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CALENDULA OFFICINALIS EFFECTS ON EXPRESSION BFGF AND TGFβ1 IN FIBROBLASTS

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ABSTRACT

Background: Calendula officinalis L. belongs to the asteraceae family which has various components such as triterpenes, saponins, tannins and volatile oils and are used for medicinal purposes like dermatitis and wound healing, but the mechanism of this effect is not fully known. Scince TGFβ1 and bFGF are two important factors that are expressed in fibroblast cells. Our study aim to evaluate the effect of methanolic extract of Calendula officinalis on the proliferation and expression of angiogenesis growth factors (TGFβ1 and bFGF) in mouse embryonic fibroblast cells.
Methods: The cells were exposed to different concentrations of methanolic extract of Calendula officinalis at different times and cell proliferation and expression of TGFβ1and bFGF were measured at the level of gene and protein by real time-polymerase chain reaction (real-time-PCR) and enzyme-linked immunoslobent assay (ELISA) respectively.
Results: The results showed that Calendula officinalis extract were not toxic for cells in study concentrations and increased gene expression of TGFβ1 and bFGF after 12 hours, but after 24 hours, the expression of these genes were down-regulated.
Conclusion: The results of research showed that changing patterns of expression TGFβ1 and bFGF were similar and the level of changes depend to dose and time.

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Introduction

The use of plants for medical purposes has a long history. Calendula officinalis L. (Asteraceae) is popularly known as pot marigold or common marigold belongs to the asteraceae family that is usually grown as a garden plant in Iran. Calendula officinalis has differnet components such as Carotenoids (flavoxanthin, Lutein, Rubixanthin, β-carotene, γ-carotene and lycopene), Polysaccharides, Proteins, Fatty acids, Flavonoids, Triterpenoids and Saponins. Different studies have been shown that it possesses a wide range of activities such as immunostimulant, wound healing, antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anti-HIV, hepatoprotective and anticancer [1-4].

Wound healing is a complex process that various cells and multiple growth factors take part in this process[5, 6]. This process is dependent on the migration and proliferation of endothelial cells to form new vessels [5]. Effects of important growth factors such as TGFβ1 and bFGF on angiogenesis, has been studied [7]. TGFβ1 peptide possesses various function such as cell proliferation and differentiation control, apoptosis in different types of cells. TGFβ1 also has the ability of absorbing chemicals, neutrophils, macrophages and fibroblasts, leading them to the wound area [8]. It has been shown that TGFβ1 activates angiogenesis by stimulating vascular smooth muscle cell migration[9]. The importance of this factor in creating and maintaining the vascular system has been proved by numerous genetic studies. TGFβ1 and its receptors leads to

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impaired fetal death due to impaired vessel formation [10, 11]. TGFβ1 induces the maturation of Retinoic acid-inducible gene I (RIGI), stimulates the formation and intensification of interactions between epithelial cells and the basement membrane of the mural cells. Additionally TGFβ1 regulates angiogenesis through angiogenesis factors such as VEGF and other activities affecting the expression of PDGF through Smad proteins[12-14]. Another important growth factor in the tissue repair process is bFGF, (FGF 2 or FGF-β). This factor helps to control migration of endothelial cells and fibroblasts, which are responsible for angiogenesis and collagen formation of the epithelial layer[5, 13, 15].
According to the scientific evidence of the effectiveness of marigold plant in the treatment of dermatologic disorders, the importance of angiogenesis in wound healing, and the increase use of traditional remedies, more research in this area is necessary in other to understand the mechanism of this plant, so the aim of this study, is to determine the effect of marigold extract on proliferation and expression of angiogenesis factors (TGFβ1 and bFGF) at the level of gene and protein in fibroblasts cells.

2. Materials And Methods

2.1. Preparation of Calendula officinalis extract

Flowers of Calendula officinalis were collected from Lorestan Agricultural and Natural Resources Research and Education Center, during the months of November and December, 2015. The collected plant material was authenticated at Lorestan University of Medical Sciences, Razi Herbal Medicines Research Center. To preparation of extract, 20 grams of dried Calendula officinalis flowers was soaked in 120 ml of 50% methanol for 72 hours in a dark. Then it was centrifuged and passed through the filter and dried at room temperature. The yield of the extract was 8.7% w/w. The extract was stored at -20°C until further use.

1. 2.2. Isolation and culture of mouse embryonic fibroblasts (MEFs):

Isolation and culture of mouse embryonic fibroblasts (MEFs) were performed according to the protocol described by Jozefczuk et al [16].

2.3. MTT assay

The evaluation of viability was performed using MTT assay, as previously described[17]. The cells were incubated in a 96-well plates for 24h after that were exposed with different concentration of Calendula officinalis (5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml and 50 µg/ml) for 12, 24, 48 and 72h at 37°C and 5% CO_2. Then 20 µl of MTT (5 mg/ml, Sigma) in PBS solution added into each of the well, and the plate was further incubated for 4 h. The medium was removed and 200 µl of DMSO added into each well for solubilizing the formazan. The absorption was measured using an ELISA reader. Cell viability expressed as a percentage of absorbance values in treated cells to that in control cells.

2. 2.4. Determination of the expression of levels of TGFβ1 and bFGF

Expression levels of TGFβ1 and bFGF were analyzed using real time–PCR assay as described previously[17]. The cells were treated methanolic Calendula officinalis extract (5 µg/ml and 10 µg/ml) for 12 and 24 h periods. Total RNA isolated from the untreated and treated cells using the Total RNA Purification Kit (Jena Bioscience, Germany) according to the instruction of the manufacturer's. CDNA was synthesized with 1 µg total RNA using the cDNA Synthesis Kit (Roche, Germany). Real-time quantitative PCR was performed with Rotor-Gene 6000 and SYBR-Green quantitative PCR (qPCR) kit (Jena Bioscience, Germany). A PCR reaction mixture of 20 µl containing 10 µl of 2X SYBR Green master mix, 1.6 µl of the cDNA template, 0.6 µl of each primer (10 pmol/µl) and 7.2 µl of deionized water. Cycling conditions were as follows: 1 cycle denaturation of 95 ºC for 2 min, followed by 40 cycles of 95 ºC for 15 s hold, annealing temperature at 60 ºC for 40 s and extension at 72 ºC for 30 s. The sequences of the primers and their characteristics were previously presented[17].

3. 2.5. Determination of TGFβ1 and bFGF protein expression by ELISA

The cells were treated to methanolic extract of Calendula officinalis (5 µg/ml, 10 µg/ml) for 12 and 24 h periods then supernatants from treated and untreated were collected and freezed at -20°C. TGF-β1and bFGF protein concentrations were measured by ELISA kits (eBioscience USA, Cat. 88-8350 and RayBiotech Cat. ELH-bFGF-001) based on kits protocols[17].

2.6. Statistical analysis

The total expression ratio of the genes of interest at two concentrations were compared between treatment groups and untreated (control) group using a randomization test implemented in the relative expression software tool (REST), which is an Excel-based application for comparing and statistically analyzing the relative expression results in qRT-PCR [18] Protein expression in the two groups were compared by Wilcoxon using SPSS software. Differences were considered significant at ($P<0.05$).

3. Results

3.1. Effect of Calendula officinalis extract on cell viability

The results of MTT assay showed that methanolic extract of Calendula officinalis was non-toxic to the cells (Fig 1). Since the results showed that the concentrations of 5µg/ml and 10 µg/ml were more suitable for cell proliferation so the expression of growth factors were evaluated in these concentrations.

4. 3.2. TGFβ1 and bFGF gene expression

The results of gene expression for TGFβ1 in treated groups compared to control (untreated) group indicated increase of gene expression after 12 hours but the reduction of gene expression agter 24 hours. This increase and decrease were significant for concentrations of 10 µg/ml. (Table 1).

Gene expression of bFGF in treated cells compared to control showed a similar patterns with TGFβ1 expression (Table 2).

3.3. TGFβ1 and bFGF protein expression

Analysis of TGFβ1 protein expression showed an increase TGFβ1 gene expression in the fibroblasts at different concentrations at 12 hours after treatment compare to the control, but at 24 hours after treatment reduced. These results were significant for concentration of 10 µg/ml (Fig 2). Protein expression of bFGF in treated cells compare to control showed similar pattern with that TGFβ1 protein but results were significant for concentrationrol (Fig 3)

4. Discussion

The results showed that extracts of Calendula officinalis is non-toxic at different concentration and can increase proliferation of mouse embryonic fibroblast cells. This results are similar to others[19, 20]. The main findings our study were that calendula extracts at first may up-regulate the expression of TGFβ1 and bFGF but after that down-regulate the expression of these genes.

Calendula Officinalis flowers are used to treatment of dermatologic disorders and wound healing [21]. Calendula offcinalis flower has many different compounds such as Phenolic compounds (flavonoids and phenolic acids) which have the role of antioxidant and antimicrobial, some important polycarbohydrates which play a role in tissue soldering and controlling cellular permeability, saponins, carotenioids and triterpenic alcohols, poly unsaturated fatty acids, such as calendic acid, saturated hydrocarbons, proteins and amino acids, vitamin C which is necessary for collagen formation, proper immune function, and as a tissue antioxidant and mineral substances which influences the process of wound repair as cofactor in various enzyme systems[22-24].

Several scientific evidence have be collected regarding the effectiveness of this plant in the increase of the rate of re-epithelialization, granulation tissue formation and the regeneration of dermal collagen in skin wounds[25].

It has anti-inflammatory, antibacterial, anti-fungal, anti-viral, angiogenic and fibroplastic properties that all are important to treat of wound, first-degree burns, contusions, and skin rashes [26, 27].It seems its anti-inflammatory properties because of inhibition of synthesis of cytokines, Cox-2 and prostaglandins [27].

It has been shown that Quercetin, one of the active components in Calendula, can inhibit collagen degradation, matrix metalloproteinase (MMP) activity and decrease the expression of tumor necrosis factor-α (TNF-α), interleukin-1β, IL-6 and IL-8[17, 20]

Several studies have been shown that Calendula officinalis flowers can induce neovascularization in the chorioallantoic membrane (CAM) model of chicken fertilized egg and skin wound[25, 28].

Fibroblast cells have a crucial role in the process of wound healing. Proliferation of fibroblasts and their migration into the wound area, synthesis of extracellular matrix (EXM) and the expression of thick actin bundles as myofibroblasts are important function of these cells in the wound healing[29, 30]. These cells also produce important growth factors such as TGFβ1 and bFGF that have important role in cell division, cell migration, cell differentiation, protein expression, and enzyme production[31]. They have potential ability to heal wound through stimulation of angiogenesis factors and cellular proliferation which affects the ECM production and degradation through their chemotactic role on inflammatory cells and fibroblasts[31].

However, fibroblast cells and growth factors have critical role in wound healing but hyper proliferation of fibroblasts and increase of expression of growth factors can cause formation of hypertrophic scar that is a major challenge in clinical practice. It has been shown that up-regulation of consistently of TGFβ1 stimulate produces fibrotic disease and blocking the expression this factor can suppress fibrotic process[32, 33].

It seems that must be a special pattern in the expression of growth factors and proliferation fibroblasts in order to inhibit hypertrophic scar. The results of our study have shown that calendula may by increase of growth factors (TGFβ1 and bFGF) at the first 12 h stimulate wound healing and by decease of these factors at 24h may suppress fibrotic process, however more studies are needed to conform this speculation.

5. Conclusion

The results showed that Calendula officinalis can effect on wound healing process by increase of proliferation and change of expression of growth factors (TGFβ1 and bFGF) in fibroblast cells in a dose and time dependent manner.

Conflict of interest

There is no conflict of interest in this paper

Acknowledgments

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References

1. Arora R, Majee C. Anti diabetic and antihyperlipidemic effect of hydro-alcholic extract of Calendula Officinalis. Int Res J Pharm 2011; 2(1): 61-5.
2. Babaee N, Moslemi D, Khalilpour M, et al. Antioxidant capacity of calendula officinalis flowers extract and prevention of radiation induced oropharyngeal mucositis in patients with head and neck cancers: a randomized controlled clinical study. DARU Journal of Pharmaceutical Sciences 2013; 21(1): 1.
3. Kalvatchev Z, Walder R, Garzaro D. Anti-HIV activity of extracts from Calendula officinalis flowers. Biomedicine & pharmacotherapy 1997; 51(4): 176-80.
4. Ukiya M, Akihisa T, Yasukawa K, Tokuda H, Suzuki T, Kimura Y. Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (Calendula officinalis) flowers. Journal of Natural Products 2006; 69(12): 1692-6.
5. Guo Sa, DiPietro LA. Factors affecting wound healing. Journal of dental research 2010; 89(3): 219-29.
6. Sadava EE, Krpata DM, Gao Y, Rosen MJ, Novitsky YW. Wound healing process and mediators: Implications for modulations for hernia repair and mesh integration. Journal of biomedical materials research Part A 2014; 102(1): 295-302.
7. Atiba A, Nishimura M, Kakinuma S, et al. Aloe vera oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor-β and fibroblast growth factor production. The American Journal of Surgery 2011; 201(6): 809-18.
8. Ferrari G, Cook BD, Terushkin V, Pintucci G, Mignatti P. Transforming growth factor-beta 1 (TGF-β1) induces angiogenesis through vascular endothelial growth factor (VEGF)-mediated apoptosis. Journal of cellular physiology 2009; 219(2): 449-58.
9. Ma J, Wang Q, Fei T, Han J-DJ, Chen Y-G. MCP-1 mediates TGF-β–induced angiogenesis by stimulating vascular smooth muscle cell migration. Blood 2007; 109(3): 987-94.
10. Larsson J, Goumans MJ, Sjöstrand LJ, et al. Abnormal angiogenesis but intact hematopoietic potential in TGF-β type I receptor-deficient mice. The EMBO journal 2001; 20(7): 1663-73.
11. Kim SI, Kwak JH, Na H-J, Kim JK, Ding Y, Choi ME. TGF-β1 activates TAK1 via TAB1-mediated autophosphorylation, independent of TGF-β receptor kinase activity in mesangial cells. Journal of Biological Chemistry 2009: jbc. M109. 007146.
12. Sánchez-Elsner T, Botella LM, Velasco B, Corbí A, Attisano L, Bernabéu C. Synergistic cooperation between hypoxia and transforming growth factor-β pathways on human vascular endothelial growth factor gene expression. Journal of Biological Chemistry 2001; 276(42): 38527-35.
13. Mason JC, Lidington EA, Ahmad SR, Haskard DO. bFGF and VEGF synergistically enhance endothelial cytoprotection via decay-accelerating factor induction. American Journal of Physiology-Cell Physiology 2002; 282(3): C578-C87.
14. Ponce CC, Chauffaille MdLLF, Ihara SSM, Silva MRR. Increased angiogenesis in primary myelofibrosis: latent transforming growth factor-β as a possible angiogenic factor. Revista brasileira de hematologia e hemoterapia 2014; 36(5): 322-8.
15. Hom DB, Unger GM, Pernell KJ, Manivel JC. Improving surgical wound healing with basic fibroblast growth factor after radiation. The Laryngoscope 2005; 115(3): 412-22.
16. Jozefczuk J, Drews K, Adjaye J. Preparation of mouse embryonic fibroblast cells suitable for culturing human embryonic and induced pluripotent stem cells. JoVE (Journal of Visualized Experiments) 2012; (64): e3854-e.
17. Hormozi M, Assaei R, Boroujeni MB. The effect of aloe vera on the expression of wound healing factors (TGFbeta1 and bFGF) in mouse embryonic fibroblast cell: In vitro study. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2017; 88: 610-6.
18. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic acids research 2002; 30(9): e36.
19. Fronza M, Heinzmann B, Hamburger M, Laufer S, Merfort I. Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. Journal of ethnopharmacology 2009; 126(3): 463-7.
20. Kaur N, Fernandez R, Sim J. Effect of Aloe vera on glycemic outcomes in patients with diabetes mellitus: a systematic review protocol. JBI database of systematic reviews and implementation reports 2017; 15(9): 2300-6.

21. Leach MJ. Calendula officinalis and Wound Healing: A Systematic Review. Wounds: a compendium of clinical research and practice 2008; 20(8): 236-43.
22. Butnariu M, Coradini CZ. Evaluation of Biologically Active Compounds from Calendula officinalis Flowers using Spectrophotometry. Chem Cent J 2012; 6: 35.
23. Kumar S, Yadav A, Yadav M, Yadav JP. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.) Burm.f. BMC research notes 2017; 10(1): 60.
24. MacKay D, Miller AL. Nutritional support for wound healing. Alternative medicine review : a journal of clinical therapeutic 2003; 8(4): 359-77.
25. Patrick K, Kumar S, Edwardson P, Hutchinson J. Induction of vascularisation by an aqueous extract of the flowers of Calendula officinalis L. the European marigold. Phytomedicine 1996; 3(1): 11-8.
26. Parente LML, Lino Júnior RdS, Tresvenzol LMF, Vinaud MC, de Paula JR, Paulo NM. Wound healing and anti-inflammatory effect in animal models of Calendula officinalis L. growing in Brazil. Evidence-based complementary and alternative medicine 2012; 2012.
27. Preethi KC, Kuttan G, Kuttan R. Anti-inflammatory activity of flower extract of Calendula officinalis Linn. and its possible mechanism of action. Indian J Exp Biol 2009; 47(2): 113-20.
28. Parente LM, Andrade MA, Brito LA, et al. Angiogenic activity of Calendula officinalis flowers L. in rats. Acta Cir Bras 2011; 26(1): 19-24.
29. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008; 453(7193): 314-21.
30. Schafer M, Werner S. Transcriptional control of wound repair. Annual review of cell and developmental biology 2007; 23: 69-92.
31. Ganapathy N, Venkataraman SS, Daniel R, Aravind RJ, Kumarakrishnan VB. Molecular biology of wound healing. Journal of pharmacy & bioallied sciences 2012; 4(Suppl 2): S334-7.
32. Isaka Y TM, Ando Y, Nakamura H, Kaneda Y, Imai E, Hori M. . Transforming growth factor-beta 1 antisense oligode-oxynucleotides block interstitial fibrosis in unilateral ureteral obstruction. Kidney Int 2000; 58: 1885-92.
33. Song L, Tian Y, Xu Z-J, Zhang C-P. Adrenaline inhibited cell proliferation and regulated expression of TGF-beta1 and bFGF in cultured human hypertrophic scar fibroblasts via alpha-receptor. 2008.

Figures:

Fig. 1. Effect of different concentrations of methanol extract of Calendula on mouse embryonic fibroblast cell viability. Cells were treated with different concentration of methanol extracts of Calendula for 12, 24, 48 and 72 h and cell viability was measured by MTT assay.

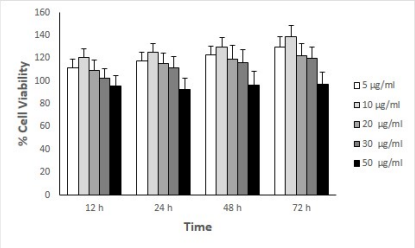
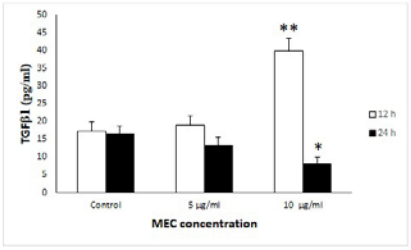
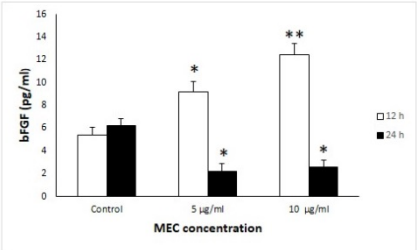


Fig. 2: Effect of various concentrations of methanol extract of Calendula the expression of TGFβ1 protein in mouse embryonic fibroblast cell culture supernatants. Cells were treated with different concentration of methanol extract of Calendula (5 and 10 µg/ml) at different time intervals treatment (12 and 24 hours) and the expression of TGFβ1 protein was assessed by ELISA.



*P<0.05, **P<0.01

Fig. 3. Effect of various concentrations of methanol extract of Calendula of bFGF protein in mouse embryonic fibroblast cell culture supernatants. Cells were treated with different concentration of methanol extract of Calendula (5 and 10 µg/ml) at different time intervals treatment (12 and 24 hours) and the expression of bFGF protein was assessed by ELISA.



*P<0.05, **P<0.01

Tables:

Table 1: The total expression ratio of the gene of TGFβ1 in mouse embryonic fibroblast cell treated with various concentration of methanol extract of Calendula (5 and 10 µg/ml) relative to control group is presented in each time (12 and 24 hours after treatment). The statistic test for significance is randomization re-allocation test, implemented in the relative expression software tool. Significant down or up regulations of the genes one highlighted.

	12 hours after treatment		24 hours after treatment	
	5 µg/ml	10 µg/ml	5 µg/ml	10 µg/ml
Relative expression	1.3	2.1	0.7	0.62
Standard error	±0.23	±0.24	±0.2	±0.13
P - value	0.265	0.001	0.661	0.001
Fold increase/decrease	+1.3	+2.1	-1.06	-2.5

Table 2: The total expression ratio of the gene of bFGF in mouse embryonic fibroblast cell treated with various concentration of methanol extract of Calendula (5 and 10 µg/ml) relative to control group is presented in each time (12 and 24 hours after treatment). The statistic test for significance is randomization re-allocation test, implemented in the relative expression software tool. Significant down or up regulations of the genes one highlighted.

	12 hours after treatment		24 hours after treatment	
	5 µg/ml	10 µg/ml	5 µg/ml	10 µg/ml
Relative expression	1.5	2.1	0.52	0.34
Standard error	±0.25	±0.27	±0.17	±0.19
P - value	0.231	0.001	0..275	0.001
Fold increase/decrease	+1.5	+2.1	-1.9	-2.96