The Efficacy of *Malus Domestica* Stem Cell Extract Cream for The Reduction of Peri Orbital Wrinkles

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ABSTRACT

Eye wrinkle, one of the most common signs of aging, despite its unharmed feature, may make one feel self-conscious because having wrinkle-free eyes is accepted to be attractive. Wrinkles are caused by intrinsic aging, environmental stress, repetitive facial expressions, and genetic, and also believed to be influenced by free radicals. Among various treatments available, the researcher would like to emphasize on the *Malus Domestica* apple stem cell extract, whose topical application act as an antiaging and antiwrinkle agent by rejuvenation the skin cells at the cellular level although the exact mechanism is unknown. This research was a split face study, conducted in Mae Fah Luang University Clinic, Bangkok, with 16 volunteers, aged between 35 to 50 years, applied *Malus Domestica* apple stem cell extract cream and standard cream to the designated sides of the face twice daily for 8 weeks. The results were measured by Cutometer® MPA 580, Corneometer, VISIA complexion system for skin elasticity, skin hydration status and wrinkle score respectively. After 8 weeks of the study, the data showed significant result in skin hydration as well as skin elasticity, which gradually increased from 4^{th} week to 8^{th} week at p=0.05. The results demonstrated that *Malus Domestica* apple stem cell extract cream positively promotes the skin moisture and elasticity to a satisfying extent and thus a promising anti- wrinkle topical intervention, without any side effects.

Keywords: *Malus Domestica,* stem cell, wrinkle, aging, free radicals

1. Introduction

Wrinkles are one of the most common cutaneous manifestations of aging signs and known as a rhytide, a fold, ridge or crease in the skin. The wrinkles are mainly caused by intrinsic or natural aging and extrinsic aging, in which photoaging or chronic sun exposure is the primary source. Wrinkles can also be caused by repeated activity of facial muscles over a long period of time (hyperdynamic lines) or redundant skin due to depletion of collagen and elasticity.

There is no cure and no fountain of youth to combat all of the signs of aging. However, there are various approaches to reverse aging and wrinkles; including of non-invasive treatment, mainly topical application of

vitamins, antioxidants, peptides, anti-inflammatories and herbal extracts, invasive procedures such as botulinum toxin injection, dermal fillers injection, and non-ablative lasers and cosmetic surgery.

Amongst the treatment guideline available, the safest and easiest treatment option is topical medication. Nowadays, many topical skin care and topical medications are using products based on herbal and natural plant extracts, which are effective and harmless to people but on the other hand, leading to use utilization of herb becomes leaving many waste products.

In recent years, stem cell based therapy which has been paid special attention in cosmetic and anti-aging purposes in recent years due to their ability in the process of stimulation and proliferation of the epidermal skin stem cells (Schmid, Schürch, Blum, Belser, & Zülli, 2008) (Trehan, Michniak-Kohn, & Beri, 2017) (MARTYNA MORUå1, 2014).

Stem cells play an important role in skin vitality. They are located in the basal layer of the skin and have the properties of self-renewal, the ability to go through numerous cycles and cell divisions while maintaining the undifferentiated state and differentiation, the capacity to differentiate into other cell types of the same tissue. Stem cells also have a limited life expectation. Environmental and intrinsic stress factors mainly effect the skin stem cells. Therefore, as we age, the number and activity of skin stem cells is reduced gradually. Thus, to delay preliminary aging, skin stem cells have to be protected and supported.

As is declared that plant stem cells can protect human stem cells, stimulate skin regeneration and prevent skin aging. (Trehan, Michniak-Kohn, & Beri, 2017).They are found to be compatible with human skin , can protect human skin stem cells ,maintains the self-renewal capacity of human skin stem cells , increase longevity of human skin stem cells ,replenish, regenerate and repair damaged DNA , delay aging of essential cells , stimulate duplication of human skin stem cells , protect the skin from ozone/pollution damage and UV damage.

The apple fruit *Malus Domestica* from a rare Swiss Apple called *Uttwiler Spatlauber*, an old cultivar which is still alive in Switzerland but no longer cultivated which is well known for its excellent storage ability, it can stay fresh-looking up to four months after being harvested, long after other varieties have become wrinkled. And the stem cell extract has been proven to show many beneficial properties *Malus Domestica* apple fruit stem cell extract act as an anti-aging and anti-wrinkle agent by rejuvenating at cellular level although the exact mechinsm is unknown.The stem cell extract cream is rich in epigenetic factors which are important to protect and maintain the function of skin stem cells. Also, the apple *Uttwiler Spätlauber* is known to contain metabolites which insure the longevity of cells which is used to delay the aging of skin cells. *Malus Domestica* stem cell extract had been concluded that it possesses properties of stimulating and renewal of human stem cells in previous studies. It maintains the power of skin cell regeneration at the cellular level thus resulting in delay of aging and decrease wrinkles. Therefore, the researcher is keen to study that the topical *Malus Domestica* stem cell extract can improve the skin in treating the periorbital wrinkles.

2. Objectives of the study

The objective of this research is to study the efficacy of *Malus Domestica* apple stem cell extract cream for the treatment of periorbital wrinkles.

3. Materials and methods

The study was comparative, double-blind, randomized, controlled and split face clinical trial. A total of 16 volunteers, between 35 and 50 years of age, were selected and informed consent were taken in both written and verbal forms at the start of the study. Healthy volunteers aged between 35 to 50 years, who wanted their eyes wrinkles treated and those who were not using any anti-wrinkle eye creams within 6 months were included in the st udy.

Volunteers who had received any aesthetic treatment for eye wrinkles including dermabrasion, chemical peeling or laser resurfacing, Botulinum toxin type A injection in previous 6 months, and, or filler injection during the previous year around the eyes area, skin allergies or hypersensitivity to *Malus Domestica* cream, pregnant women, lactating women, those who had any associated medical illness such as coagulopathy, poorly controlled diabetes mellitus, photosensitivity and immunosuppression, pregnant or breast feeding women, those with history of strong UV exposure in some career or leisure, those on poor wound healing or abnormal scarring were excluded in this study. Patch tests was performed to determine any irritation or allergy caused by creams. Patch test was performed by applying *Malus Domestica* stem cell extract on the arms of the volunteers under water-proof patch test then left in place for 24 hours. During the test, volunteers should not be sweating excessively or exposing strong sunlight and then examine for any reaction such as redness or inflammation in 36-46 hours. Volunteers with any allergic reaction will be treated until fully recovered and will be excluded.

The randomization was done by physician unrelated with the research. After randomization, the volunteers will be given two tubes of cream, *Malus Domestica* extract cream in one tube and standard cream in the other, both two tubes had the identical package, color, texture and smell, and labelled as ‘right’ and ‘left’. Dermatologists and participants who have to evaluate the results will be blinded. The physician, who are unrelated with the study, will establish which side of the face will be treated with cream A and cream B by using Random Sequence Generator from the Website (http**://** www**.**random**.**org/sequences/) and will conceal the sequences in an opaque envelopes. The volunteers were instructed to apply one finger-tip unit of each cream, from the bottle labelled “right side” will be applied to the right side of the face and the other labelled “left side” will be applied to the left side of the face, twice daily ; morning and evening, for 8 weeks duration. They came for assessments at 0^{th}, 4^{th}, 8^{th} weeks.

Sample Size Calculation

The sample size calculation was done by using 2 means and standard deviation (SD) of the measurement from previous research "Formulation and in vivo evaluation for anti-aging effects of an emulsion containing basil extract using non- invasive biophysical techniques" (Rasul, A., & Akhtar, N, 2011).

Biophysical Techniques

The measurements of skin elasticity and hydration were recorded indoor with the room temperature range 20 to 24 degree Celsius and relative humidity range.

Cutometer® MPA 580

This is the standard equipment to measure skin elasticity and hydration.

Corneometer®

This device is used to measure hydration of the epidermis especially stratum corneum.

VISIA® Complexion Analysis System

is an equipment for the facial skin scanning and captures key visual information by using multi-spectral imaging and analysis system.

Facial wrinkles assessment was done by 3 different dermatologists with the photos taken by VISIA®.

Statistical Analysis

The medical record data and outcomes of the volunteers in this research were conducted in Mae Fah Luang University Dermatology Clinic and recorded by using SPSS 23 software and Microsoft Excel 2010.

4. Results

In this study, 16 volunteers were enrolled; the mean age was 42.06±4.92 years (range 34-50 years). There were no subjects with underlying disease, food allergy, drug allergy and treatment within 4 weeks before study. The mean of exposure to sunlight duration was 40.63±11.53 minute. Majority of the subjects had combination skin (62.5%), dry skin (25.0%) and oily skin (12.5%), respectively.

4.1 Results of skin moisture measured by Corneometer

Table 4.1 Statistical analysis of skin moisture at under eyes and crow's feet between *Malus Domestica* stem cell extract cream versus Standard cream base on 0^{th}, 4^{th}, 8^{th} week

	Malus Domestica stem cell extract cream	Standard cream base	Mean difference	p-value (a)
	Mean±SD	Mean±SD		
Under eyes				
Week 0	56.66±11.45	57.93±7.95	1.27	0.526
Week 4	62.37±13.46	60.07±11.51	2.31	0.418
Week 8	67.28±11.55	60.28±10.47	6.99	0.024*
p-value (b)	<0.001*	0.220		
Crow's feet				
Week 0	56.49±12.53	55.87±13.07	0.63	0.472
Week 4	58.44±13.91	56.89±13.54	1.55	0.514
Week 8	68.35±14.96	61.33±12.04	7.02	<0.001*
p-value (b)	<0.001*	0.002*		

Note. Data were analyzed between group with Paired t-test (a), within group with Repeated measure ANOVA (b) p-value Significant at *p<0.05 **p<0.001

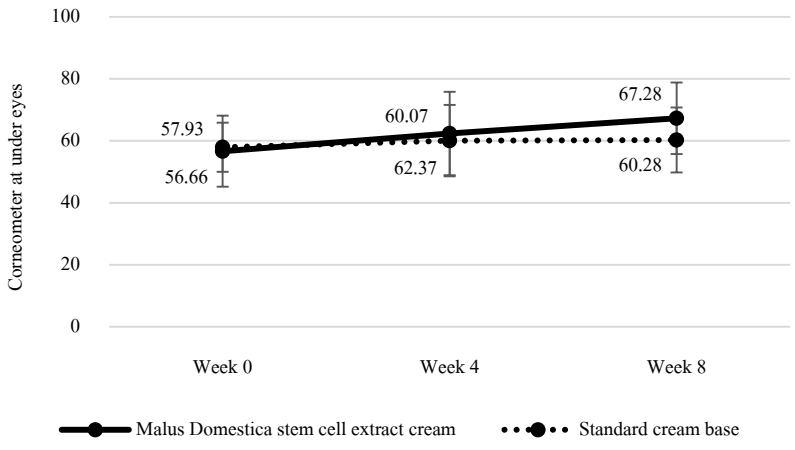

Figure 4.1 Linear graph show comparison of skin moisture at under eyes in each visit between *Malus Domestica* stem cell extract cream versus Standard cream base

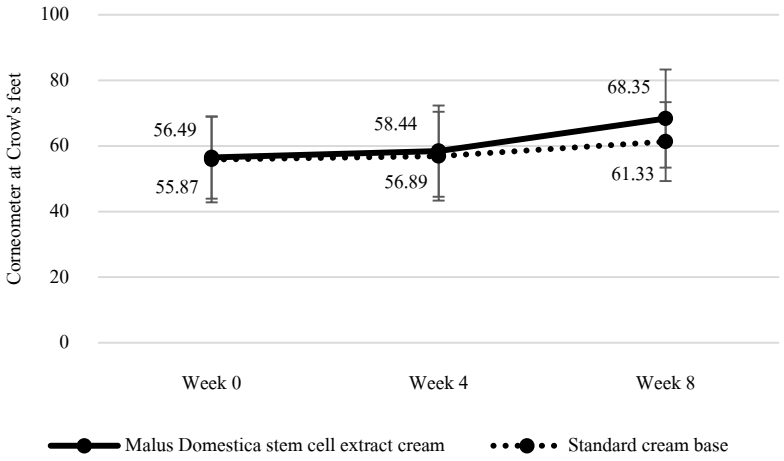

Figure 4.2 Linear graph show comparison of skin moisture at crow's feet in each visit between *Malus Domestica* stem cell extract cream versus Standard cream base

According to the statistical analysis results from table 4.1 and figure 4.1 – 4.2, the mean of skin moisture at under eyes in *Malus Domestica* stem cell extract cream on 0^{th} week was 56.66±11.45, 4^{th} week was 62.37±13.46 and 8^{th} week was 67.28±11.55. The mean of skin moisture at under eyes in each visit increase statistically and significant at the level of 0.05 ($p<0.001$). For Standard cream base, the results at 0^{th} week was 57.93±7.95, 4^{th} week was 60.07±11.51 and 8^{th} week was 60.28±10.47. The mean of skin moisture at under eyes in each visit was not statistically significant difference ($p=0.220$). The comparison of skin moisture at under eyes between *Malus Domestica* stem cell extract cream versus Standard cream base; It was found that mean of skin moisture at under eyes of *Malus Domestica* stem cell extract cream was significantly higher than Standard cream base at 8^{th} week ($p=0.024$).

The mean of skin moisture at crow's feet in *Malus Domestica* stem cell extract cream on 0^{th} week was 56.49±12.53, 4^{th} week was 58.44±13.91 and 8^{th} week was 68.35±14.96. The mean of skin moisture at crow's feet in each visit statistically significant increase at the level of 0.05 ($p<0.001$). For Standard cream base, the results at 0^{th} week was 55.87±13.07, 4^{th} week was 56.89±13.54 and 8^{th} week was 61.33±12.04. The mean of skin moisture at crow's feet in each visit statistically significant increase at the level of 0.05 ($p=0.002$). The comparison of skin moisture at crow's feet between *Malus Domestica* stem cell extract cream versus Standard cream base; It was found that mean of skin moisture at crow's feet of *Malus Domestica* stem cell extract cream was significantly higher than Standard cream base at 8^{th} week ($p<0.001$).

When comparing skin moisture score under the eye between the follow-up phase for both groups, it was found that the skin moisture at under eyes in *Malus Domestica* stem cell extract cream at 4^{th} and 8^{th} week was higher than 0^{th} week and at 8^{th} higher than 4^{th} week statistically significant at the level of 0.05. For Standard cream base, skin moisture was not statistically significant difference between visits. The skin moisture at crow's feet in

Malus Domestica stem cell extract cream at 8th week higher than 0th week, at 8th week higher than 4th week statistically significant at the level of 0.05. For Standard cream base, the skin moisture at 8th week was higher than 0th week and at 8th week was higher than 4th week, statistically significant at the level of 0.05.

4.2 Results of skin elasticity measured by Cutometer

Table 4.2 Statistical analysis of skin elasticity at under eyes and crow's feet between *Malus Domestica* stem cell extract cream versus Standard cream base on 0th, 4th, 8th week

	Malus Domestica stem cell extract cream	Standard cream base	Mean difference	p-value (a)
	Mean±SD	Mean±SD		
Under eyes				
Week 0	0.7929±0.1254	0.7978±0.0843	0.0048	0.825
Week 4	0.8377±0.0773	0.7946±0.0777	0.0431	0.139
Week 8	0.8746±0.0769	0.8194±0.0754	0.0552	0.015*
p-value (b)	0.002*	0.106		
Crow's feet				
Week 0	0.7466±0.1206	0.7728±0.0754	0.0262	0.213
Week 4	0.8107±0.0534	0.7985±0.0654	0.0122	0.497
Week 8	0.8108±0.0745	0.7935±0.0716	0.0174	0.054
p-value (b)	0.012*	0.144		

Note. Data were analyzed between group with Paired t-test (a), within group with Repeated measure ANOVA (b)
p-value Significant at *p<0.05 **p<0.001

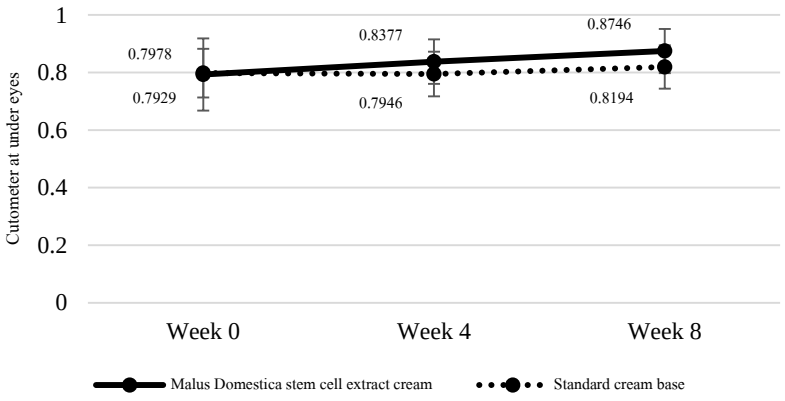

Figure 4.3 Linear graph show comparison of skin elasticity at under eyes in each visit between *Malus Domestica* stem cell extract cream versus Standard cream base

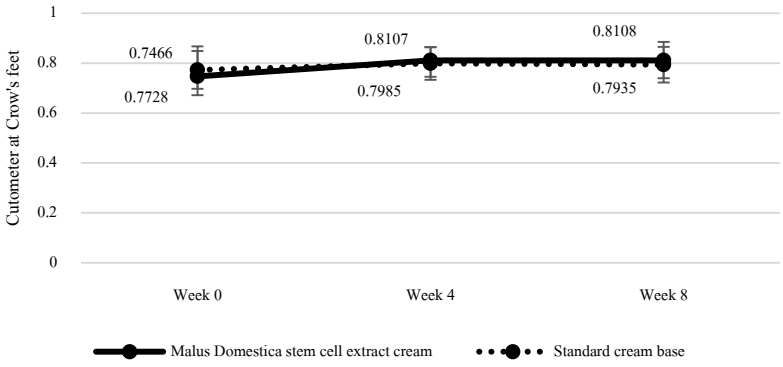

Figure 4.4 Linear graph show comparison of skin elasticity at crow's feet in each visit between *Malus Domestica* stem cell extract cream versus Standard cream base

According to the statistical analysis results from table 4.2 and figure 4.3 – 4.4, mean of skin elasticity at under eyes in *Malus Domestica* stem cell extract cream on 0^{th} week was 0.7929±0.1254, 4^{th} week was 0.8377±0.0773 and 8^{th} week was 0.8746±0.0769. The mean of skin elasticity at under eyes in each visit increased statistically and was significant at the level of 0.05 (p=0.002). For standard cream base, the results at 0^{th} week was 0.7978±0.0843, 4^{th} week was 0.7946±0.0777 and 8^{th} week was 0.8194±0. The mean of skin elasticity at under eyes was not statistically significant difference (p=0.106). The comparison of at skin elasticity between *Malus Domestica* stem cell extract cream versus Standard cream base; It was found that the mean of skin elasticity at under eyes of *Malus Domestica* stem cell extract cream was significantly higher than standard cream base at 8^{th} week (p=0.015).

The mean of skin elasticity at crow's feet in *Malus Domestica* stem cell extract cream on 0^{th} week was 0.7466±0.1206, 4^{th} week 0.8107±0.0534 and 8^{th} week 0.8108±0.0745. The mean of skin elasticity at crow's feet in each visit statistically significant increased at the level of 0.05 (p=0.012). For standard cream base, the results at 0^{th} week was 0.7728±0.0754, 4^{th} week was 0.7985±0.0654 and 8^{th} week was 0.7935±0.0716. The mean of skin elasticity at crow's feet in each visit not statistically significant difference (p=0.144). The comparison of skin elasticity at crow's feet between *Malus Domestica* stem cell extract cream versus standard cream base; it was found that mean of skin elasticity at crow's feet of *Malus Domestica* stem cell extract cream and standard cream base was not statistically significant difference at all visits.

When comparing skin elasticity score under the eye between the follow-up phase for both groups, it was found that skin elasticity at under eyes in *Malus Domestica* stem cell extract cream at 8^{th} week higher than 0^{th} week statistically significant at the level of 0.05. For standard cream base, the skin elasticity was not statistically significant difference between visits.

The skin elasticity at crow's feet in *Malus Domestica* stem cell extract cream at 4^{th}week and 8^{th} week was higher than 0^{th} week and was statistically significant at the level of 0.05. For standard cream base, the skin elasticity had no statistically significant difference between visits.

4.3 Facial assessment wrinkle score by 3 dermatologists

Table 4.3 Statistical analysis of Facial assessment wrinkle score (FAWS) at under eyes and crow's feet between *Malus Domestica* stem cell extract cream versus Standard cream base on 4th, 8th week

	Malus Domestica stem cell extract cream	Standard cream base	p-value (a)
	Median (IQR)	Median (IQR)	
Week 4	2 (1, 2)	2 (1.25, 2)	0.739
Week 8	2 (2, 2)	2 (2, 2)	0.705
p-value (b)	0.180	0.317	

Data were analyzed between group with Wilcoxon signed rank test (a), within group with Firedman test (b)

* $p<0.05$

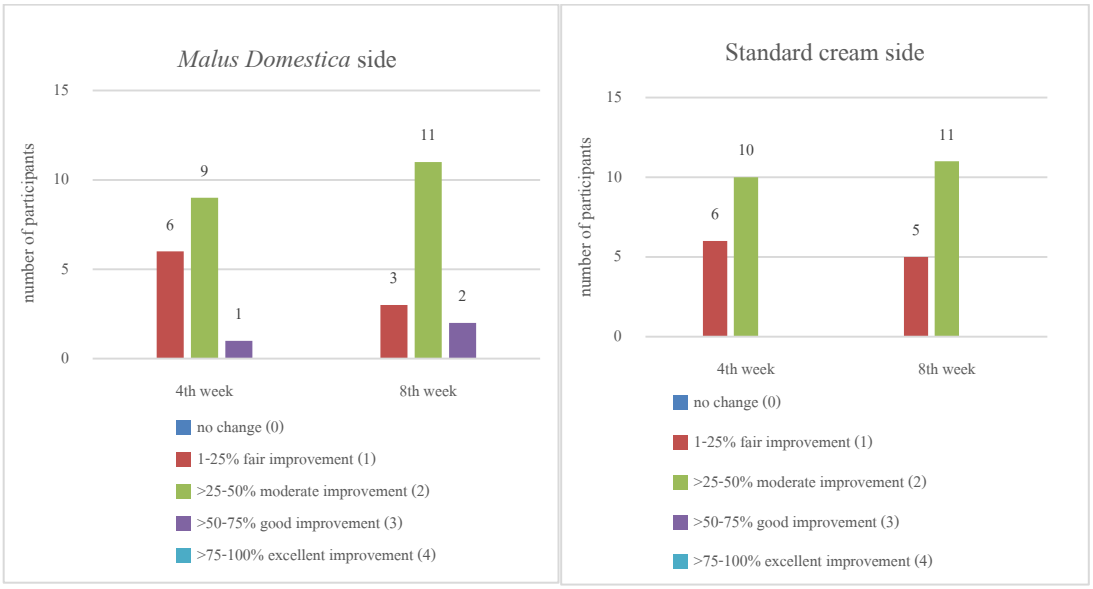

Figure 4.5 Bar chart reveals comparism of dermatologists' satisfaction score of *Malus Domestica* cream treated side and standard cream treated side

According to the statistical analysis results from table 4.3and figure 4.5, median of FAWS in *Malus Domestica* stem cell extract cream on 4th week was 2 (1, 2) and 8th week 2 (2, 2). The median of FAWS in each visit showed no statistical significant difference at the 0.05 level (p=0.180). For Standard cream base, the results were at 4th week was 2 (1.25, 2) and 8th week 2 (2, 2). The median of FAWS in each visit not statistically significant difference (p=0.061). The comparison of FAWS between *Malus Domestica* stem cell extract cream versus Standard cream base, it was found that median of FAWS at crow's feet of *Malus Domestica* stem cell extract cream and Standard cream base was not statistically significant difference at 4th and 8th visits.

5. Discussion and Conclusion

According to the study, all of the volunteers were between 35 to 50 years old, female more than male. The average age was 42.06±4.92 years. There were no volunteers who had treatment for eye wrinkles within 4 weeks before study. All volunteers had no underlying disease, food allergy, drug allergy and they all can avoid the sun exposure for a certain period. Majority of the subjects had combination skin, dry skin and oily skin by 62.5% ,25% and 12.5% respectively.

From all the results, the efficacy of the *Malus Domestica* stem cell extract cream for the reduction of peri orbital wrinkles is summarized as follows;

Firstly, topical *Malus Domestica* did not caused any allergy since patch test was shown negative and there were no reported cases of allergy throughout the study. Thus, it is concluded that topical *Malus Domestica* stem cell extract cream can be safely applied around peri orbital area.

Furthurmore the topical *Malus Domestica* extract cream can increase the skin moisture measured by Corneometer, both at crow's feet and under eye area, significantly higher than the standard cream at the level 0.05 ($p<0.001$), at 4^{th} week and 8^{th} week. It was also found that *Malus Domestica* cream significantly improved the skin moisture between follow up phases compared to the standard cream. This study was attempted on finding the efficacy of *Malus Domestica* stem cell extract in related to increasing skin moisture around peri orbital area, and the results showed significant increase in moisture in *Malus Domestica* cream treated side comparing with the standard cream base. The skin moisture result was much better in *Malus Domestica* treated side compared to the cream base treated side.

Moreover, the skin elasticity was significantly increasing since the 4^{th} week (when $p<0.05$), measured by Cutometer, and it was significantly effective until week 8. This study was the attempt on finding the efficiency of *Malus Domestica* stem cell extract cream in related to skin elasticity around the peri orbital area, the skin flexibility level might be increased from the elevation of skin layer fluid and improvement of collagen and elastin underneath the skin. Thus, *Malus Domestica* significanty improved the skin elasticity in comparison with the cream base.

Next to the patients' satisfaction, *Malus Domestica* stem cell extract cream treated side had significant difference in patients' satisfaction compared with the standard cream treated side.

Suggestions

From the above study, it can be assumed that:

1. The duration of the study was 8^{th} week, which the improvement of wrinkles has just begun, it might be worthy to continue the study for a longer period of time.
2. The research data may be tested to improve *Malus Domestica* stem cell extract cream in increasing the

   effectiveness of skin elasticity and skin moisturization compared with other antioxidant cream and

3. The data from this study can be used as a database for further research about skin rejuvenation, smoothness and moisturization.
4. Moreover, this study may assist the usage of *Malus Domestica* stem cell extract for skin rejuvenation, skin diseases and other cosmetic purposes.

5.1 Conclusion

The results after 8 weeks trail demonstrated that *Malus Domestica* stem cell extract cream was able to show gradual significant improvement from 4^{th} to 8^{th} week, in skin moisture as well as skin elasticity in comparison with the standard cream. The results demonstrated that *Malus Domestica* apple stem cell extract cream positively promoted the skin moisture and elasticity to a satisfying extent to the participants without any allergy. Therefore, it is concluded that the topical *Malus Domestica* stem cell cream extract in treating the peri orbital wrinkles has shown to be a safe, interesting and promising anti- wrinkle topical intervention.

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