkisi olmamıştır. Çalışmanın sonuçları, diğer maddelerle birlikte mezenkimal kök hücreler üzerindeki arı zehirinin etkisini araştırarak veya arı zehirinin saflaştırma sürecini geliştirerek iyileştirilebilir. Arı zehiri bileşenlerinin etki mekanizmalarını daha iyi anlamış olsak da konuyla ilgili hala cevaplanmamış birçok soru bulunmaktadır. Arı zehrinin yara iyileşmesi üzerindeki etkisinin belirlenmesinin hem veteriner hekimlikte hem de beşeri hekimlikte yara tedavisini kolaylaştırmada avantaj sağlayacağı düşünülmektedir.

How to cite this article: Çınar ÖÖ, Sevin S. Wound healing effects of bee venom on adipose tissue derived mesenchymal stem cells. Vet Hekim Der Derg 94 (1): 59-66, 2023. DOI: 10.33188/vetheder.1183380.

* *Sorumlu Yazar e-posta adresi / Corresponding Author e-mail address:* ozgenc@ankara.edu.tr

1. Introduction

Honeybees are hymenopteran insects that belong to the genus *Apis* and are known for producing and storing honey and other chemicals that could be valuable to humans. Bee products used for the human benefit include honey, propolis, bee pollen, bee bread, beeswax, and bee venom.

Bee venom (BV), also known as apitoxin secreted by bee venom glands, is one of the bee products has a wide spectrum of biological activity (1). BV is an odorless, colorless liquid that contains a hydrolytic combination of proteins with an acid pH (4.5 to 5.5) that bees utilize to defend themselves against predators. BV includes 88 percent water and merely 0.1 g of dry venom per drop (2). Melittin, adolapin, apamin and mast cell degranulating peptides are among the peptides that constitute the structure of bee venom. It also contains enzymes, particularly Phospholipase A2, as well as low-molecular-weight substances such as bioactive amines (such as histamine and adrenaline) and minerals (3).

Multiple therapeutic applications for BV have been established for various diseases since the initial investigations in apitherapy at the turn of the twentieth century. Due to the anti-inflammatory properties of this venom, various forms of traditional bee venom therapy have been used to relieve pain and treat chronic inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, including the administration of live stings, venom injections, and venom acupuncture (4). In addition, bee venom is a chemical that has been tried in the treatment of cancer and neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease (3). Bee products have been utilized not only in treatment but also for cosmetics. Studies have shown that bee products have a positive effect on the skin, and the use of BV in wound healing underlines their therapeutic significance (5, 6).

Mesenchymal stem cells (MSCs) are one of the adult stem cells and were first isolated from the bone marrow (7). They have also been isolated from various tissues such as adipose tissue, which is an efficient source of MSCs. MSCs have been regarded as a promising therapeutic option for many injured tissues besides having therapeutic effects for many diseases. The presence of MSCs in tissues, coupled with their significant roles in normal wound healing, implies that MSCs may be beneficial for wound healing. Thus, a great number of studies have been conducted to investigate MSCs and their secretions' therapeutic potential for difficult-to-treat wounds (8). We believe that the beneficial qualities of MSCs for wound healing may be enhanced by BV's effect. However, to the best of our knowledge, the wound healing effects of BV on canine adipose tissue derived MSCs have not been reported to date. Therefore, in this study, we investigated the potential on wound healing properties of adipose tissue-derived MSCs exposed to different doses of bee venom. It is thought that determining the effect of bee venom on wound healing will provide an advantage in facilitating wound treatment in both veterinary medicine and human medicine.

2. Material and Methods

Bee venom collection, preparation and determination

Samples of bee venom from *Apis mellifera* anatoliaca colonies located in Muğla province of Türkiye were taken in September 2021. The sample was collected using a Beesas bee venom collector (BeeSas beekeeping, Turkey). To collect bee venom, a sharp lancet was used to scrape the glass plates off after the bees' venom had fallen on them. After being freeze-dried, the bee venom was kept in a freezer at 20 °C until analysis. Bee venom content analysis was carried out by Muğla Sıtkı Koçman University Food Analysis Application and Research Center. Using an Agilent 1260 HPLC with a binary pump and degasser unit, together with an Agilent 1200 Series autosampler, autoinjector, column oven, and variable wavelength detector, component analysis of apamine, phospholipase A2, and melittin were carried out (VWD). The Poroshell C18 column performed the separation. The optimum separation temperature was 20 °C and the column flow rate was 1 ml/min. The absorbance was measured at 218 nm (9).

Cell culture

Explant culture method was preferred for MSC isolation from canine adipose tissue. Adipose tissue (1cm3) split into small pieces in a sterile petri dish under laminar flow and kept at 37 °C with 5% CO^2 for 20 minutes. The cells were maintained in medium containing 20% Fetal bovine serum (Biowest, USA), 2% L-Glutamine, 1% Penicillin, Streptomycin, Amphotericin (Lonza, USA) and 77% Modified Eagle Medium (Lonza, USA) (10).

Cell viability assay

Methyl thiazolyl tetrazolium (MTT) was used as a substrate to examine the impact of bee venom on the cytotoxicity of MSCs. In 96-well plates, MSCs were cultivated at a density of $10x10^4$ cells per well. BV (10 mg/ml) was prepared by dissolving with PBS and filtration via a 0.22 µm pore size. Bee venom concentrations of 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm were applied to cells for 24 hours. Following the incubation, 100 µL of solution containing MTT reagent (5 mg/mL) that dissolved with PBS and low glucose serum-free medium mixed at a ratio of 1:10 by volume was added. A microplate reader (Sunrise, Tecan GmbH, Austria) was used to measure the number of formazan salts at 570 nm after the formazan salt generated after 4 hours of incubation was solubilized with sodium dodecyl sulfate (10%, 100 µL).

Wound healing test

Following isolation MSCs, $10x10^4$ cells were grown in 6-wells and incubated for 24 hours under cell culture conditions to achieve a density of between 70% and 80% confluence. A 1 mL pipette tip was used to scrape the monolayer cells across the well's center. Cells were gently washed with PBS twice. Fresh medium was added with four concentrations of either bee venom (5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm). Cells were incubated for 24 h under cell culture conditions. Cells were rinsed three times with PBS and photographed every two hours for 16 hours. Gap sizes were measured with Leica Application Suit software. The area of the wound was measured in every group and the percentage of the scratch area was compared with the control.

Statistical analysis

To evaluate how BV concentrations affected the results of the MTT analyses, a two-way analysis of variance was used. Tukey test was used as an advanced test for the factors that were found to be significant. Analysis was done with GraphPad Prism software and data are presented as mean ± standard deviation. Statistical significance was expressed as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

2. Results

Determination of bee venom

Table 1 displays the analysis results (%) of the sample of bee venom that was obtained. Using HPLC-VWD, apamin, phospholipase, and melittin concentrations in bee venom were calculated to be 4.05%, 14.36%, and 70.98%, respectively.

Table 1: The concentrations of components of bee venom.

Tablo 1: *Arı zehiri bileşenlerinin konsantrasyonları.*

Sample	Apamin (%)	Phospholipase A2 (%)	Melittin (%)
Sample 1	4.05	14.36	70.98

Cell viability assay

Cell viability test was performed on mesenchymal stem cells exposed to 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm concentrations of bee venom. A calibration curve was drawn using the absorbance values that correspond to the 500, 1000, 2000, 3000, 4000, 6000, 8000, and 12000 MSCs numbers. According to the calibration curve, the cell numbers were calculated. The number of cells in the control group was significantly higher than in all other experimental groups. When the groups were evaluated within themselves, it was seen that the cell counts decreased significantly at 20 ppm and 40 ppm doses compared to the other groups. Among the 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm doses, the group with the highest cell count was the group exposed to 2.5 ppm bee venom. This elevation in the 2.5 ppm group was only significant compared to the 0.312 ppm group.

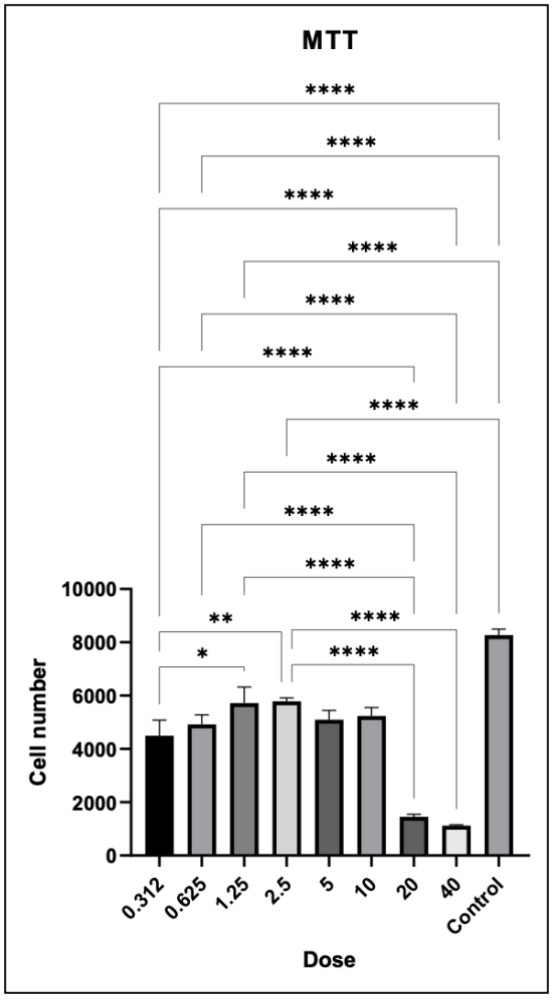

Figure 1: MSC numbers exposed to 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm concentrations of bee venom obtained from the absorbance values of the MTT test (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).

Şekil 1: *MTT testinin absorbans değerlerinden elde edilen arı zehirinin 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm ve 0.312 ppm konsantrasyonlarına maruz kalan mezenkimal kök hücre sayıları (* P < 0,05, ** P < 0,01, *** P < 0,001, **** P < 0,0001).*

Wound healing test

Wound healing test was performed on mesenchymal stem cells exposed to 5 ppm, 2.5 ppm, 1.25 ppm and 0.625 ppm concentrations of bee venom. While the wound line became confluent at the 10th hour in the control group, healing was completed at the 14th hour in all other experimental groups.

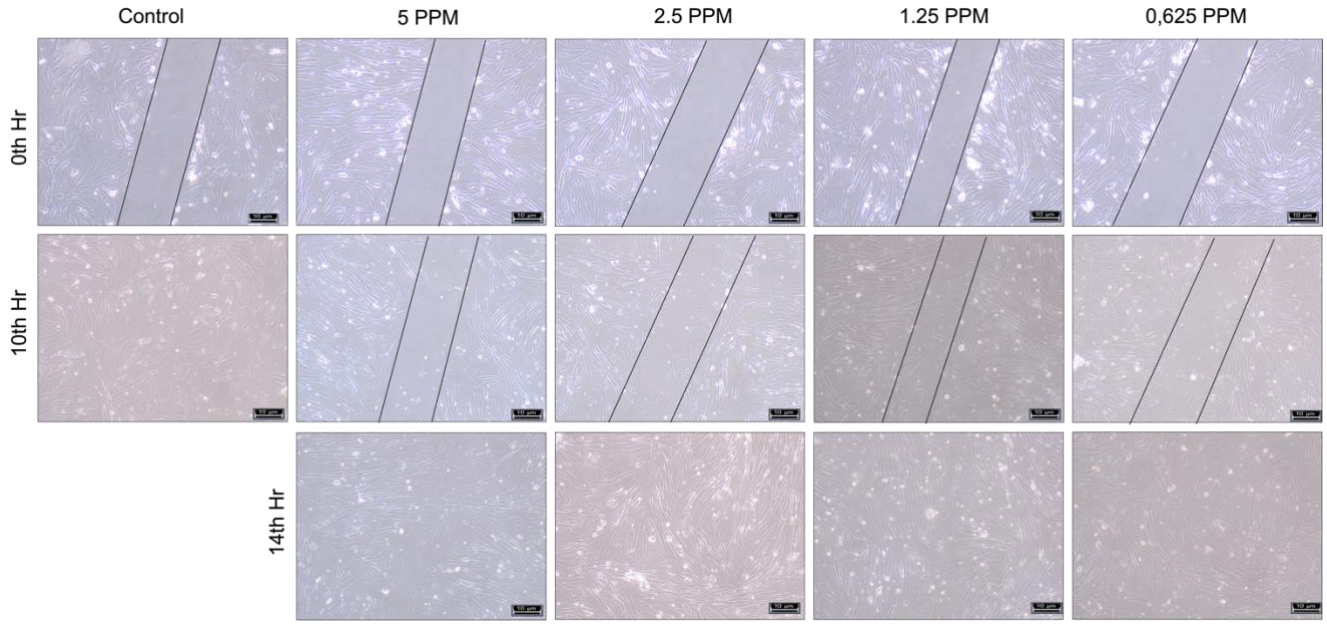

Figure 2: Wound healing test results of 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and control groups.
Şekil 2: *5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm ve kontrol gruplarının yara iyileşmesi analizi sonuçları.*

3. Discussion and Conclusion

Bee venom is the most researched venom among other arthropod venoms due to its anti-inflammatory, antioxidant, antifungal, antiviral, antimicrobial and analgesic properties that positively affect the wound healing process (11). In this study, we investigated the effect of bee venom on the wound healing ability of adipose tissue-derived MSCs. We also focused on which doses of BV are more effective in wound healing on MSCs. For this purpose, we selected doses of 40 ppm, 20 ppm, 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm, and 0.312 ppm for cell viability analysis on MSCs (12).

Cell proliferation and wound healing test results of our study have shown that bee venom has no proliferative effect on mesenchymal stem cells in the specified dose range. However, *in vivo* studies are showing the positive effects of bee venom on wound healing by suppressing activating transcription factor-3 and inducible nitric oxide synthase and mobilizing bone marrow-derived endothelial progenitor cells (13). It has been reported that a combination of bee venom and chitosan causes a decrease in wound size and an increase in epithelial proliferation in the wound model (14). Studies in diabetic mice have shown that BV treatment significantly improves wound closure by increasing type I collagen and stimulating angiogenesis (15, 16, 17, 18). Contrary to all these studies, there are data that bee venom has a direct antitumor effect *in vivo* and *in vitro* (12, 19, 20, 21). Researchers have focused on the therapeutic potential for cancer treatment of Melittin which is the principal active component of bee venom. According to various *in vitro* and *in vivo* studies Melittin has effects on the cellular functions of cancerous cells such as proliferation, apoptosis, metastasis, angiogenesis, and cell cycle (3, 22, 23, 24, 25). Several earlier studies have shown that BV has promising anticancer effects on a variety of human cancer cell types, including lung cancer (26), breast cancer (27), ovarian cancer (28), prostate cancer (29), melanoma (30) as well as leukemia (31). The fact that bee venom did not have a proliferative effect even at lower doses compared to the control group in our study suggests that bee venom can be studied in cancer

lines. However, while bee venom destroys cancer cells, the possibility of suppressing healthy cells in the environment may be a disadvantage for cancer studies.

In conclusion, BV at doses of 40 ppm and 20 ppm showed cytotoxic effects on mesenchymal stem cells and decreased metabolic activity of cells at doses of 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm and 0.312 ppm. BV has no wound healing effect on canine adipose tissue derived MSCs in the selected dose range. Even if we have a better understanding of the mechanism of action of BV, it is necessary to develop the purification method of bee venom and to investigate the effect on MSCs by separating the peptides and enzymes in BV. The effect of bee venom on MSCs should be investigated in detail with *in vivo* studies. Investigating the effect of bee venom on MSCs in combination with other substances that support the cells' proliferative properties may improve the study's results.

Conflict of Interest

The author declared that there is no conflict of interest

Funding

This research received no grant from any funding agency/sector.

Authors' Contributions

Motivation / Concept: Özge ÖZGENÇ ÇINAR
Design: Özge ÖZGENÇ ÇINAR, Sedat SEVİN
Control/Supervision: Özge ÖZGENÇ ÇINAR, Sedat SEVİN
Data Collection and / or Processing: Özge ÖZGENÇ ÇINAR
Analysis and / or Interpretation: Özge ÖZGENÇ ÇINAR, Sedat SEVİN
Literature Review: Özge ÖZGENÇ ÇINAR, Sedat SEVİN
Writing the Article: Özge ÖZGENÇ ÇINAR
Critical Review: Özge ÖZGENÇ ÇINAR, Sedat SEVİN

Ethical Statement

An ethical statement was received from the authors that the data, information, and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules

References

1. Somwongin S, Chantawannakul P, Chaiyana W. Antioxidant activity and irritation property of venoms from Apis species. Toxicon 2018;*145*:32-39.
2. Bellik Y. Bee venom: its potential use in alternative medicine. Anti-infective agents 2015;*13*: 3-16.
3. Moreno M, Giralt E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. Toxins 2015;*7*:1126-1150.
4. Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier JM, Fajloun Z. Bee venom: Overview of main compounds and bioactivities for therapeutic interests. Molecules 2019;24(16):2997-2298.
5. Hozzein WN, Badr G, Al Ghamdi AA, Sayed A, Al-Waili NS, Garraud O. Topical application of propolis enhances cutaneous wound healing by promoting TGF-beta/Smad-mediated collagen production in a streptozotocin-induced type I diabetic mouse model. Cell. Physiol. Biochem. 2015;37(3):940-954.
6. Goharshenasan P, Amini S, Atria A, Abtahi H, Khorasani G. Topical application of honey on surgical wounds: A randomized clinical trial. Complement. Med. Res. 2016;*23*(1):12-15.
7. Friedenstein AJ, Piatetzky SII, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol. 1966;16: 381-390.

8. Huang YZ, Gou M, Da LC, Zhang WQ, Xie HQ. Mesenchymal stem cells for chronic wound healing: current status of preclinical and clinical studies. Tissue Engineering Part B: Reviews 2020;*26*(6):555-570.

9. Frangieh J, Salma Y, Haddad K, Mattei C, Legros C, Fajloun Z, El Obeid D. First Characterization of The Venom from Apis mellifera syriaca, A Honeybee from The Middle East Region. Toxins 2019;11:191-192.

10. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose Tissue: Implications for cell-based therapies. Tissue Engineering 2001;7: 211-228.

11. Kurek-Górecka A, Komosinska-Vassev K, Rzepecka-Stojko A, Olczyk P. Bee venom in wound healing. Molecules 2020;*26*(1): 148.

12. Gülmez Y, Aydın A, Can İ, Tekin Ş, Cacan E. Cellular toxicity and biological activities of honey bee (Apis mellifera L.) venom. MARMARA Pharm. J. 2017; 21(2): 251-260.

13. Han S, Lee K, Yeo J, Kim W, Park K. Biological effects of treatment of an animal skin wound with honeybee (Apis melifera. L) venom. J. Plast. Reconstr. Aesthet. Surg. 2011;64(3): 67-72.

14. Amin MA, Abdel-Raheem IT, Madkor HR. Wound healing and anti-inflammatory activities of bee venom-chitosan blend films. J Drug Deliv Sci Technol . 2008;18(6): 424-430.

15. Amin MA, Abdel-Raheem IT. Accelerated wound healing and anti-inflammatory effects of physically cross linked polyvinyl alcohol–chitosan hydrogel containing honey bee venom in diabetic rats. Arch. Pharm. Res. 2014; 37(8): 1016-1031.

16. Badr G, Hozzein WN, Badr BM, Al Ghamdi A, Saad Eldien HM, Garraud O. Bee venom accelerates wound healing in diabetic mice by suppressing activating transcription factor-3 (ATF-3) and inducible nitric oxide synthase (iNOS)-mediated oxidative stress and recruiting bone marrow-derived endothelial progenitor cells. J. Cell. Physiol. 2016; 231(10): 2159-2171.

17. Hozzein WN, Badr G, Badr BM, Allam A, Al Ghamdi A, Al-Wadaan MA, Al-Waili NS. Bee venom improves diabetic wound healing by protecting functional macrophages from apoptosis and enhancing Nrf2, Ang-1 and Tie-2 signaling. Mol. Immunol. 2018;103: 322-335.

18. Al-Waili N, Hozzein WN, Badr G, Al-Ghamdi A, Al-Waili H, Al-Waili T, Salom K. Propolis and bee venom in diabetic wounds; a potential approach that warrants clinical investigation. Afr. J. Tradit. Complement. Altern. Med. 2015;12(6): 1-11.

19. Liu X, Chen D, Xie L, Zhang R. Effect of honey bee venom on proliferation of K1735M2 mouse melanoma cells in-vitro and growth of murine B16 melanomas in-vivo. J. Pharm. Pharmacol. 2002;54(8): 1083-1089.

20. Oršolić, N. Bee venom in cancer therapy. Cancer Metastasis Rev. 2012; 31(1): 173-194.

21. Oršolić N, Knežević A, Šver L, Terzić S, Hackenberger BK, Bašić I. Influence of honey bee products on transplantable murine tumors. Vet. Comp. Oncol. 2003;1(4): 216–226.

22. Liu CC, Hao DJ, Zhang Q, An J, Zhao JJ, Chen B, Yang, H. Application of bee venom and its main constituent melittin for cancer treatment. Cancer Chemother. Pharmacol. 2016;78(6): 1113-1130.

23. Gajski G, Garaj-Vrhovac V. Melittin: a lytic peptide with anticancer properties. Environ Toxicol Pharmacol 2013;36: 697-705.

24. Jo M, Park MH, Kollipara PS, An BJ, Song HS, Han SB, Kim JH, Song MJ, Hong JT. Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. Toxicol Appl Pharmacol 2012; 258:72-81.

25. Jamasbi E, Batinovic S, Sharples RA, Sani MA, Robins- Browne RM, Wade JD, Separovic F, Hossain MA. Melittin peptides exhibit different activity on different cells and model membranes. Amino Acids 2014;46: 2759-2766.

26. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I, Lee JS, Kim KA, Kim EH, Kim CJ. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. J Pharmacol Sci 2003;91: 95-104.

27. Ip SW, Liao SS, Lin SY, Lin JP, Yang JS, Lin ML, Chen GW, Lu HF, Lin MW, Han SM. The role of mitochondria in bee venom-induced apoptosis in human breast cancer MCF7 cells. In Vivo 2008;22: 237-245.

28. Jo M, Park MH, Kollipara PS, An BJ, Song HS, Han SB, Kim JH, Song MJ, Hong JT. Anti-cancer effect of bee

venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. Toxicol Appl Pharmacol 2012;258: 72-81.

29. Park MH, Choi MS, Kwak DH, Oh KW, do Yoon Y, Han SB, Song HS, Song MJ, Hong JT. Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-kappaB. Prostate 2011;71: 801-812.

30. Tu WC, Wu CC, Hsieh HL, Chen CY, Hsu SL. Honeybee venom induces calcium-dependent but caspase-independent apoptotic cell death in human melanoma A2058 cells. Toxicon 2008;52: 318-329.

31. Moon DO, Park SY, Heo MS, Kim KC, Park C, Ko WS, Choi YH, Kim GY. Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt. Int Immunopharmacol 2006;6: 1796-1807.