ejpmr, 2018,5(12), 67-72

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECTS OF STRONTIUM CHLORIDE HEXAHYDRATE IN *CAENORHABDITIS ELEGANS* INDIVIDUALS AS A MODEL FOR SKIN AGING

[1]*Rukiye Güner, [2]Sibel Berksoy Hayta, [3]Necati Özpınar, [4]Melih Akyol, [5]Mustafa Tosun and [6]Sedat Özçelik

[1,2,4,6]Deparment of Dermatology, School of Medicine Cumhuriyet University, 58140 Sivas, Turkey.
[3]Deparment of Parasitology, School of Medicine Cumhuriyet University, 58140 Sivas, Turkey.
[5]Dermatology Clinic in Local Hospital, 58000 Sivas, Turkey.

***Corresponding Author: Dr. Rukiye Güner**

Deparment of Dermatology, School of Medicine Cumhuriyet University, 58140 Sivas, Turkey.

Article Received on 23/10/2018 **Article Revised on 02/11/2018** **Article Accepted on 22/11/2018**

ABSTRACT

Backround: Collagen destruction is related to the presence of matrix metalloproteinase (MMP) that are released by epidermal keratinocytes and dermal fibroblasts and the MMP-1 expression stimulated by ultraviolet radiation is provided by the protein kinase C-dependent (PKC)-dependent activation of transient receptor potential vanilloid 1 (TRPV-1) in human keratinocytes and subsequent calcium (Ca2+) transition. Strontium is a an alkaline-earth metal with chemical and biological behaviors similar to calcium. **Objective:** To investigate whether strontium chloride hexahydrate has anti-aging effects on Caenorhabditis elegans. Materials and Methods: After treatment with strontium solutions, the lifespan, physical development and pharynx pumping numbers of *C.elegans* were determined and compared with the control group. **Results:** An increase in strontium-exposed *C.elegans* depending on the dosage increase in their lifespans was specified. İt was determined that physical development in strontium-exposed *C. elegans* decreased depending on the dosage. It was specified that pharynx pumping count per minute decreased depending on the strontium dosage**. Conclusion:** Strontium chloride hexahydrate slowed down the developmental process of *C.elegans* and extended their lifespans. Strontium chloride hexahydrate may have a positive effect on skin aging in humans with similar mechanisms.

KEYWORDS: Strontium, anti-aging, calcium, Caenorhabditis elegans, lifespan, skin aging.

INTRODUCTION

Skin aging shows itself by increasing wrinkles, prolapse, and laxity and develops through exogenous and endogenous processes. Ultraviolet radiation is an important factor because it causes the destruction of collagen, which is the basic element of extracellular matrix. The effect of oxidative stress on skin aging is well-known.[1,2]

In recent years, new information regarding the role of calcium channels on skin aging has started to take place in the literature.[3-8] Collagen destruction is related to the presence of matrix metalloproteinase (MMP) that are released by epidermal keratinocytes and dermal fibroblasts and the MMP-1 expression stimulated by ultraviolet radiation is provided by the protein kinase C-dependent (PKC)-dependent activation of transient receptor potential vanilloid 1 (TRPV-1) in human keratinocytes and subsequent calcium (Ca^{2+}) transition.[4,5,8]

Strontium is a an alkaline-earth metal with chemical and biological behaviors similar to calcium.[9-11] Strontium is a bad calcium imitator and may change over with calcium in the bone.[12,13]

Caenorhabditis elegans (C.elegans) is apathogen nematode that lives in the soil around the plant roots. A major part of the genes shows a great similarity to the human genes. This nematode can be used as a model in the studies regarding natural immunity, wound healing, and skin aging.[14-21]

The purpose of this study is to assess the effects of *C.elegans* as an aging model on vital functions and physical development by taking the hypothesis of MMP activation inhibition as a basis by reducing the Ca^{2+} influx of strontium chloride hexahydrate within the cell.

MATERIAL AND METHOD

Approval of local ethical committee for animal experiments was taken before conducting the study.

Preparation of NGM, the living environment of *C.elegans*

In the experiment, N2 wild type *C.elegans* and *Escherichia coli* (*E.coli*)OP50 strain were used and they were obtained from the University of Minnesota, Caenorhabditis Genetic Center (CGC).

2,5 gr Peptone, 3 gr NaCl, 20 gr Agar were dissolved in 1 L distilled water and cooled to 55°C after they were autoclaved at 125°C for 15 minutes. 1mL $MgSO_4$(1M), 1 mL Cholesterol (5 mg/mL), 1 mL $CaCl_2$ (1M), and 25 mL KPO_4 buffer (pH:7), which were previously prepared and filtered by using 0.2 µm porous filters, were added to the medium and became homogenized. For the study, 5% (1st dosage), 2.5% (2nd dosage), 1% (3rd dosage), 0.5% (4th dosage) and 0.1% (5th dosage) strontium solutions were prepared and 1 ml from each solution was added to the petri dishes containing 10 ml Nematode Growth Medium (NGM). After the homogenization was achieved, NGMs were ensured to be cooled and solidified. *E.coli* OP50 strain prepared was added to the solidified NGM and dried in a sterile cabinet. Control group was fed in a medium with no strontium.

In the analysis of the lifespan, NGMs to be used were prepared as explained above. However, in order to prevent the egg development of *C.elegans* during the lifespan analysis, fluorodeoxyuridine (FUDR) was added to the NGM during the preparation stage. Among the synchronized *C.elegans*; 20 of them were transferred to each petri dish which were prepared with specified dosages of strontium. Until all the *C.elegans* in all the petri dishes were died, the live animals were counted every day at the same time and compared with the control group.

Without adding fluorodeoxyuridine (FUDR) to the NGM's containing strontium at the same dosages which were prepared in order to evaluate the responses of *C. elegans* against the external mechanical stimuli, their pharynx pumping count per minute and their physical development; 20 mature *C.elegans* were transferred to the petri dishes and also *C.elegans* in the form of L1 was transferred in order to evaluate the physical development. Petri dishes were examined as compared with the control group every day in terms of the physical development of *C.elegans*.

Studies were repeated three times by working at 22°C with 5 petri dishes.

Statistical assessment

Results of control and experimental groups were compared to the difference tests (t-Test) and $p<0.05$ value was accepted as statistically significant. Statistical analyses of data were carried out by using SPSS (Statistical Package for Social Sciences) program. Version 16.0 of the program was used.

RESULTS

Figure 1 illustrate lifespan analyses in the strontium-exposed *C.elegans*. According to Figure 1, an increase in strontium-exposed *C.elegans* depending on the dosage increase in their lifespans was specified. It was found that this increase was significant in 1st, 2nd and 3rd dosages and it was weaker in 4th and 5th dosages.

Figure 2 show Examination of physical development in strontium-exposed *C.elegans*. According to Figure 2, it was determined that physical development in strontium-exposed *C. elegans* decreased depending on the dosage. In the examination of strontium-exposed *C.elegans* in the form of L1 at the end of day 3, it was determined that physical development slowed down as strontium dosage increased and data was compared according to the control group. While physical development in strontium dosage has significantly slowed down in the first and second strontium dosages, no statistical difference was observed in the fifth dosage compared to the control.

It was specified that pharynx pumping count per minute decreased depending on the strontium dosage. It was observed that pharynx movements decreased when the dosage increased. As the pharynx movements at first and second dosages significantly decreased, data were found close to the control at fourth and fifth dosages. Additionally, it was observed that the responses of strontium-exposed *C.elegans* against the external mechanical stimuli slowed down depending on the dosage. These results show parallelism with the slowness in the pharynx movements.

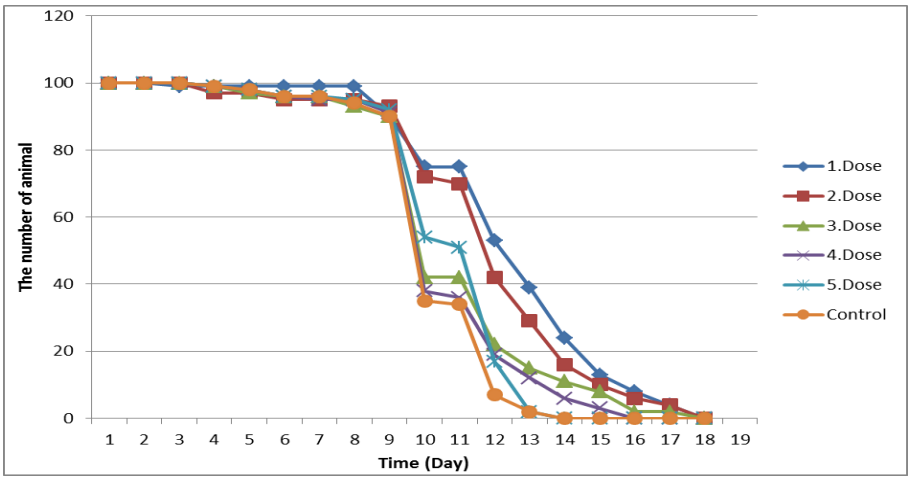

Figure 1: Lifespan of strontium-exposed *C.elegans*.

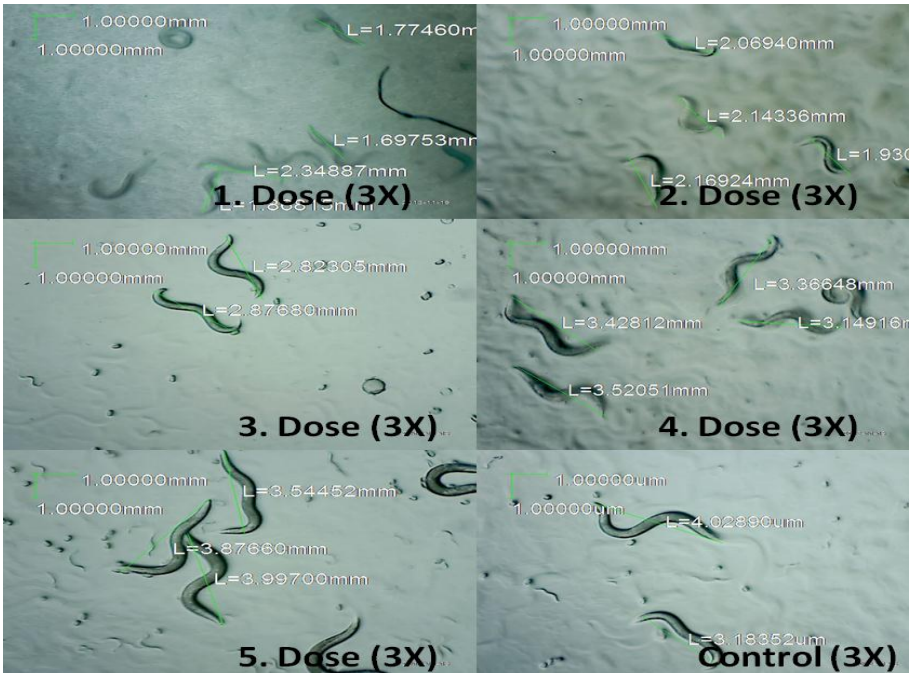

Figure 2: Examination of physical development in strontium-exposed *C.elegans.*

DISCUSSION

Skin aging is divided into two as the intrinsic aging, occurred within a natural process which is known as biological or chronological aging and is genetically programmed, and as extrinsic aging (photoaging) caused by especially the chronic ultraviolet (UV) exposure and environmental factors. As a result of long-term ultraviolet exposure, metalloproteinase activity increases and this causes collagen damage. MMP-1 expression is provided by the protein kinase C (PKC)-dependent activation of transient receptor potential vanilloid 1 (TRPV-1) in human keratinocytes and the subsequent transition of calcium (Ca^{2+}).[4,5,8]

Strontium (Sr^{+2}) is an alkaline-earth metal. Strontium compounds (especially strontium chloride and strontium ranelate) are used in industry and medicine.[11] Strontium is a bad calcium imitator and it causes some biological effects by accumulating in the human body, bones, and teeth because its chemical and biological behavior is similar to calcium.[9,10,12,13] Strontium compounds also have effects on the skin. Strontium chloride can be used in cosmetics and dermatology due to its anti-irritant and anti-allergic effects in the skin and mucosa.[22] In the study of Akyol et al., cytotoxic and proliferative effects of strontium chloride on the fibroblast cell culture were evaluated and it was shown that it had no negative effect on the cell viability in any of 20%, 10%, 5%, 2.5%, 1.25%, 0.6%, and 0.3% test concentrations.[22]

C.elegans, which is an earth nematode, provides very important advantages in biological and medical researches because of its economical usage, easy maintenance, rapid result gaining and short life cycle (15-20 days). Since *C.elegans* provides also an advantage of observing and analyzing effect of a drug on the whole organism, we encounters *C.elegans* as an appropriate model organism in the studies. Since homologue of 60-80% of human genes is present in *C.elegans*, numerous discoveries related to mammalians have been performed by using this model organism.[14-21]

Lifespan of *C.elegans* is determined by numerous factors such as neuroendocrine signals, nutritional factors, mitochondrial functions etc. The clarification of the counterparts of these mechanisms in human will be guiding for the prevention of aging-associated diseases.[23,24]

Matrix metalloproteinase (MMP) released from the epidermal keratinocyte and dermal keratinocytes are responsible for collagen destruction. MMPs are matrix breaking enzymes which play an important role in various destructive processes such as inflammation, tumor invasion, and skin aging.[4,25] MMP levels increase as a result of various stimulations such as cytokine release, UV, and oxidative stress.[26,27] While extracellular Ca^{2+} increase in keratinocytes causes MMP-9 gene release, inhibition of Ca^{2+} influx causes the reduction of MMP-1 mRNA level. The regulation of intracellular Ca^{2+} level affects the MMP-1 secretion.[6,28]

Ca^{2+} influx caused by the UV-induced TRPV1 channels is firstly defined in epidermal keratinocytes.[29] As a result of UV exposure, TRPV1 channel expression increases and thus an increase in both intrinsic and extrinsic aging is observed.[4]

Ca^{2+}/calmoduline complex (CaM) takes place in various cellular functions such as the cell growth, proliferation, and migration.[30] Intracellular Ca^{2+} increase stimulated by UV is important in the activation of cofactor source

for PKC and the Ca^{2+}/calmoduline pathway. Intracellular Ca^{2+} chelator or Ca^{2+}/calmoduline complex antagonists are important for the protection from photoaging.[31]

Heat and UV increase the TRPV1 expression in the skin. TRPV1 inhibitors (capsazepine) reduce the UV-induced MMP expression. TRPV1 levels in elders are found high. This may be related to the indicators in elders such as skin aging, senile pruritus, and neurogenic inflammation. TRPV1 inhibitors can be used in order to be protected from photoaging caused by repetitive UV exposure.[32]

Temporary increase of intracellular Ca^{2+} plays a signal role in various situations such as contraction, secretion, fertilization, proliferation, metabolism, heart beat and memory.[33] Numerous cellular functions are controlled by the intracellular concentration of Ca^{2+} ion. However, long-term increase of Ca^{2+} concentration (greater than 10μM) is dangerous for the cell. Harmful effects that may occur due to the high calcium in the cell are eliminated through the buffering effect of calcium-binding proteins (CaBP) (such as calmoduline).[34]

Ca^{2+} buffering feature of calbindin provides the limitation of Ca^{2+} influx to mitochondria and depolarization and thus prevents the cell to undergo apoptosis.[35]

"Calpain" is a Ca^{2+}-dependent proteolytic enzyme that is related to the diseases of central nervous system. Increase of intracellular Ca^{2+} level causes over-activation of calpain. Thus the decomposition of cell skeleton and membrane proteins increases and cell death occurs by the degradation of the integrity of nerve cells.[36]

In this study, we investigated the effects of strontium chloride hexahydrate preventing Ca^{2+} influx in the cell on the lifespans, physical development, and pharynx pumping count of *C.elegans* individuals at various concentrations by considering that Ca^{2+} takes place in various pathways in aging.

In this study, it was specified that strontium chloride hexahydrate preventing the Ca^{2+} influx inside the cell significantly increased the lifespan of *C.elegans* individuals depending on the dosage increase. Again compared to the control group, a significant increase was determined in the lifespans of the groups to which strontium chloride hexahydrate was administered.

In the examination of the effects of strontium chloride hexahydrate on the vital functions of *C.elegans* individuals; it was determined that physical developments of *C.elegans* exposed to strontium chloride hexahydrate significantly decreased depending on the dosage. In the examination of *C.elegans* exposed to strontium in the form of L1 at the end of day three; it was determined that as the strontium dosage increased, physical development slowed down and data were compared with the control group. While it was observed that physical development at first and second strontium dosages prominently slowed down, no statistical difference was determined at fifth dosage compared to the control.

Because the pharynx in *C.elegans* is a neuromuscular organ, it has its own stimulation conduction system and function principles such as heart in vertebrates, it is an important organ in order to measure neuromuscular effects of active substance thought to be studied. [37] In this study, pumping count of this organ per minute at each strontium dosage was determined in order to measure the neurologic effects of strontium. Accordingly, it was specified that pharynx pumping count per minute decreased depending on the strontium dosage. As the dosage increased, pharynx movements slowed down. While the pharynx movements significantly decreased at first and second dosages, data at fourth and fifth dosages were found to be close to the control. Additionally, it was observed that the responses of *C.elegans* exposed to strontium against the external mechanical stimuli decreased depending on the dosage. These results show parallelism with the retardation in the pharynx movements.

CONCLUSION

Strontium chloride hexahydrate slowed down the developmental process of *C.elegans* and extended their lifespans. Strontium chloride hexahydrate may have a positive effect on skin aging in humans with similar mechanisms. For this reason, further studies are required to explain the efficiency and action mechanism of strontium chloride hexahydrate on skin aging.

REFERENCES

1. Treiber N, Maity P, Singh K, Ferchiu F, Wlaschek M, Scharffetter-Kochanek K. The role of manganese superoxide dismutase in skin aging. Dermato-Endocrinology, 2012; 4: 232-5.
2. Sparavigna A, Tenconi B, De Ponti I. Antiaging, photoprotective, and brightening activity in biorevitalization: a new solution for aging skin. Clin Cosmet Investig Dermatol, 2015; 10: 57-65.
3. Hwang YP, Oh KN, Yun HJ, Jeong HG. The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. J Dermatol Sci., 2011; 61: 23-31.
4. Lee YM, Kim YK, Chung JH. Increased expression of TRPV1 channel in intrinsically aged and photoaged human skin in vivo. Exp Dermatol, 2008; 18: 431-6.
5. Lee YM, Kim YK, Kim KH, Park SJ, Kim SJ, Chung JH. A novel role for the TRPV1 channel in UV-induced matrix metalloproteinase (MMP)-1 expression in HaCaT cells. J Cell Physiol, 2009; 219: 766–75.
6. Mukhopadhyay S, Munshi HG, Kambhampati S, Sassano A, Platanias LC, Stack MS. Calcium-induced matrix metalloproteinase 9 gene expression

is differentially regulated by ERK1/2 and 38 MAPK in oral keratinocytes and oral squamous cell carcinoma. J Biol Chem, 2004; 279: 33139–46.
7. Kohn EC, Jacobs W, Kim YS, Alessandro R, Stetler-Stevenson WG, Liotta LA. Calcium influx modulates expression of matrix metalloproteinase-2 (72-kDa type IV collagenase, gelatinase A). J Biol Chem, 1994; 269: 21505–11.
8. Li WH, Lee YM, Kim JY, Kang S, Kim S, Kim KH, et al. Transient receptor potential vanilloid-1 mediates heat-shock-induced matrix metalloproteinase- 1 expression in human epidermal keratinocytes. J Invest Dermatol, 2007; 127: 2328–35.
9. Başçetin E, Atun G. Adsorption behavior of strontium on binary mineral mixtures of Montmorillonite and Kaolinite, Applied Radiation and Isotopes, 2006; 64: 957-64.
10. Chegrouche S, Mellah A, Barkat M, Removal of strontium from aqueous solutions by adsorption onto activated carbon: kinetic and thermodynamic studies. Desalination, 2009; 235: 306-18.
11. Concise International Chemical assessment document 77. Strontium and strontium compounds. First draft prepared by Mr Peter Watts, Toxicology Advice & Consulting Ltd, Sutton, England; and Mr Paul Howe, Centre for Ecology and Hydrology, Monks Wood, England. World Health Organization, 2010.
12. Schroder SL, Knudsen CM, Volk EC. Marking salmon fry with strontium chloride solutions. Canadian Journal of Fisheries and Aquatic Sciences, 1995; 52: 1141–1149.
13. Chowdhury MJ, Van Ginneken L, Blust R. Kinetics of waterborne strontium uptake in the common carp, Cyprinus carpio, at different calcium levels. Environmental Toxicology and Chemistry, 2000; 19(3): 622-30.
14. Hertweck M, Hoppe T, Baumeister R. C. elegans, a model for aging with highthroughput capacity. Exp Gerontol, 2003; 38(3): 345-6.
15. Corsi AK. A Biochemist guide to C. elegans. Anal Biochem, 2006; 359(1): 1-17.
16. Zhang Y, Lv T, Li M, Xue T, Liu H, Zhang W, et al. Anti-aging effect of polysaccharide from Bletilla striata on nematode Caenorhabditis elegans. Pharmacogn Mag, 2015; 11(43): 449-54.
17. Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolkow CA.. Blueberry polyphenols increase lifespan and thermotolerance in Caenorhabditis elegans. Aging Cell, 2006; 5(1): 59-68.
18. Olsen A, Vantipalli MC, Lithgow GJ. Using Caenorhabditis elegans as a model for aging and age-related diseases. Ann N Y Acad Sci., 2006; 1067: 120-8.
19. Tan L, Wang S, Wang Y, He M, Liu D. Bisphenol A exposure accelerated the aging process in the nematode Caenorhabditis elegans. Toxicology Letters, 2015; 235: 75-83.
20. Xu S, Chisholm AD. A Gαq-Ca^{2+} signaling pathway promotes actin-mediated epidermal wound closure in C. elegans. Curr Biol, 2011; 21: 1960-7.
21. Chisholm AD, Xu S. The Caenorhabditis elegans epidermis as a model skin. II: differentiation and physiological roles. Wiley Interdiscip Rev Dev Biol, 2012; 1: 879-902.
22. Tosun M, Berksoy Hayta S, Akyol M, Özçelik S. Changes emerging with calcinerin inhibition in Caenorhabditis elegans individuals as an aging model. Cumhuriyet Medical Journal, 2017; 39(3): 518-24.
23. Berksoy Hayta S, Durmuş K, Altuntaş EE, Yildiz E, Hisarciklıo M, Akyol M. The reduction in inflammation and impairment in wound healing by using strontium chloride hexahydrate. Cutan Ocul Toxicol, 2018; 37(1): 24-8.
24. Akyol M, Polat Akın Z, Özçelik S, Kaya Ö. The effects of strontium chloride on viability of mouse connective tissue fibroblast cells. Cumhuriyet Med J., 2013; 35: 33-8.
25. Guarente L, Kenyon C. Genetic pathways that regulate aging in model organisms. Nature, 2000; 408: 255–62.
26. Klass MR. A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. Mech Age Dev., 1983; 22: 279-86.
27. Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene specific transcription factors. Arthritis research, 2002; 4: 157-64.
28. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med, 1997; 337: 1419-28.
29. Varani J, Warner RL, Gharaee-Kermani M, Phan SH, Kang S, Chung JH et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J Invest Dermatol, 2000; 114: 480-6.
30. Sudbeck BD, Pilcher BK, Pentland AP, Parks WC. Modulation of intracellular calcium levels inhibits secretion of collagenase 1 by migrating keratinocytes. Mol Biol Cell, 1997; 8: 811–24.
31. Nakagaki T, Oda J, Koizumi H, Fukaya T, Yasui C, Ueda T. Ultraviolet action spectrum for intracellular free Ca^{2+} increase in human epidermal keratinocytes. Cell Struct Funct, 1990; 15: 175–9.
32. Mercure MZ, Ginnan R, Singer HA. CaM kinase II delta2-dependent regulation of vascular smooth muscle cell polarization and migration. Am J Physiol Cell Physiol, 2008; 294: C1465–7.
33. Hwang YP, Kim HG, Han EH, Choi JH, Park BH, Jung KH, et al. N-Acetylglucosamine suppress collagenases activation in ultraviolet B-irradiated human dermal fibroblasts: Involvement of calcium

ions and mitogen-activated protein kinases. J Dermatol Sci., 2011; 63: 93-103.
34. Lee YM, Kang SM, Chung JH. The role of TRPV1 channel in aged human skin. J Dermatol Sci., 2012; 65: 81-5.
35. Akalın G, İncesu Z. The effects of 1,3 bıs (heteroaryl substıtued) benzene derıvatıves on apoptosıs of n-ras transformed mouse skeletal muscle. J. Fac. Pharm Ankara, 2005; 34: 1- 15.
36. Tandoğan B, Ulusu N. Calcium Binding Proteins. Turk J Biochem, 2005; 31: 36-40.
37. Kook SY, Jeong H, Kang MJ, Park R, Shin HJ, Han SH, et al. Crucial role of calbindin-D28k in the pathogenesis of Alzheimer's disease mouse model. Cell Death and Differentiation, 2014; 21: 1575-87.
38. Wu H-Y, Tomizawa K, Matsui H. Calpain-calcineurin signaling in the pathogenesis of calcium-dependent disorder. Acta Med., 2007; 61: 123-37.
39. Mango SE. The C. elegans pharynx: a model for organogenesis. WormBook: The online review of C. elegans biology (Internet), Available from: www.ncbi.nlm.nih.gov › NCBI › Literature › Bookshelf, 2005.