Dicle Tıp Dergisi / Dicle Med J (2024) 51 (2) : 233-240

www.diclemedj.org

Original Article / Özgün Araştırma

Investigation of the İn Vitro Effect of Vanillic Acid on Wound Healing via FN1 and COL1α1 Genes

Yesim Yeni[1]

1 Malatya Turgut Ozal University, Faculty of Medicine, Department of Medical Pharmacology, Malatya, Turkey

Received: 12.01.2024; Revised: 22.03.2024; Accepted: 28.05.2024

Abstract

Objective: Wound healing is characterized by the removal of dead/damaged tissue, the formation of new tissue, and finally, the restoration of the damaged tissue to its original function, starting from the moment of tissue injury. Vanillic acid (VA) is an important component of wheat bran and can heal wounds thanks to its antioxidant potential. This work aimed to investigate the dose-dependent effects of VA (1-2-4-8-16 and 32 µg/ml) in an in vitro way using a wound healing pattern in fibroblast cells.

Methods: The MTT test was performed to determine cell viability 48 hours after VA application to the cells in which the wound model was created (except for the control and wound groups). The cells were examined morphologically with an inverted microscope. ELISA and Real-Time PCR analyses were performed to determine changes in oxidative stress parameters and FN1 and COL1α1 gene expressions.

Results: The highest percentage closure rate of fibroblast cells in the in vitro wound pattern analysis and the highest percentage of cell viability by MTT analysis were determined in the VA-32 treated group. Morphological images showed that the evaluated gene expressions increased in fibroblast cells in a VA dose-dependent manner.

Conclusion: Our findings demonstrated for the first time that VA promotes cell migration and proliferation by regulating oxidative stress and FN1A and COL1α1 genes. The results of this work are thought to pioneer the use of VA in in vivo wound healing studies.

Keywords: 8-OHdG, Fibroblast, MTT, Vanillic acid

DOI: 10.5798/dicletip.1501313

Correspondence / Yazışma Adresi: *Yesim Yeni, Faculty of Medicine, Department of Pharmacology, Malatya Turgut Ozal University, 44210 Battalgazi-Malatya, Turkey e-mail: yesim.yeni@ozal.edu.tr*

Vanilik Asidin Yara İyileşmesi Üzerindeki İn Vitro Etkisinin FN1 ve COL1α1 Genleri Aracılığıyla Araştırılması

Öz

Amaç: Yara iyileşmesi, dokuda hasarın oluşmasıyla başlayan ölü/hasarlı yapının uzaklaştırılması, yeni dokunun oluşumu ve hasarlanmış dokunun fonksiyonlarını yerine getirmeleriyle karakterizedir. Vanilik asit (VA), buğday kepeğinin önemli bir bileşeni olup antioksidan potansiyeli sayesinde yarayı iyileştirebilmektedir. Bu çalışmada; fibroblast hücrelerinde yara iyileşmesi modeli oluşturarak VA'nın doz (1, 2, 4, 8, 16, and 32 µg/ml) bağlı etkilerinin in vitro metodlarla incelenmesi amaçlanmıştır.

Yöntemler: Yara modeli oluşturulan hücrelere (kontrol ve yara grubu haricinde) VA uygulaması yapıldıktan 48 saat sonra hücre canlılığını belirlemek için MTT testi yapıldı. İnvert mikroskop ile hücrelerin morfolojik olarak incelendi. Oksidatif stres parametrelerindeki değişiklikleri ve FN1 ve COL1α1 gen ekspresyonlarını belirlemek için de ELİSA ve Real-Time PCR analizi yapıldı.

Bulgular: In vitro yara modeli testinde fibroblast hücrelerinin en yüksek yüzde kapanma oranı ve MTT testi ile en yüksek hücre canlılık yüzdesi VA-32 uygulanan grupta tespit edildi. Morfolojik görüntülerinde ise, değerlendirilen gen ekspresyonlarının fibroblast hücrelerinde VA dozuna bağlı olarak arttığını gösterdi.

Sonuç: Bulgularımız ilk kez VA'nın oksidatif stresi ve FN1A ve COL1α1 genlerini düzenleyerek hücre göçünü ve çoğalmasını desteklediğini göstermiştir. Bu çalışmanın sonuçlarının, yapılacak olan in vivo yara iyileşmesi araştırmalarında VA'nın kullanımına öncülük edeceği düşünülmektedir.

Anahtar kelimeler: 8-OHdG, Fibroblast, MTT, Vanillik asit.

INTRODUCTION

A wound is defined as the disruption of normal anatomical integrity and function in the body by physical damage caused by any agent[1]. Wound healing is a duration that starts from the moment of tissue injury and includes results such as the removal of dead/damaged tissue, formation of new tissue, and finally remodeling of damaged tissue to fulfill its functions[2,3]. The wound healing process consists of four stages including hemostasis, inflammation, proliferation, and maturation[1,3]. Fibroblasts proliferate at the wound site and transform into myofibroblasts. They form a new connective tissue shape by secreting collagen (COL) and fibronectin (FN) to form the temporary extracellular matrix. COL accumulation, which starts after the formation of the cellular matrix, supports extracellular tissue[4].

FN is an extracellular matrix protein that determines cell adhesion, spreading, migration, proliferation, and apoptosis[5]. FN plays complementary roles in forming and regulating tissues during development and in the three phases of wound recovery (inflammation, proliferation, and remodeling). FN is active at all phases of wound recovery[6,7]. FN mediates a wide range of wounds and tissues: skin, periodontal tissues, bone, heart valves, corneal wound recovery, the tongue, and the growth of peripheral neurites[8,9].

Wheat dandruff is the resource of structurally diverse bioactive composites like phenolic acids, minerals, and polyphenols[10]. Vanillic acid (VA), a phenolic acid, is an important composite of wheat dandruff. It has been reported to have antioxidant, neuroprotective, and hepatoprotective activities[11,12]. VA is the oxidized form of vanillin and is found in high concentrations in vanilla beans and Angelica sinensis, a plant used in traditional Chinese medicine[13]. Free radical production and oxidative stress play a key role in the development of these illnesses, and VA can heal these illnesses thanks to its antioxidant

potential[14]. In addition, several studies have shown that VA has various pharmacological activities like antimicrobial, anti-inflammatory, antihypertensive, antioxidant, and inhibition of snake venom activity[15]. Based on former works, VA decreases free radical formation and lipid peroxidation, enhancing mitochondrial function, scavenging free radicals, and increasing antioxidant status[16,17].

It has been supported by many studies that VA exhibits anti-oxidative activity. However, its effect on wound healing has not been investigated. In this study, the effects of VA, a potential wound healing agent in vitro, on the cellular wound repair mechanism were investigated by considering changes in FN1 and COL1α1 gene levels.

METHODS

Cell culture

For our study, adult human dermal fibroblast, HDFa (PCS-201-012™) as the healthy cell, was purchased from ATCC. The cells were resuspended in fresh Dulbecco's modified Eagle's medium, 10% fetal bovine serum, and antibiotic 1% (penicillin, amphotericin B, and streptomycin) (St. Louis, MO, U.S.A). Cells were cultured in 6-, 24- and 96-well plates and stored in an incubator under optimal conditions (5% CO_2; 37°C)[18].

Drug administration

When the cells reached 85% confluence, they were seeded into 6 (for Real-Time PCR), 24 (for wound healing assay), and 96 well plates (for MTT). Experimental groups were defined as control, wound control, VA (1-2-4-8-16, and 32 µg/ml) were administered. The samples were incubated under optimal conditions until the first wound in the experimental groups was closed.

Wound assay

The wound test was used to appraise the migration ratio of VA in the fibroblast cell line. Fibroblast cells were seeded in a 24-well plate and incubated to 100% confluence. At the end of day 5, each well was aspirated with a sterile plastic pipette tip (yellow tip-100 µl). Cell debris was aspirated with phosphate buffer solution (St. Louis, MO, USA). They were then exposed to distinct doses of VA (1-2-4-8-16 and 32 µg/ml). Images from the central area of the wound were taken at 0-48 hours using an inverted microscope (Leica Microsystems, Germany) at ×20 magnification to assess cell migration. All experiments were performed in triplicate[19].

MTT assay

10 µL MTT (5 mg/ml concentration) was supplemented to every well and incubated for 4 hours (5% CO_2; 37 °C) to perform the MTT assay. To dissolve formazan crystals, the medium was lifted after 4 hours and 100 µL DMSO was supplemented. Cell viability was measured by optical density at 570 nm using a Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, USA) and cell viability was calculated as %18.

Biochemical Analyses

Cell medium was gathered 1 day after toxicity administration and assayed according to the manufacturer's instructions.

Total oxidant status (TOS) The evaluation and the method used the TOS ELISA kit (Rel Assay Diagnostic® Company, Turkey). 500 µL of Reagent1 solution was supplemented to the wells containing the sample, and the initial absorbance value was read at 530 nm, for evaluated TOS assay. Then 30 µL of Reagent 2 solution was supplemented to the same well. After 10 minutes at room temperature, the second absorbance value was read. TOS values were calculated as H_2O_2 Equiv mmol/L-1.

Total antioxidant status (TAC) The evaluation and the method used the TAC ELISA kit (Rel Assay Diagnostic® Company, Turkey). 500 µL of Reagent1 solution was supplemented to the wells containing the sample, and the initial

absorbance value was read at 660 nm, for evaluated TAC assay. Then 75 µL of Reagent 2 solution was supplemented to the same well. 75 µL Reagent 2 solution was added and the second measurement was made at 660 nm. TAC values were calculated as Trolox Equiv/mmol L-1.

Lactate dehydrogenase (LDH) The evaluation and the method used the LDH ELISA kit (Cayman Chemicals, USA). Triton X-100 (10%) and 20 µL Assay buffer were supplemented to cells seeded in 96-well plates. Then it was incubated at room temperature for 1 hour. The cells were centrifuged at 400xg for 5 minutes. 100 µL of cell supernatant were transferred to new 96-well assay plates. LDH reaction solution was supplemented to each well and incubated on the plate with gentle shaking on an orbital shaker for 30 minutes at 37°C. LDH levels were measured with the absorbance optical density value at 490 nm.

8-hydroxy-2 ' deoxyguanosine (8-OHdG) The evaluation and the method used the 8-OHdG ELISA kit (Cell Biolabs, USA). Optical density was measured spectrophotometrically at 450 nm wavelengths. 8-OHdG activity was expressed as pg/mL.

Glutathione (GSH) The evaluation and the method used the GSH ELISA kit (E-EL-0026/GSH; Elabscience, USA). Optical density was measured spectrophotometrically at 450 nm wavelengths. The obtained results were given as % value.

Gene expression

A High Pure RNA Isolation commercial kit was used for RNA. The Transcriptor First Strand cDNA Synthesis (Roche, Germany) commercial kit was used to transform isolated RNAs into cDNA. The sequences of the gene-specific PCR primers are shown below.

β-actin

Forward: 5'- CACCATTGGCAATGAGCGGTTC-3'

Reverse: 5'- AGGTCTTTGCGGATGTCCACGT-3'

FN1

Forward:5′-ACAACACCGAGGTGACTGAGAC−3′

Reverse: 5′- GGACACAACGATGCTTCCTGAG-3′

COL1α1

Forward: 5′-GATTCCCTGGACCTAAAGGTGC-3′

Reverse: 5′- GCCTCTCCATCTTTGCCAGCA-3′

Then, 3 µL cDNA, 0.75 µL gene primer, and 3 µL master were supplemented to each tube for PCR of the cDNAs synthesized for β-actin, FN1, and COL1α1 gene measurements. 13.25 µL ultrapure water was supplemented to the mixture and the final volume was completed to 20 µL. After 5 min at 95 °C, expression levels were measured by beginning a cycle of 45 cycles (at 95 °C for 10 s and 60 °C for 30 s). Results are expressed as relative fold change compared to control. Using the ΔΔCt method (x), gene expressions were normalized to β-actin and expressed as fold change relative to control[20].

Ethical Approval

Ethical approval isn't necessary because commercially present cell lines are used in an in vitro study.

Statistical Analyses

Statistical analyses were made using one-way ANOVA and Tukey HSD method (SPSS 22 software). *p<0.05* was considered a statistically important difference in all tests. Results were offered as mean and standard deviation.

RESULTS

Effects of VA on cell viability

The results of MTT cell viability analysis performed 48 hours after 1-2-4-8-16 and 32 µg/mL VA applications to fibroblast cells were compared with the wound group (52.84%) and calculated as percentage cell viability. Accordingly, the results were found to be 69.85, 71.96, 79.90, 82.49, 88.41 and 95.59%, respectively. It was found that VA applications increased fibroblast viability and proliferation

depending on dose and duration. The results were statistically significant (*p<0.05, p<0.01*) (Figure 1).

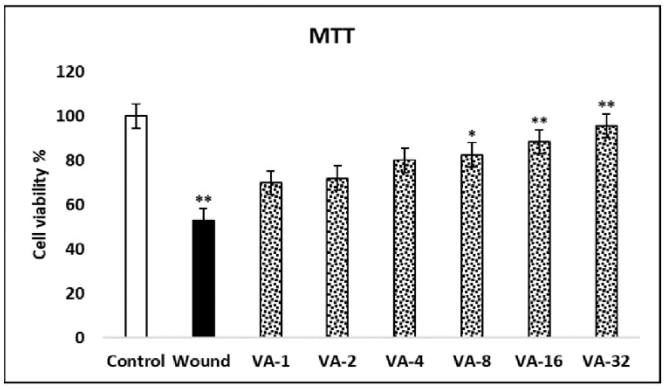

Figure 1. Dose-dependent MTT analysis graph of VA application in fibroblast cells. **p<0.05* vs. Wound group, ***p<0.01* vs. Wound group.

Effects of VA on cell morphology

When fibroblast cells were grown under appropriate conditions, it was observed that they had spindle-shaped morphology and tended to grow overlappingly. When the fibroblast cells covered the culture dish in a monolayer, the wound areas formed with the help of a sterile pipette tip were photographed at 0, 24, 36, and 48 hours and their closure was observed. It was observed that the spindle-shaped morphology of fibroblast cells did not change with different doses and durations of VA applications. According to these results, it was determined that the cells were not exposed to any stress conditions as a result of the experimental treatments. In addition, in the VA 16 and 32 µg/ml groups, it was observed that the wound line was closed at the 36th hour. In the wound group, the wound line was observed even at the 48th hour (Figure 2).

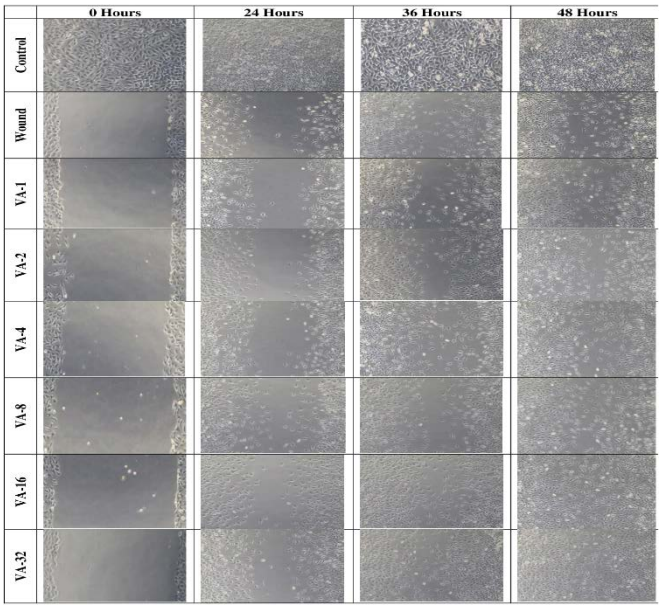

Figure 2. Inverted microscopic images of wound closure areas of fibroblast cells after VA applications.

Effects of VA on oxidative stress

In the wound group, TAC (7.59 mmol Trolox equivalent/L) and GSH (20.14%) activities were significantly decreased, while LDH (98%), TOS (63.65 H2O2 Equiv mmol/L-1), and 8-OHdG (0.594 pg/ml) levels were importantly raised compared to the control group. The results of VA groups (1-2-4-8-16, and 32 µg/ml), TAC (7.73-9.29-10.93-11.01-11.34, and 13.13 mmol Trolox equivalent/L, respectively), GSH (24.41-27.18-29.06-30.46-32.64, and 35.89%, respectively), LDH (63.53-61.49-50.81-47.67-42.24, and 30.12%, respectively), TOS (53.17-47.24-45.79-27.37-26.89, and 16.27 H2O2 Equiv mmol/L-1, respectively), and 8-OHdG levels (0.389-0.296-0.289-0.259-0.251, and 0,236 pg/ml, respectively) were given. In VA groups, TAC, GSH, LDH, TOS, and 8-OHdG activities were close to the control (Figure 3). These results are correlated with MTT. It was determined that cytotoxicity was eliminated due to VA repairing the scar tissue and contributing to cell proliferation (*p<0.05, p<0.01*).

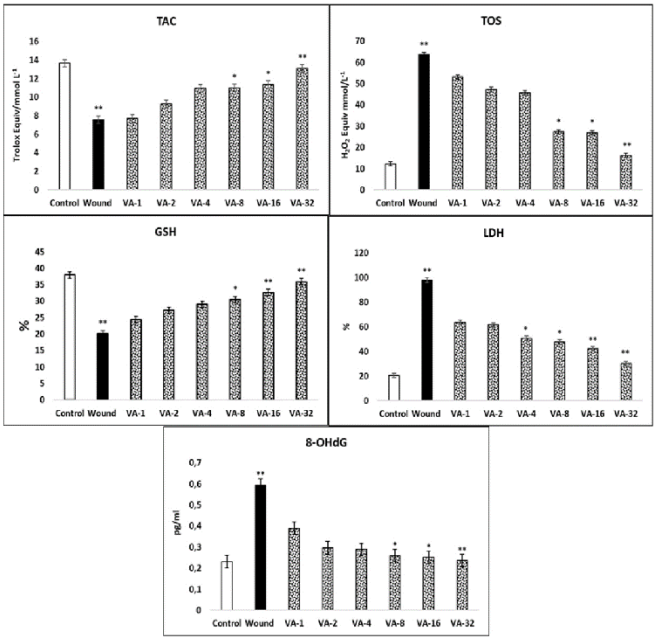

Figure 3. Oxidative Stress Results of Application Group. *$p<0.05$ vs. Wound group, **$p<0.01$ vs. Wound group.

VA regulates the expression of FN1 and COL1α1 gene

While FN1 (0.3-fold) and COL1α1 (0.4-fold) gene expressions were importantly reduced in the wound group, these gene expressions were importantly raised with the administration of VA. With the onset of healing in the wound group, the regulation of FN1 (0.4-0.4-0.5-0.6-0.7 and 0.8-fold, respectively) and COL1α1 (0.4-0.5-0.6-0.7-0.8 and 0.9-fold, respectively) gene expression increased in the VA groups (1-2-4-8-16 and 32 µg/ml). FN1 and COL1α1 are expressed together with the repair of scar tissue. According to the results we obtained, it was determined that there was a 0.9-fold increase in VA 32 µg/ml concentration. The control group was accepted as 1 and the other groups were compared with the control group. The data acquired were statistically important (*$p<0.05$, $p<0.01$*) (Figure 4). Similarly, an approximately 0.8-fold increase in FN1 gene level was determined at 32 µg/ml concentration.

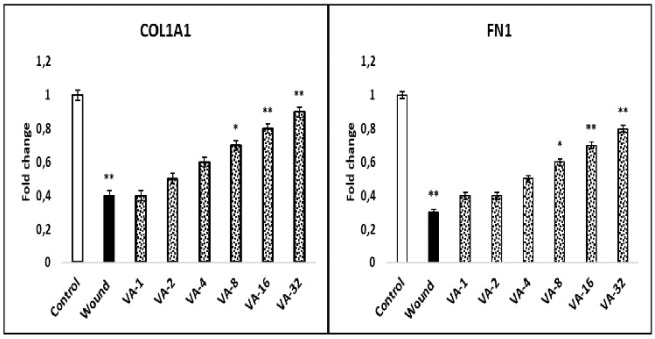

Figure 4. Gene Expression of Application Group. *$p<0.05$ vs. Wound group, **$p<0.01$ vs. Wound group.

DISCUSSION

During the wound-healing process, local and blood-borne fibroblasts proliferate and migrate to wound sites to form wound granulation tissue. Fibroblasts provide the formation of a new extracellular matrix under the influence of various cellular factors and some fibroblasts differentiate into myofibroblasts to help the wound contract[20]. However, various substances with antioxidant activity have also been proven to be beneficial in wound healing[22,23]. Previous in vitro and in vivo studies disclosed that VA derived from wheat bran promoted hair growth[24,25]. Kang et al. showed that VA increased the proliferation of dermal papilla cells by activating Wnt/β-catenin and PI3K/Akt paths[26]. The antioxidant effect of VA showed a healing effect on the wound. It provided the closure of the wound line in a shorter time compared to the wound group. In addition, the gradual decrease of oxidative stress, which rose in the wound group, and the VA group triggered the healing of the wound area in a shorter time. During wound healing, FN is one of the first and most abundant extracellular matrix components that accumulate in that area[27,28]. FN triggers fibril formation together with extracellular matrix formation. Accumulation of FN matrix in wounds stimulates collagen and contributes to wound contraction. In addition, FN can bind to other cells to further stabilize the extracellular matrix[7]. In this study, the effect of VA on the healing duration of fibroblast cells

was investigated. According to our findings, it was determined that increased oxidative stress and cellular cytotoxicity in the wound area were eliminated by VA and the wound line was closed faster than the normal process.

In vitro studies have shown that FN polymerization leads to the composition and stability of the extracellular matrix and cell-matrix adhesion[7,29]. FN polymerization enables the accumulation of COL types I and III in the extracellular matrix and results in the stabilization of COLI matrix fibrils. A study by Shi and Sottile showed that membrane-type matrix metalloproteinase 1 promotes extracellular matrix FN turnover by regulating the cleavage of large FN fibrils and subsequent endocytosis of α5β1 integrin[30]. They also showed that inhibition of FN polymerization accelerated myofibroblast migration.

Type I collagen is the most expressed collagen in the skin, tracked by type III and type IV, which conduce to the stability of the epidermis and are responsible for tension[31]. In this study, it was confirmed that VA significantly raised COL1α1 expression in wound cells. In contrast, the decrease in migration of fibroblasts in the wound group led to a decrease in COL1α1. Recent studies have shown that reduction in collagen expression in early granulation tissue promotes myofibroblast differentiation and increased scar deposition in cutaneous wounds[32]. In their Type 1 diabetes study, Black et al. reported that a 40% reduction in collagen deposition delayed the wound healing process[33]. Another study demonstrated that overexpression of collagen in fibrotic lungs is a key factor in tissue dysfunction[34].

CONCLUSION

Our findings show for the first time that VA increases migration and/or proliferation of fibroblasts by regulating oxidative stress and FN1 and COL1α1 genes. This activity may be related to the production of FN1 and COL1α1, which are considered important targets for modulation of the tissue repair duration and play an important role in the wound healing process. It is thought that the results of this study will lead to the use of VA in vivo wound healing research.

Ethics Committee Approval: Ethical approval isn't necessary because commercially present cell lines are used in an in vitro study.

Conflict of Interest Declaration: The author declares that he has no conflict of interest.

Financial Support: This study was not supported by any funding.

REFERENCE

1. Parsak CK, Sakman G, Çelik Ü. Yara iyileşmesi, yara bakımı ve komplikasyonları. Arşiv Kaynak Tarama Dergisi 2007;16(2):145-59.

2. Venus M, Waterman J, McNab I. Basic physiology of the skin. Surgery (Oxf) 2011; 29: 471-4.

3. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 2009; 37: 1528-42.

4. Sen CK, Roy S. Redox signals in wound healing. Biochim Biophys Acta 2008; 1780:1348-61.

5. Jarnagin WR, Rockey DC, Koteliansky VE, et al. Expression of variant fibronectins in wound healing: cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis. J Cell Biol 2013; 127:1-12.

6. Bielefeld KA, Amini-Nik S, Whetstone H, et al. Fibronectin and β-catenin act in a regulatory loop in dermal fibroblasts to modulate cutaneous healing. J Biol Chem 2011; 286: 27687-97.

7. Lenselink EA. Role of fibronectin in normal wound healing. Int Wound J 2015; 12: 313-6.

8. Martino MM, Tortelli F, Mochizuki M, et al. Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. Sci Transl Med 2011; 3: 1-10.

9. Stevenson L, Phillips F, O'sullivan K, et al. Wheat bran: its composition and benefits to health, a European perspective. Int J Food Sci Nutr 2012; 63:1001–13.

10. Itoh A, Isoda K, Kondoh M, et al. Hepatoprotective effect of syringic acid and vanillic acid on concanavalin a-induced liver injury. Biol Pharm Bull 2009; 32: 1215–9.

11. Sinha AK, Sharma UK, Sharma N. A comprehensive review on vanilla flavor: extraction, isolation and quantification of vanillin and others constituents. Int J Food Sci Nutr 2008; 59: 299–326.

12. Amin FU, Shah SA, Kim MO. Vanillic acid attenuates Aβ1-42-induced oxidative stress and cognitive impairment in mice. Sci Rep 2017; 7: 40753.

13. Ingole A, Kadam MP, Dalu AP, et al. A review of the pharmacological characteristics of vanillic acid. J Drug Delivery Ther 2021; 11(2–S): 200-4.

14. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018; 13: 757–72.

15. Kaur J, Gulati M, Singh SK, et al. Discovering multifaceted role of vanillic acid beyond flavours: nutraceutical and therapeutic potential. Trends Food Sci Technol 2022; 122: 187-200.

16. Yalameha B, Nejabati HR, Nouri M. Cardioprotective potential of vanillic acid. Clin Exp Pharmacol Physiol 2023; 50(3): 193–204.

17. Lashgari NA, Roudsari NM, Momtaz S, et al. Regulatory mechanisms of vanillic acid in cardiovascular diseases: a review. Curr Med Chem 2023; 30(22): 2562-76.

18. Altinoz MA, Yılmaz A, Taghızadehghalehjoughı A, et al. Ulipristal-temozolomide hydroxyurea combination for glioblastoma: in-vitro studies. J Neurosurg Sci 2022; 66:1-14.

19. Genc S, Cıcek B, Yenı Y, et al. Wound healing potential of quinic acid in human dermal fibroblasts by regulating the expression of FN1 and COL1α genes. Tr J Nature Sci 2022; 11(4): 63-9.

20. Yeni Y, Cakir Z, Hacimuftuoglu A, et al. A selective histamine H4 receptor antagonist, JNJ7777120, role on glutamate transporter activity in chronic depression. J Pers Med 2022;12(2): 246.

21. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol 2015; 173(2): 370-8.

22. Fitzmaurice SD, Sivamani RK, Isseroff RR. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. Skin Pharmacol Physiol 2011; 24(3):113-26.

23. Kunkemoeller B, Kyriakides TR. Redox signaling in diabetic wound healing regulates extracellular matrix deposition. Antioxid Redox Signal 2017; 27(12): 823-38.

24. Kang JI, Kim SC, Kim MK, et al. Effects of dihydrotestosterone on rat dermal papilla cells in vitro. Eur J Pharmacol 2015; 757: 74–83.

25. Lian X, Zhao J, Wu X, et al. The changes in glucose metabolism and cell proliferation in the kidneys of polycystic kidney disease mini-pig models. Biochem Biophys Res Commun 2017; 488: 374–81.

26. Kang J, Choi YK, Koh YS, et al. Vanillic acid stimulates anagen signaling via the pı3k/akt/β-catenin pathway in dermal papilla cells. Biomol Ther (Seoul) 2020; 28(4): 354–60.

27. Fayet C, Bendeck MP, Gotlieb AI. Cardiac valve interstitial cells secrete fibronectin and form fibrillar adhesions in response to injury. Cardiovasc Pathol 2007; 16(4): 203-11.

28. Liu C, Rinderknecht H, Histing T, et al. Establishment of an ın vitro scab model for ınvestigating different phases of wound healing. Bioengineering (Basel) 2022; 9(5): 191.

29. Koyuncu A, Koç S, Akdere OE, et al. Investigation of the synergistic effect of platelet-rich plasma and polychromatic light on human dermal fibroblasts seeded chitosan/gelatin scaffolds for wound healing. J Photochem Photobiol B 2022; 232:112476.

30. Shi F, Sottile J. MT1-MMP regulates the turnover and endocytosis of extracellular matrix fibronectin. J Cell Sci 2011; 124 (Pt 23): 4039-50.

31. Trojanowska M, LeRoy EC, Eckes B, et al. Pathogenesis of fibrosis: Type 1 collagen and the skin. J Mol Med (Berl) 1998; 76: 266–274.

32. Volk SW, Wang Y, Mauldin EA, et al. Diminished type III collagen promotes myofibroblast differentiation and increases scar deposition in cutaneous wound healing. Cells Tissues Organs 2011; 194: 25-37.

33. Black E, Vibe-Petersen J, Jorgensen LN, et al. Decrease of collagen deposition in wound repair in type 1 diabetes independent of glycemic control. Arch Surg 2003; 138: 34-40.

34. Molina-Molina M, Machahua-Huamani C, Vicens-Zygmunt V, et al. Anti-fibrotic effects of pirfenidone and rapamycin in primary IPF fibroblasts and human alveolar epithelial cells. BMC Pulm Med 2018; 18: 63.