

A comparative study on the anti-wrinkle benefits of an active complex (Palmitoyl Tripeptide-38, *Silybum marianum* fruit extract, Hyaluronic acid (HA), and *Chenopodium quinoa* seed extract)

Nooshin Hashemi, Ehsan Eslahi, Sara Shamaei

R&D Department, Unit 9 & 10, Kaj Building, No.347, Assadabadi Ave, Mahloran cosmetic company, Tehran, Iran

Abstract. *Background:* recently, a wide range of natural polymers and plant extracts have been used to slow down aging and ameliorate the skin by reducing fine lines and wrinkles. *Aims:* This research investigates the efficacy of hyaluronic acid, palmitoyl tripeptide-38, and two rich herbal extracts in the form of a topical anti-wrinkle cream. *Methods:* the complex which was formulated using some active ingredients, was applied twice daily for 3 months in a group composed of 30 women, in the crow's feet region. The improvement of the appearance of wrinkles in that area was assessed by a self-evaluation and the GAIS (Global aesthetic improvement) scale. In addition, the wrinkle depth and volume, skin moisture, and transepidermal water loss (TEWL) were measured using the Visioface, Tewameter, and Cutometer devices at baseline and after 6 and 12 weeks of treatment. *Results:* there were no noticeable adverse reactions including erythema, irritation, or allergic reactions after the treatment period. Clinical measurements showed an improvement in the subjective evaluation of the skin's appearance by the GAIS scale in 14 and 17 of the participants in grade 1 of this scale after 6 and 12 weeks of treatment. The wrinkles' volume and depth were significantly reduced ($p < 0.05$). In addition, the skin's moisture and gross and net elasticity increased after 12 weeks of treatment ($p < 0.05$). The skin transepidermal water loss also decreased to -22% after 12 weeks of application ($p < 0.05$). *Conclusions:* the results of this study revealed that this topical cream, serves as a safe and reliable option for skin hydration and exerts powerful anti-aging benefits compared to the base formula.

Key words: anti-wrinkle, active complex, skin moisture, transepidermal water loss, visioface, cutometer

Introduction

Skin aging is caused by two contributing factors, including endogenous and exogenous factors of aging. As the skin structure changes, the skin loses its natural elasticity and becomes more fragile¹. Human skin consists of three huge and complex layers: the epidermis, dermis, and subcutaneous layer, which act as a barrier by protecting the skin and separating the inner space of the body from the surrounding one². In the aging process, depending on the causing factor, both

the epidermal and dermal layers are affected. As we age, the skin cells' proliferation slows down, and the dermal layer's diameter becomes thinner. Over time, as the dermis layer becomes thinner, the amount of collagen and elastin fibers in the skin and the skin's ability to retain moisture also decreases. These factors, along with the reduction of the secretion of sebum in the skin, lead to skin aging³. Collagen, which serves as the major component in the Extracellular matrix (ECM) of the dermis layer, becomes fragmented and degraded by free radicals and their effects on the formation of

the reactive oxygen species. By reducing the amount of collagen in the dermis layer and reducing the interaction between the ECM and fibroblasts, the skin's elasticity decreases causing dermal aging. The epidermal layer also becomes thinner, presenting fine wrinkles⁴.

The skin barrier prevents water evaporation from the subcutaneous layers and modifies and maintains the body's temperature by regulating the homeostasis of the body⁵. Fine lines and wrinkles are usually formed due to internal factors such as genetic factors and environmental factors such as exposure to ultraviolet and infrared radiation, pollutants, and the impact of facial expressions such as prolonged frowning and the improper use of cosmetic products. In addition to the external factors, some internal factors such as skin type can also cause the formation of wrinkles in different populations, and the accumulation of these factors can lead to the development of wrinkles and their deepening over time⁶. The most accepted theories explaining the aging process as a result of external factors are based on DNA damage and the subsequent skin repair process⁷.

Because of the deconstruction of the skin barrier caused by these factors, there are changes in the parameters determining the strength and the stability of the skin's barrier, including the pH of the skin surface, the amount of water in the superficial layer of the skin, and the amount of water evaporated from the surface of the epidermal layer. In this situation, skin pH and water evaporation from the epidermal layer of the skin can increase which leads to roughness and fragility of the skin⁸. All these changes are undesirable and mostly cause aging and the formation of fine-lines. In conclusion, aging and UV radiation are the two most important causes of skin aging and wrinkling⁹. Furthermore, the exposure to UV rays is one of the main causes of premature skin aging. These rays break down the collagen fibers in our skin and produce elastin in an abnormal form. Ultraviolet rays damage the skin, as a result of which some enzymes are produced that change the structure of collagen in the skin, and the repetition of this process eventually leads to wrinkling^{10,11}.

In recent years, various cosmetic products with different mechanisms of action have been used to improve skin elasticity and resistance to fight exogenous and endogenous aging factors. Today, synthetic ingredients inspired by natural peptides such as peptides,

vitamins, and herbal extracts have the potential to improve the skin's appearance and prevent aging. In general, these ingredients have antioxidant, anti-inflammatory, moisturizing, and firming effects on the skin, preventing the formation of wrinkles and the progression of the aging process¹².

For this purpose, the appearance of the skin was studied, and different parameters were calculated through an objective evaluation and biophysical techniques. The skin was evaluated by applying a cream containing the active ingredients (Palmitoyl tripeptide-38, Silybum marianum fruit extract, Hyaluronic acid (HA) with a molecular weight spectrum of 50-3000 kDa, and Chenopodium quinoa seed extract) in a group of 30 female volunteers with grade II wrinkles based on the Glogau classification of photoaging. Based on this classification, patients are categorized into photoaging types I to IV depending on the degree of skin wrinkling¹³. Summarizing, the antiwrinkle benefits of the active complex were analyzed before and after the use this complex by administering the self-appraisal questionnaire, while skin elasticity, moisture, and water evaporation were measured using the Cutometer and the Tewameter device in a randomized study in a 12-week in the treatment period. This study was further continued by utilizing the Visioface device to measure the volume and depth of the wrinkles. All the tests and measurements were assessed according to a single-blind randomized method.

Material and methods

Clinical study

In this research, the effectiveness of some active ingredients used in the formulation of a cream intended to improve fine lines and deep wrinkles was evaluated using the Visioface[®] purchased from (CK electronic company, Germany), Cutometer[®] dual MPA 580 Courage & Khazaka (from CK electronics, Germany), and Tewameter[®] TM300 (EnviroDerm Services, United Kingdom) devices. All these devices were purchased directly from these companies. Depth and volume of the skin fold, Skin elasticity, water evaporation, and skin moisture were measured, and the results were shown further ahead. This clinical

evaluation involved thirty volunteers with a mean age of 30 to 50 years and moderate to advanced levels of skin wrinkles. There were no signs of adverse effects including itching, erythema, burning, or flaking of the skin during the treatment period. All the participants requested to apply the active cream complex on their crow's feet wrinkles twice a day.

This cream is composed of two parts of a basic formulation and active ingredients but in the control group, only the base formulation was applied. Active complex ingredients comprise Palmitoyl tripeptide-38 purchased from Sederma company (France), *Silybum marianum* fruit extract from Mibelle Biochemistry (Switzerland), and Hyaluronic acid from ROELMI. HPC company (Italy), and a *Chenopodium quinoa* seed extract from COBIOSA company (Spain). The base formulation in the active and control group is composed of Glyceryl Stearate Citrate purchased by Evonik company (Germany), an Acrylate Copolymer purchased by Sigma-Aldrich (Germany), Glycerin, and Cetearyl Alcohol purchased by KLK OLEO company (Malaysia), and Phenoxyethanol purchased by the Schülke company (Germany). All active and base ingredients are directly purchased from these companies.

The formula of base:

Glyceryl Stearate Citrate: 1-2%
 Acrylate Copolymer: 1.5-3.5%
 Glycerine: 3%
 Cetearyl Alcohol: 2.5%
 Phenoxyethanol: 1%
 Distilled water: q.s 100%

The devices applied in this research include the pH meter (Benchtop Meter, PH500, CLEAN, China), mixer (MS 280D, MTOPS, Korea), beaker, digital scale (PS 600.R2, RADWAG, Poland), heater stirrer (MS300HS, MTOPS, Korea), Cutometer® Dual MPA 580, Visioface® 1000 D and the Tewameter® (Courage+Khazaka electronic GmbH, Germany). All the devices were directly purchased from these companies.

This study was conducted in a group involving 30 women with a mean age of 30-50 years showing

the II level of wrinkles based on the Glogau photoaging classification¹³.

The formulation of the antiwrinkle cream is designed using the active ingredients named Palmitoyl tripeptide-38 in the concentration of 1% (w/w) in high purity (>97%), *Silybum marianum* fruit extract at 1% (w/w) in the purity of >97%, pure hyaluronic acid (>98%) at 1% (w/w) with a molecular weight spectrum of 50-3000 kDa, and *Chenopodium quinoa* seed extract with high purity (>97%) in 0.5% (w/w) as an active complex in a total concentration of 3.5% (w/w) in a base formulation.

The formula prepared for active formulation:

Glyceryl Stearate Citrate: 1-2%
 Acrylate Copolymer: 1.5-3.5%
 Glycerine: 3%
 Cetearyl Alcohol: 2.5%
 Palmitoyl tripeptide-38: 1%
Silybum marianum fruit extract: 1%
 Hyaluronic acid: 1%
Chenopodium quinoa seed extract: 0.5%
 Phenoxyethanol: 1%
 Distilled water: q.s 100%

A base cream formulated by the mentioned base materials was also used as a control. To evaluate the antiwrinkle effects of the active complex, the participants were requested to apply these complexes in a fingertip unit on the crow's feet region of their faces twice daily for 12 weeks. The study protocol was conducted based on the ethical rules of the Declaration of Helsinki and according to the Good Clinical Practice (GCP). It should be mentioned that the patients which have used any topical or injectable antiaging treatment during the last year, people who consumed isotretinoin in the past three months, patients with severe acne or smoking history, and patients who had a deep chemical peeling or laser therapy over the past 6 months, were excluded from this study¹².

Measurements

The subjective and objective analysis of the skin was performed in the study group using non-invasive instruments Visioface, Cutometer, and Tewameter.

These devices are used to measure the skin parameters such as wrinkle depth and volume, skin elasticity, skin moisture, and transepidermal water loss. The measurements were done on the base time (before applying the cream), the 6th and 12th weeks after using the active complex for evaluating the wrinkle depth and volume of the skin, and the skin's elasticity. Furthermore, the improvement of wrinkles was assessed using the GAIS scale in the subjective method (global aesthetic improvement scale) (Table 1) after the 6th and 12 weeks of application, and each volunteer requested to rate the improvement level as a measure of her satisfaction using a five-grade Likert scale (as shown in 1 = dissatisfied, 2 = slightly satisfied, 3 = relative satisfaction, 4 = satisfied and 5 = too much satisfied). For evaluating the skin moisture and the TEWL content, each volunteer was instructed to use the cream for 12 weeks, twice a day. The probable changes in skin moisture and the TEWL content were measured after the 2nd, 4th, 6th, 8th, 10th, and 12th week of applying the active complex in comparison with the base formulation.

The percent change in the skin's moisture and water loss affecting the skin's appearance before and after applying this complex was calculated using the following equation¹⁵:

$$\text{Percentage change} = \frac{[(A - B)]}{B} \times 100$$

In this equation, A is the individual value of each parameter and B is the base value of each parameter at zero hours.

Statistics

To evaluate the significance of test analysis, Two-tailed significance tests were used in all cases. A statistical analysis of different skin parameters (skin moisture, TEWL, depth and volume of wrinkles, and skin elasticity) was assessed using the IBM SPSS statistical software version 16.0 (independent sample t-test for measuring the skin moisture and the TEWL content of the skin, and one-sample t-test for other measured parameters). In all the results, a P-value <0.05 is considered significant.

Results

The subjective evaluation of skin using the GAIS scale showed an improvement in 14 and 17 participants in grade 1 of this scale, 8 and 9 participants in grade 2, and 8 and 4 participants in grade 3 respectively after 6 and 12 weeks of treatment, compared to the baseline value in a qualitative test (Figure 1).

The volunteers' self-evaluation of skin parameters showed the average satisfaction level ranging from 3.5 to 5 based on the Likert scale.

The objective analysis of skin wrinkles was done using the devices as mentioned prior. For this purpose, the skin parameters such as depth and volume of

Table 1. The GAIS scale^{12,14}.

GAIS grade	Global aesthetic improvement scale
Very much improvement	Excellent result of implantation in these patients
Much improve	Marked improvement, but patients don't show the excellent results and a touch-up are needed to improve the results
Improved	A clear improvement was seen compared to the initial condition
Unaltered condition	Skin condition is the same as the initial condition but still, a touch-up is needed or recommended
Worsened	Skin appearance is worse than the initial condition

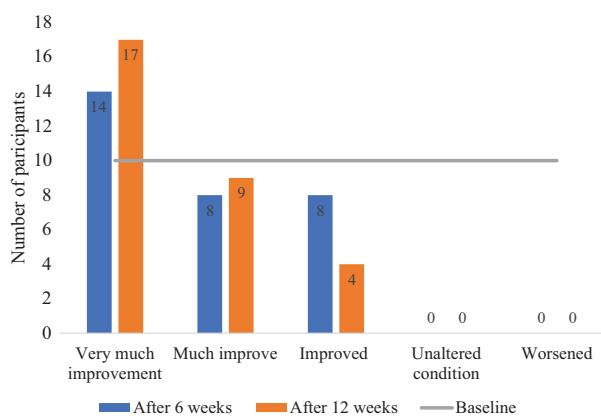


Figure 1. Subjective evaluation of skin by GAIS scale.

wrinkles were measured by the Visioface device before and after 6 and 12 weeks of using this complex. The results of skin evaluation by Visioface showed a significant decrease in the volume and depth of the wrinkles (Figures 2A, B) with the P-values of 0.000 and 0.009 and the Std. Deviation of 0.31 and 7.75 respectively for wrinkle depth (Figure 2B) and volume (Figure 2A). The wrinkle volumes reduced from 54.2 to 46.9 and 38.7 after the 6th and 12th weeks, respectively. Furthermore, there was a decrease in the wrinkles' depth from 8.75 to 8.13 after 12 weeks of application.

Furthermore, the skin's moisture content or the percent of variation in the skin's hydration level was measured by the Cutometer apparatus before and after the 2nd, 4th, 6th, 8th, 10th, and 12th week of using the active cream complex. The improvement in skin moisture was seen in all volunteers represented in Figure 3 (P-value = 0.003) with the Std. Deviation of 0.54. Skin elasticity was also measured by comparing the values before and after the 6th and 12th weeks of skin treatments using the active complex (Figure 4).

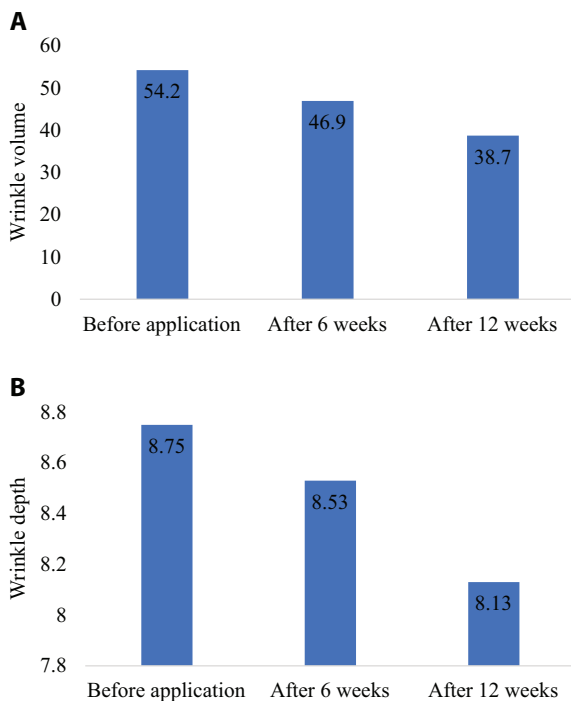


Figure 2. A. Wrinkle volume before and after 6 and 12 weeks of applying the active complex. B. Wrinkle depth before and after the 6 and 12 weeks of applying the active complex.

In this research, the gross and net elasticity of the skin increased after the 6th and 12th week of treatment. The gross elasticity showed a significant increase after the 6th and 12th week of treatment using this complex (P-value = 0.024 and 0.001 and the Std. Deviation of 0.035 and 0.05, respectively). Furthermore, the net elasticity of the skin showed a significant increase after the 6th and 12th week of the treatment (P-value= 0.02 and 0.003, and the Std. Deviation of 0.1 and 0.05 respectively).

Trans epidermal water loss is a further assay that was performed to evaluate the amount of water evaporated from the skin and the hydration rate before and after the treatment of the skin through the active complex cream which shows the strength of the skin barrier (Figure 5). As seen in Figure 5, after applying this complex, the amount of TEWL decreased in the follow-up sessions, and showed a significant decrease after the 6th and 12th week of application (P-value = 0.000 and Std. Deviation of 0.89). In addition, the skin's hydration rate increased after using the active complex at the end of the 6th and the 12th week.

Discussion

Facial skin aging and wrinkles are one of the main concerns of the cosmetics industry these days. The skin is composed of the compact extracellular matrix (ECM) composed of collagen and elastin fibers¹⁶.

Both internal and external factors affect the onset of skin aging and photoaging is an important factor among them. All these factors contribute to the loss of skin elasticity and firmness, causing visible signs of aging¹⁷.

Improvement of skin firmness and the reconstruction of the skin's matrix are the main achievements of the cosmetic active ingredients. If the skin is well hydrated and nourished, the elasticity of the skin will be increased. Furthermore, protecting the skin from free radical damage could reduce the signs of aging and wrinkling. To improve the skin's parameters and signs of aging, different active ingredients have been used in the formulation of cosmetic products to soothe the skin and reduce the wrinkles' depth¹⁸.

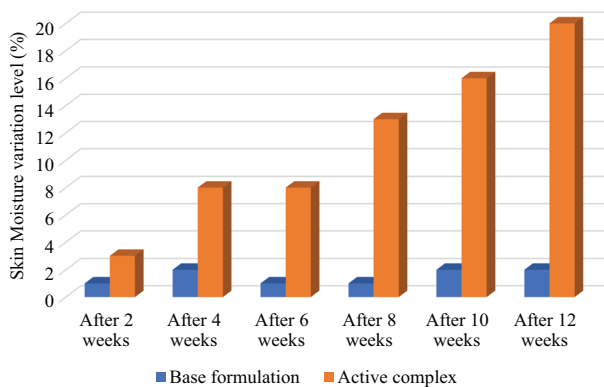


Figure 3. Skin moisture percentage changes before and after using the active complex.

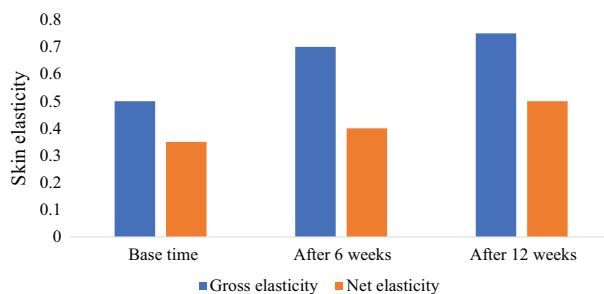


Figure 4. Skin elasticity parameters in base time, after 6 and 12 weeks of using the active complex.

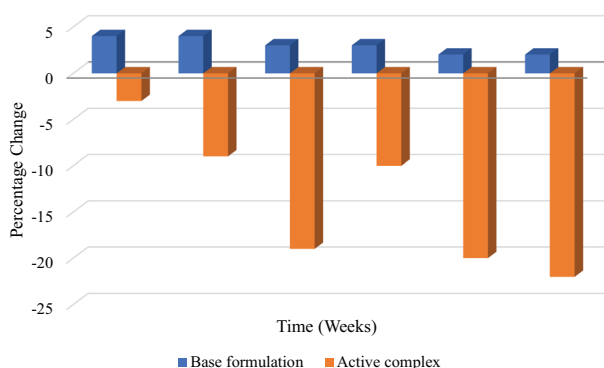


Figure 5. TEWL percentage change before and after using the base formulation and the active cream complex.

In this research, various plant extracts combined with some peptides and hyaluronic acid in the form of an active complex cream revealed improvements in skin wrinkles, and helped tighten and moisturize the skin.

This product has been found to significantly reduce fine lines and wrinkles. For this, a complex of active ingredients composed of Palmitoyl tripeptide-38,

Silybum marianum fruit extract, Hyaluronic acid, and *Chenopodium quinoa* seed extract were formulated in a basic cream and applied to reduce the fine lines and wrinkles in the study group.

Peptides are known for their effectiveness in stem cell regeneration. Epidermal and dermal stem cells are also key cells for continuous skin rejuvenation¹⁹.

For example, Palmitoyl tripeptide-38 is a member of peptides that plays an important role in keratinocyte differentiation and is an active ingredient that increases dermal density and skin elasticity. In recent studies, this active ingredient has also been used to improve skin firmness and as an antiwrinkle, moisturizer, or anti-aging ingredient. For example, in a study of 37 female subjects, the effectiveness of a moisturizer composed of some herbal extracts and palmitoyl tripeptide-38 was evaluated, and the results of the questionnaires showed the favorable effects of the topical formulation after application. To increase the efficacy of the formulation, hyaluronic acid is used as an active moisturizing ingredient penetrating deep into the skin. HA is a non-sulfate compound composed of repeating units of N-acetyl-D-glucosamine and D-glucuronide B. When used, hyaluronic acid's ability to retain large amounts of water keeps the skin hydrated, and this helps to prevent the development of wrinkles by filling in the existing wrinkles to reduce their appearance^{20,21}.

UV rays are one of the major external factors that cause extrinsic aging due to the upregulation of collagen-degrading enzymes (MMP-1 and the gelatinase MMP-2). These enzymes trigger the breakdown of collagen and further increase skin fragility. In the skin, hyaluronic acid act as a radical scavenger of free radicals in the skin and as an antioxidant agent in physical conditions. Studies showed that the D-glucuronic acid unit of hyaluronic acid can capture the reactive oxygen species and reduce radical toxicity^{22,23}. Thus, hyaluronic acid acts as an excellent moisturizer and antioxidant, having powerful anti-aging effects.

Milk thistle or *Silybum marianum* extract, which is from the Asteraceae family, is another active ingredient used in the active cream formulation. *Silybum marianum* seeds contain silymarin flavonoids which have incredible radical scavenging and anti-aging properties. Based on recent studies, the flavonoid content of this herbal extract makes it a suitable candidate

for reducing wrinkles. Furthermore, the polyphenolic compounds in the seed extract provide antioxidant, anti-inflammatory, and immunomodulatory properties that improve the appearance of the skin and soothe the fine lines²⁴.

Chenopodium quinoa is composed of polyphenols, proteins, and essential fatty acids with moisturizing and antioxidant effects which improve the dermal thickness and the differentiation of keratinocytes and additionally represent an inhibitory effect for collagenase activity. The seed extract of this plant had an antioxidant potential leading to the absorption of UV rays and further slowing down the progression of wrinkle formation²⁵.

In this research, all volunteers completed the study before the end of a 12 week period of application. After using the active complex cream, the Likert scale was used for the self-evaluation of the volunteers. This scale is defined as a range of satisfaction from dissatisfied to very satisfied²⁶.

The results of the self-evaluation study showed a satisfaction level of 3.5 to 5 based on this scale. All volunteers scored an improvement in the general characteristics of the crow's feet wrinkles, and the results showed a completely moderate improvement in skin wrinkles.

Furthermore, improvement in volunteer skin folds was determined and assessed by the GAIS scale. GAIS scale is a global aesthetic improvement scale that indicates an improvement in skin aging, skin appearance, and general condition after using a specific product. In this study, the results of using the test complex were compared as a subjective analysis of the skin at baseline, after 6 and 12 weeks. For this purpose, the GAIS scaling method is used by a dermatologist to assess the improvement of skin aging during this period. Based on this scale, most volunteers showed "improved" and "very much improved" ratings after 6 and 12 weeks of application.

The volume and depth of the wrinkles were assessed using the non-invasive Visioface device (Figures 2A, B). The results of this study showed a significant improvement in reducing the depth and volume of wrinkles. The improvement of these factors is due to the antioxidant effect of flavonoids from the *Silybum marianum* extract, and polyphenols present in

Chenopodium quinoa seed extract. These compounds could uptake the free radicals produced by UV rays and further stimulate collagen synthesis and repair damaged collagen and elastin fibers.

The elastic fibers of the skin are closely linked to the collagen fibrils which constitute the main part of the extracellular matrix of the skin. These parts are the main compartment of the dermis layer and can be affected by external and internal aging factors. Hence, this study measured skin elasticity parameters including the net and gross elasticity, with the Cutometer device before and after 6 and 12 weeks of using the active complex (Figure 4). Gross elasticity is defined as the ability of the skin to return to its original state after vacuum release from the probe (R2), and net elasticity (R5) is defined as the ratio of the skin's immediate retraction to its immediate distension^{27,28}.

The gross elasticity of the skin in all cases was increased to 0.7 and 0.75 compared to the initial value of 0.5. The net elasticity of the skin also increased from 0.35 to 0.4 and 0.5 respectively after 6 and 12 weeks of using the active cream complex. Based on these objective results, the skin parameters of R2 and R5 significantly increased after 12 weeks. This effect might be explained by the effect of hyaluronic acid and palmitoyl tripeptide-38 active ingredients that have been proven to reinforce skin firmness and elasticity. This effect is also due to the active phenolic compounds of *Silybum marianum* extract and *Chenopodium quinoa* seed extract.

The active ingredients used in the formulation of this cream had a significant effect on the moisture content of the skin after 12 weeks of application. The results showed an improvement in skin moisture content 12 weeks after using the test complex increasing from 3% to 20%, while the results using the basic formula as a control varied between 1% and 2% during the treatment period. The improvement seen in skin moisture levels could be attributed to the role of the active moisturizing ingredients of hyaluronic acid and the flavonoid content of the herbal extracts used in this cream. Flavonoids could improve the moisture content of the skin due to the swelling of the corneocytes on the surface layer of the skin²⁹.

Moreover, a relative improvement in the skin's appearance was observed after using the cream by

measuring transepidermal water loss or TEWL after 12 weeks with a Tewameter device, and calculating the percentage change using Eq. 1. The results, as shown in Figure 5, represent a decreasing pattern of TEWL compared to the baseline to -22% after 12 weeks of application. The underlying mechanism of the TEWL reduction could be determined by the moisturizing effect of hyaluronic acid and the flavonoid content of the applied plant extracts. In addition, the peptide used in this formulation rejuvenates the skin and, based on previous studies, further improves and strengthens the skin barrier.

Conclusions

The aim of this study was to evaluate *in vivo* the anti-wrinkle effect of a cream formulated with Palmitoyl tripeptide-38, Silybum marianum fruit extract, hyaluronic acid (HA), and Chenopodium quinoa seed extract. This formulation has been found to have a significant effect on the volume and depth of wrinkles, skin moisture content, and skin elasticity. In the self-evaluation and dermatologist analysis of the skin based on the GAIS scale, the appearance of the skin showed signs of improvement or great improvement. The decrease in TEWL also indicates that the formulation exerts significant anti-aging effects.

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Conflict of Interest: All the authors declare that there are no conflicts of interest in this paper.

Informed Consent: An informed consent was obtained from all subjects involved in the study.

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Correspondence:

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Ehsan Eslahi

Phone number: (+98)2188600935

Fax number: (021)88212946

E-mail: e.eslahi@mahloran.com