Hourolein Arab[1] 0000-0003-1674-7208, *Sajjad Sadeghi*[2] 0000-0003-1579-394X
Mohammad Ali Shahtalebi[3†] 0000-0002-9284-562X, *Amir Hosein Siadat*[4] 0000-0003-3201-7037
Bita Tahvilian[3] 0000-0002-8095-8424, *Mohammad Reza Mofid*[5] 0000-0002-0347-8152

[1] *Department of Clinical Biochemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Hezar Jerib Avenue, Isfaha 73461-81746, Iran*
[2] *Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Valiasr avenue, Sari 33971-4815, Iran*
[3] *Department of Pharmaceutics, School of Pharmacy and pharmaceutical science, Isfahan University of Medical Sciences, Hezar Jerib Avenue, Isfahan 73461-81746, Iran*
[4] *Department of Dermatology, School of Medicine, Skin Diseases and Leishmaniasis Research Center, Al-Zahra Hospital, Isfahan University of Medical Sciences, Hezar Jerib Avenue, Isfahan 73461-81746, Iran*
[5] *Department of Clinical Biochemistry, School of Pharmacy and Bioinformatics Research Center, Isfahan University of Medical Sciences, Hezar Jerib Avenue, Isfahan 73461-81746,*
+98 3137927047 *mofid@pharm.mui.ac.ir*

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Anti-wrinkle effect of an emulsion based on the hexapeptide argireline. Pilot study

Przeciwzmarszczkowe działanie emulsji na bazie heksapeptydu argireliny. Badanie pilotażowe

ABSTRACT

One of the symptoms of skin aging is the appearance of wrinkles on the face, which have a significant impact on appearance, self-esteem, and quality of life, especially in women. Delaying this process has been the subject of much research for many years.

The study aimed to evaluate the effectiveness of oil-in-water (O/W) emulsions based on argireline peptide and inexpensive raw materials available in Iran. Their anti-wrinkle effect was evaluated in twenty women.

According to the results obtained, the use of the emulsion contributed to a reduction in wrinkles without causing an allergic reaction. The argireline peptide has anti-aging properties and may be a suitable choice for improving the appearance of the skin.

Keywords: argireline, anti-aging, facial wrinkles, lotion, cosmetics

STRESZCZENIE

Zmarszczki to jeden z najbardziej widocznych objawów starzenia się skóry. Wpływają nie tylko na wygląd, ale też na poczucie pewności siebie i ogólny komfort życia, szczególnie wśród kobiet. Od lat trwają intensywne badania nad metodami, które pozwoliłyby ten proces spowolnić lub złagodzić jego efekty.

Celem pracy była ocena skuteczności emulsji typu olej w wodzie (O/W) przygotowanej na bazie peptydu argireliny oraz niedrogich i dostępnych w Iranie surowców. Oceniano ich działanie głównie pod kątem przeciwzmarszczkowym u dwudziestu kobiet.

Wyniki badania wykazały, że stosowanie emulsji zmniejszyło widoczność zmarszczek, nie powodując przy tym reakcji alergicznych. Peptyd argireliny wykazuje działanie przeciwstarzeniowe i może skutecznie wspierać poprawę wyglądu skóry.

Słowa kluczowe: argirelina, właściwości przeciwstarzeniowe, zmarszczki, emulsja, kosmetyki

INTRODUCTION

Beauty has been a concern for humans, particularly women, for many years [1]. One of the critical issues of this innate instinct is the appearance of facial wrinkles. Aging, stress, unhealthy diet, smoking, sunlight exposure, fat accumulation, and inflammation are all risk factors associated with skin aging [2, 3]. Over time, these factors contribute to the destruction of collagen and elastin, the reduction in thickness of the topmost layer of skin, weakening of the tissue responsible for connecting and supporting other tissues, stiffness of facial muscles, resulting in the aging process, which becomes visible [4]. In today's society, the desire to maintain a youthful appearance has led to an increase in therapeutic techniques for removing or reducing facial wrinkles. These techniques fall into three categories: invasive, semi-invasive, and non-invasive. Non-invasive methods have become increasingly popular due to the fewer complications and problems they cause [5, 6].

Argireline, a synthetic acetyl hexapeptide-3, is a popular non-invasive option for reducing facial wrinkles, especially on the forehead and eye area. This peptide mimics the effects of botulinum toxin by inhibiting the secretion of acetylcholine, a chemical messenger, by destabilizing the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) complex at neuromuscular junctions and interfering with catecholamine release. Unlike botulinum toxin, argireline does not have any side effects such as ptosis, bleeding, swelling, erythema, or pain, making it a safe, needle-free alternative. It is commonly used as an active substance in skin creams [7, 8]. It can be applied topically and penetrates the skin barrier, so it does not require injections and making it suitable for use in cosmetic formulations [9].

Although cosmetic products containing argireline peptide are available in international markets, there is a lack of transparency and understanding about what precisely is included in these formulations. Specifically, the economic reasons behind their production and marketing are not well explained, and the exact ingredient compositions, such as the concentration of argireline and other components, are often not disclosed publicly. This lack of transparency makes it difficult for consumers and industry stakeholders to assess the true value, efficacy, and safety of these products, highlighting a gap between market availability and scientific or economic clarity. Consequently, efforts to elucidate these formulations are impeded by a lack of foundational research [10].

AIM

The study aimed to evaluate the effectiveness of a new anti-wrinkle emulsion containing argireline among Iranian women. Available and economical materials from the domestic market were used. The rise in consumer awareness about the ingredients, concentrations, and prices of cosmetics introduced to the market was of key importance.

MATERIALS AND METHODS

Argireline was purchased from GL Biochem Ltd. (Shanghai, China). Isopropyl myristate, glycerin monostearate, phenoxyethanol, butylene glycol, zinc sulphate, bicinchoninic acid, potassium sorbate, cetyl alcohol, tween 80 and tween 20 were procured from Merck (Germany). Vitamin E was obtained from Osve (Tehran, Iran) and, canola oil was supplied by Ladan (Behshahr, Iran). All of chemical ingredients used in the research were of analytical grade.

The study consisted of two parts: the first involved the formulation and physicochemical evaluation of an argireline peptide-based lotion, and the second focused on the clinical assessment of the prepared lotion on volunteer women. The specific steps for each part are outlined below.

Formulation and physicochemical evaluation of a lotion based on argireline peptide

Identification of the argireline peptide

The Argireline peptide was authenticated based on molecular weight by sodium dodecyl sulfate and polyacrylamide gel electrophoresis (SDS-PAGE) method.

Preparation of 0.5 g/L argireline peptide solution

Due to the excellent solubility of argireline in water, 0.05 g of the peptide sample was weighed and dissolved in pure deionized water, and the final volume reached 100 mL. To clarify, the 0.05 g/L solution was a stock solution used *in vitro*, while the 5% in the formulation refers to the mass/volume percentage of argireline added directly to the lotion during preparation.

Planning different basic formulations by experimental design software

According to the formulations made in various research, the amount of each of the four ingredients (tween 20, tween 80, butylene glycol, and cetyl alcohol) was assigned (table 1). The several volumes – minimum, mean and maximum – were determined according to their minimum and maximum concentration in the formulation, and the information was entered into the Design Expert software (version 7.0.0; Stat-Ease, Inc., Minneapolis, MN, USA.). This software analyzed the given information, established the top nine formulations (table 2) in order of priority, and then the physicochemical parameters of each preparation were examined after formulation (table 3).

Preparation of lotion formulations

To prepare the formulations according to the table 2, oil phase (isopropyl myristate, glycerin monostearate, cetyl alcohol, paraffin, canola oil) and aqueous phase (tween 20, tween 80, phenoxyethanol, butylene glycol, potassium sorbate, zinc sulphate, argireline, water) were added to separate beakers and heated to the same temperature (80°C). The two phases

Table 1 List of suggested data for experimental design software

R_x	butylene glycol (w/w)	cetyl alcohol (w/w)	tween 20 (w/w)	tween 80 (w/w)
1	2	4	1	2
2	2	1	1.5	1.5
3	4	1	2	2
4	2	2	2	1.3
5	4	2	1	1.5
6	1	4	2	1.5
7	1	1	1	1.3
8	1	2	1.5	2
9	4	4	1.5	1.3

Source: Own elaboration

Table 3 Physicochemical parameters of formulations

Formulation number	pH ± SD	Viscosity (Centipoise ± SD)	Centrifugation
1	7.5±0.11	700±1.15	Not separated
2	5.5±0.12	480±1.15	Not separated
3	6.2±0.15	240±0.9	Separated
4	5.6±0.11	400±1.10	Not separated
5	6.1±0.15	700±1.15	Separated
6	6.3±0.15	1100±1.80	Separated
7	5.4±0.40	200±1.10	Not separated
8	5.5±1.15	420±1.10	Not separated
9	5.5±0.18	180±1.10	Not separated

Source: Own elaboration

Table 2 Formulations established by experimental design software

Formulation ingredient	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
isopropyl myristate	10%	10%	10%	10%	10%	10%	10%	10%	10%
glycerin monostearate	3%	3%	3%	3%	3%	3%	3%	3%	3%
paraffin	1%	1%	1%	1%	1%	1%	1%	1%	1%
cetyl alcohol	4%	1%	1%	2%	2%	4%	1%	2%	4%
canola oil	2%	2%	1%	1%	1%	1%	1%	1%	1%
tween 20	1%	1.5%	2%	2%	1%	2%	1%	1.5%	1.5%
tween 80	2%	1.5%	2%	1.3%	1.5%	1.5%	1.3%	2%	1.3%
phenoxyethanol	1%	1%	1%	1%	1%	1%	1%	1%	1%
butylene glycol	2%	2%	4%	2%	4%	1%	1%	1%	4%
potassium sorbate	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
zinc sulphate	1%	1%	1%	1%	1%	1%	1%	1%	1%
argireline	5%	5%	5%	5%	5%	5%	5%	5%	5%
water	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100

Source: Own elaboration

were then gently mixed until homogenous and allowed to cool to 40°C [11].

Each formulation designed above was evaluated of centrifugation, viscosity, and pH tests. According to the results, formulation No. 2 (F2) was selected as the best formulation for physicochemical tests, which are presented below.

Physicochemical evaluation of a lotion based on argireline peptide formulation candidate

The following several tests were performed for the final evaluation of the candidate formulation.

- Organoleptic Characteristics: The prepared lotion was visually checked for color and homogeneity, as it was previously described (Table 4) [12].

Table 4 Physicochemical evaluation of formulation No. 2[a]

Parameters	*Results*
Physical appearance	white color lotion, completely homogeneous
Centrifuge	+++
Freeze-Thaw	+++
pH	5.85±0.18
Active ingredient content	98.07%±0.41

+: poor (significant phase separation), ++: good (minor separation), +++: excellent (no phase separation). Data are presented as mean ± SD.

Source: Own elaboration

- **Centrifuge test:** 24 hours after preparation, the lotion was centrifuged by a Hettich D-7200 (Hettich, Kirchlengern, Germany) at 3000 rpm for 30 minutes and at one-week intervals for 28 days (Table 4) [11, 13].
- **pH:** Triplicate measurement of lotion pH value was conducted by Metrohm-Switzerland digital pH meter, and their average was calculated (Table 4) [12].
- **Freeze-thaw cycle:** Immediately after the preparation of the lotion, the freezing and thawing operation was performed. 20 mL of each sample was kept at -20°C for 48 hours, and then these frozen samples were thawed at room temperature within 48 hours. This test was also done in triplicate for all the samples (Table 4) [14].

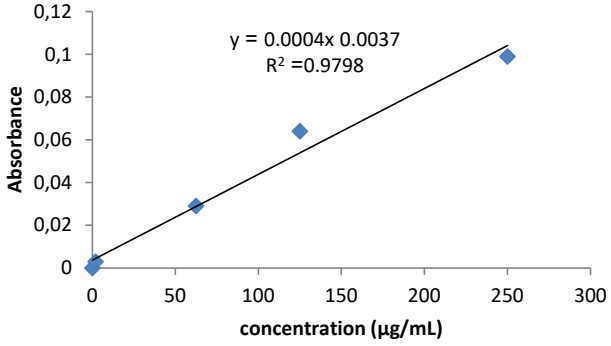

Fig. 1 Argireline standard curve in water **Source:** Own elaboration

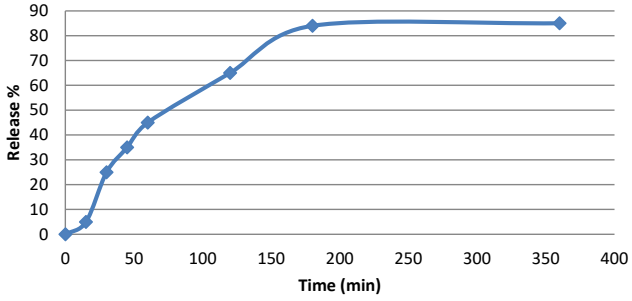

Fig. 2 *In vitro* release of argireline through CA membrane **Source:** Own elaboration

- **Rheological investigation:** The viscosity of the lotion was measured using a Brookfield DV-III Ultra viscometer at a constant temperature of 25°C and speed of 100 rpm. All of these evaluations were carried out in triplicate as the tests above [12].
- **Active ingredient content**: 5 g of the lotion were dissolved in 50 mL of purified water in screw cap tubes. Tubes were kept in a water bath and shaken for 2 hours (Gallen KAMP - Germany). Protein content of the lotions was determined by the bicinchoninic acid (BCA) protein method with a Protein Assay kit (Pars Toos, Iran) (Table 4).

In vitro release study of argireline

In vitro diffusion of argireline from 0.45-micron cellulose acetate (CA) membrane of prepared formulation (F2) was studied using a modified Franz diffusion cell (with a volume of 24 cells). 24 mL of purified water was used as the receiving chamber. 5 g of emulsion was applied to the CA membrane surface, and the membrane between the donor and receiver chambers was closed. The donor chamber was kept in contact with the receiving chamber while the temperature was set at 37°C. The magnetic stirrer stirred the solution in the receiving chamber. At predetermined time intervals, 1 mL volume of solution was removed from the receiving chamber with the help of a pipette and immediately replaced with 1 mL volume of fresh water. After preparing 4 appropriate dilutions, argireline concentration on the receptor fluid was quantified using the BCA assay described previously [12]. Linear regression was analyzed and the straight-line equation of the calibration curve was obtained, then the concentration of argireline in the withdrawn aliquots was calculated using the calibration curve. Regarding the calibration curve, standard solutions of argireline with a concentration of 5-250 µg/mL were prepared in water. The linear correlation coefficient was 0.9798, which indicates compliance with Beer Lambert's law in the range of concentrations used. The argireline standard curve is shown in fig. 1, and *in vitro* release of argireline through the CA membrane is shown in fig. 2.

Table 5 Parameters of stability test of formulation No. 2

	Condition	Initial	7 days	14 days	28 days
Physical appearance	8°C	√	√	√	√
	25°C	√	√	√	√
	40°C	√	√	√	√
Active ingredient content (mean ±SD)	8°C	98.07%±0.41	97.7±1	97.6±1.02	97±1.07
	25°C	98.07%±0.41	97.9±1.02	97.5±1.16	97.56±1.16
	40°C	98.07%±0.41	97 ± 1	96.88±1.023	96.5 ±1.07

√ = No visible changes in color, texture, or homogeneity observed.

Source: Own elaboration

Kinetic analysis of active ingredient release

To investigate the kinetics of peptide release, the data calculated from the *in vitro* release study were applied to 3 kinetic models' equations (zero, first and Higuchi), which are shown in fig. 3 [15].

Stability check test

The stability of the lotions was checked at different temperatures of 8°C, 25°C and 40°C. At intervals of 7 days for 30 days, the peptide content and the physical appearance of the desired lotion (organoleptic characteristic) were investigated (table 5) [11].

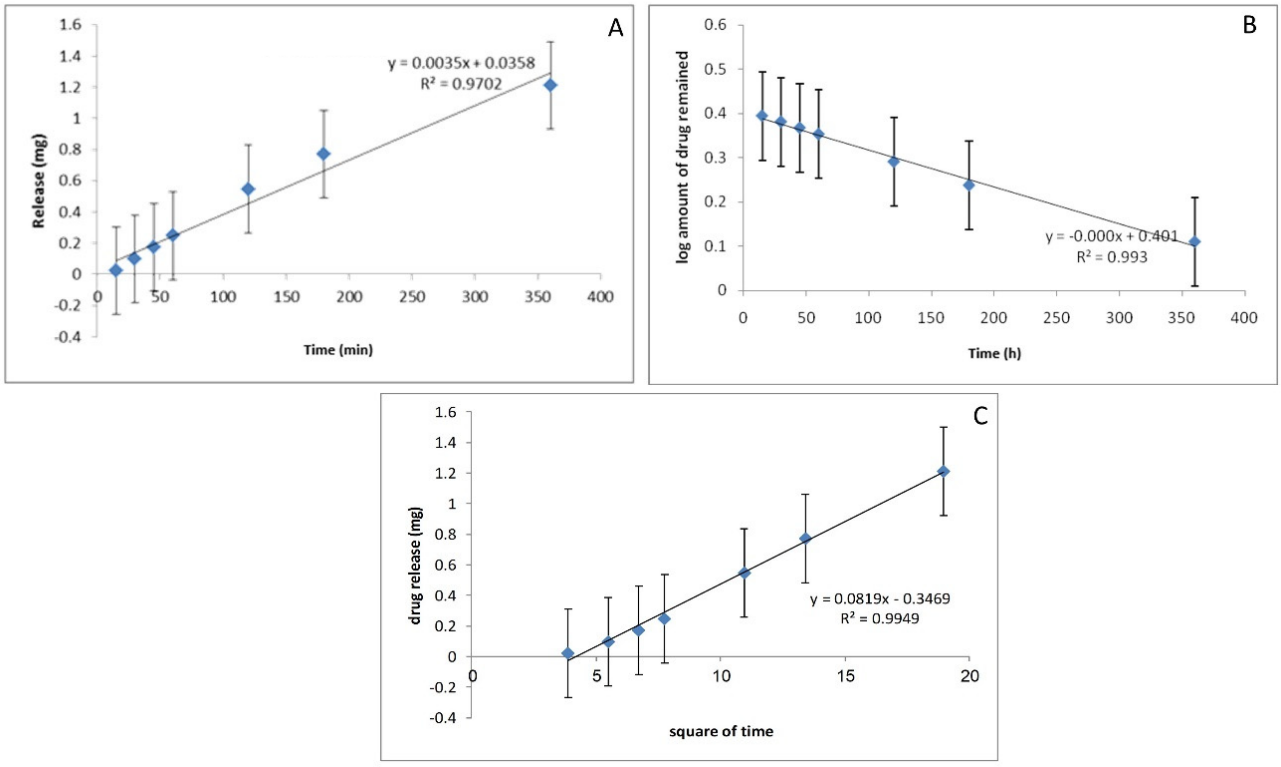

Fig. 3 Zero order (A), First order (B), Higuchi order (C) kinetic of release of lotion based on argireline peptide **Source:** Own elaboration

Clinical evaluation of the prepared lotion on volunteer women

Formulation No. 2 (F2) was used in the clinical study, both as the active formulation containing argireline and as the base for the placebo (without argireline). Formulation F2 was selected as the optimal formulation based on comprehensive physicochemical evaluations (e.g., stability, pH, viscosity, organoleptic properties), as detailed in the previous sections of the manuscript.

The study was conducted in accordance with the approved guidelines set by the Committee of registry of clinical trials at Isfahan University of Medical Sciences, Isfahan, Iran (Code: 393319). 20 women aged between 35 and 65 years who visited Dr. Siadat's clinic were enrolled to participate in a pilot study, serving as a precursor to a clinical trial. Through random selection, 10 participants received treatment with lotion based on argireline peptide, while another 10 women volunteered to join the control group, receiving a placebo lotion without argireline. Each participant was verbally briefed on the experimental procedure, and all provided signed consent forms to accompany the research. The study took place over three months in 2021.

Inclusion and exclusion criteria: The primary criterion for acceptance was having wrinkles and not participating in another similar study, at least in the last two months. The women with no history of skin sensitivity to cosmetics and health products, without skin, genetic, endocrine and hematological disorders, without both pregnancy and breastfeeding were included.

Irritancy Test

The lotions were applied to the dorsal left-hand surface of 10 women. Irritancy, erythema and edema of the relevant area were checked every hour for up to 48 hours and reported. This method was also performed on ten other women in the control group with lotion without argireline [16].

Wrinkle evaluation

The volunteers used the lotions every night on the forehead and between the eyebrows for one month. The changes before and after using the desired lotion and photos of the interesting areas for Visioface D1000 ck (Courage + Khazaka, Cologne, Germany) analysis were taken. To use this device, the head of each volunteer in the frontal part of the device was stabilized, on a certain part of the face, and then the device was set to take photos and analyze them. Finally, the device software calculated the volume, area and depth of facial skin wrinkles pixels [17]. The technical specifications of the device are provided below:

Illumination: 210 white light LEDs, Camera: Canon EOS 550D, 18 megapixels, sensor CMOS, autofocus. Objective: EF 20 mm/2.8, USM: focal.

Statistical analysis

In the statistical analysis, all data were analyzed using the SPSS version 16.0 package. After confirming the normality assumption of the data, the independent t-test was employed to compare variables between different groups (e.g., treatment vs. control), as it assesses whether the means of two independent samples differ significantly. The paired t-test was used to evaluate changes within the same group before and after the intervention. A significance level was set at a p-value ≤ 0.05 to determine statistical significance.

RESULTS

Identification of argireline peptide

The results of the SDS-PAGE188 identification of the argireline peptide are shown in fig. 4. Band No. 1 is related to the protein ladder, and No. 2 is related to the desired sample. The size of band No. 2 is less than 6000 Dalton, which corresponds to the size of the hexapeptide argireline.

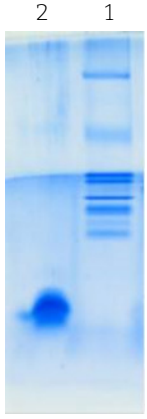

Fig. 4 Peptide identification in gel electrophoresis (1 - protein ladder, 2 - study sample)
Source: Own elaboration

Emulsion preparation

Formulation F2 was considered as the best formulation based on the analysis of experimental design software. This formulation was made from isopropyl myristate 10%, glycerin monostearate 3%, paraffin 1%, cetyl alcohol 1%, canola oil 1%, tween 20 1.5%, tween 80 1.5%, phenoxyethanol 1%, butylene glycol 2%, potassium sorbate 0.5 %, zinc sulphate 1%, argireline 5% and purified water up to 100%. Both the oil and aqueous phases were separately heated up to 80°C. Both phases were mixed with continuous stirring. Control tests were subsequently performed after cooling the emulsion to 25°C.

Quality control tests of the selected formulation

Formulation No. 2 not only had a uniform white appearance but also showed the homogeneity of micelles and internal phases under the microscope. Also, the pH of the formulation was 5.85 ± 0.18 , which is within the physiological skin pH range (4.5-6.5) and is therefore suitable for topical application. The formulation was also centrifuged after adding argireline at room temperature, after which it maintained its stability, and no breakage or separation of phases was observed. This result indicates the resistance of the product against mechanical forces. Another control test that evaluated the endurancevof the formulation in terms of appearance changes and phase separation is the freeze-thaw test, which did not show any changes in these characteristics during consecutive freezing and thawing cycles.

The *in vitro* release profile of lotion based on argireline peptide from prepared formulation for 6 hours

The diffusion mechanism was non-Fickian in accordance with the release advocacy which refers to the combination of both diffusion and erosion-controlled release rate. Based on the correlation coefficient, Higuchi kinetic (R2=0.9949) is leading, which applies to drug release by a diffusion model.

Stability studies

The lotion maintained its quality and content in different seasons of the year and in different geographic and weather conditions, because no changes in physical appearance, organoleptic characteristics and argireline content were observed at different temperatures during 28 days (table 5).

Skin irritation test

The skin irritation evaluation test on healthy skin of volunteers showed that the formulation was not a irritant, as it did not cause any adverse reactions such as erythema or edema.

The rheological study

Based on this test, the viscosity of formulation No. 2 was estimated 480 rpm.

Anti-wrinkle activity evaluation

The mean changes in the depth, volume, and area of wrinkles at the beginning and end of the trial are presented in table 6. The data includes measurements taken before and after the treatment, along with standard deviations (SD) for each group. P-values are provided to indicate the statistical significance of the differences observed between the groups and within each group before and after the treatment. For the intervention group (n=10), a paired t-test (p-value***) showed a statistically significant reduction in wrinkle volume (p=0.00), area (p=0.00), and depth (p=0.00) after the 30-day treatment with the argireline lotion. In contrast, the control group (n=10) did not show statistically significant changes in wrinkle volume (p=0.48), area (p=0.74), or depth (p=0.75). An independent t-test (p-value**) comparing the intervention and control groups after the treatment showed no statistically significant differences in volume (P=0.53), but area(P=0.14) and depth (P=0.03) were statistically significant.

Table 6 Comparison of mean results of volumes, area and depth of wrinkles in the intervention and control groups

		Before intervention (Mean ± SD*)	After intervention (Mean ± SD*)	P-value***
Volume (px^3)	Intervention (n=10) Control (n=10) P-value**	160.26±56.15 151.96±52.71 0.53	135.11±40.42 145.82±59.47 0.14	0.00 0.48
Area (px^2)	Intervention (n=10) Control (n=10) P-value**	18.80±1.49 19.40±1.81 0.181	17.33±1.49 19.33±1.84 0.00	0.00 0.74
Depth (px)	Intervention (n=10) Control (n=10) P-value**	17.47±6.96 16.90±6.49 0.75	12.63±6.67 16.77±6.98 0.03	0.00 0.75

* Standard deviation; ** Independent t-test; *** Paired t-test; Px^3 volume of wrinkles at pixel; Px^2 area of wrinkles at pixel; Px depth of wrinkles at pixel.

Source: Own elaboration

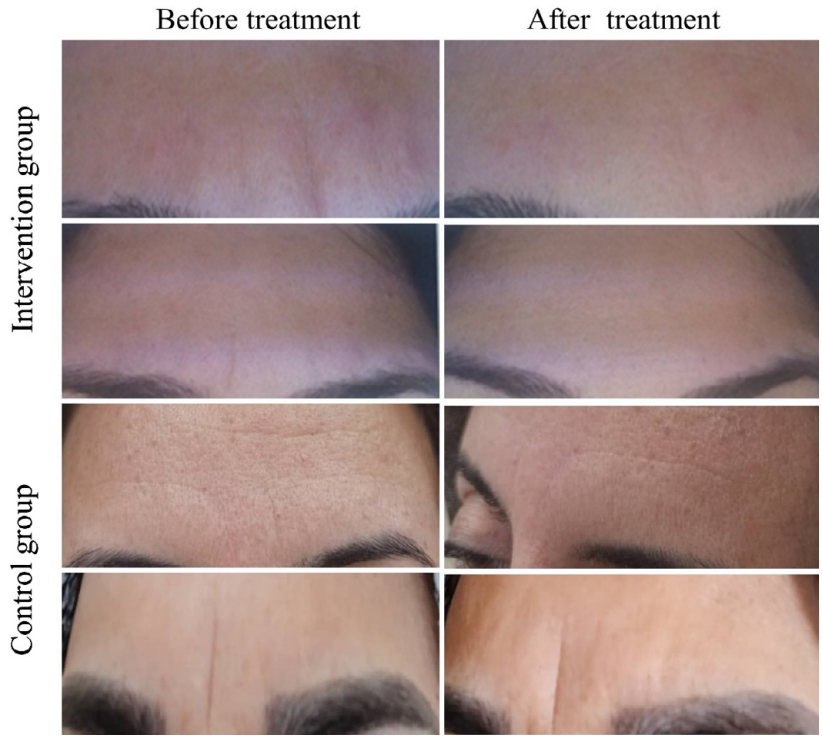

Fig. 5 Changes seen in two participants in intervention group and two participants in control group, before and after treatment withlLotion based on argireline peptide, and placebo lotion, respectively. **Source:** Own elaboration

Fig. 5 demonstrates the visible improvement in wrinkle appearance observed in two patients from the intervention group (female, aged 50, and 42 years), whereas changes in two patients from the control group (female, aged 52, and 62 years).

DISCUSSION

Nowadays, the aging demographic, with a notable increase in the average age surpassing 60 years, has spurred heightened demand among individuals seeking solutions for the mitigation of wrinkles and other age-related changes [18]. Addressing these manifestations holds promise for bolstering self-assurance and enhancing the overall quality of life for affected individuals [19, 20]. While botulinum toxin treatment has long been regarded as a leading approach for achieving favorable outcomes in the management and prevention of facial wrinkles, its invasive nature and associated complications have prompted a shift towards non-invasive skin rejuvenation techniques in recent years. Consequently, there has been growing interest in exploring alternative methods, with particular focus on investigating the efficacy of argireline peptide as a safer alternative to botulinum toxin [7, 21]. Throughout this research endeavor, a novel product featuring a distinctive formulation incorporating argireline peptide was developed. Initial investigations were conducted, followed by *in vivo* and *ex vivo* assessments, aimed at further elucidating its effectiveness and safety profile for potential therapeutic applications. From the array of prepared products, one was chosen due to its commendable physicochemical properties. The lotion formulation identified as F2 demonstrated numerous favorable properties and advantages, supported by the meticulous quality control examinations and assessments. The uniform white texture and homogeneous micelles observed in Formula F2 signified stability and consistency, essential for consumer acceptance and shelf-life stability [22], while its pH decreased within the skin's physiological range underscores the skin compatibility, thereby reducing the risk of irritation [23, 24]. Moreover, the formulation exhibited stability post-centrifugation and resilience to freeze-thaw cycles, highlighting its durability against both mechanical stress and temperature fluctuations [25, 26]. The *in vitro* release profile of argireline from formulation F2 revealed a non-Fickian release mechanism, with the prevalence of the Higuchi kinetic model. This controlled release profile was advantageous for ensuring consistent efficacy and prolonged activity of the active ingredient, thus augmenting the anti-wrinkle potency of the lotion [27]. The stability studies, spanning 28 days, demonstrated that the lotion retained its quality and composition across various environmental conditions and temperatures. This robust stability profile was crucial for ensuring product efficacy and durability, particularly in diverse climatic regions like Iran.

Every ingredient incorporated into the formulation of lotions fulfills a distinct role, collectively enhancing its efficacy and sensory attributes. By skillfully amalgamating these constituents in precise proportions, manufacturers of lotions can develop products that deliver optimal hydration, nourishment, and protective benefits to the skin, thereby addressing a multitude of skin types and concerns [28].

In formulation No. 2 of the lotion, meticulous proportions were assigned to each component to fulfill specific roles. Isopropyl myristate, constituting 10% of the formulation, was chosen to enhance skin penetration, softness and moisture. Moreover, it facilitated uniform application by improving spreadability [29, 30]. Glycerin monostearate at 3% served as an emulsifier, thickener, and stabilizer [31, 32], while 1% cetyl alcohol contributed to softening and thickening effects [33]. Canola oil, at 2%, was selected for its moisturizing properties, rich in vitamins, proteins, lipids, and antioxidants, fortifying the skin barrier and addressing various skin concerns [34, 35]. Tween 20 and tween 80, both at 1.5%, acted as emulsifiers [36], whereas phenoxyethanol and potassium sorbate at the same concentrations served as safe preservatives [37]. Water, the primary solvent, was supplemented with 2% butylene glycol [38]. Additionally, 1% paraffin enhanced texture and consistency and formed a protective barrier against moisture loss [39, 40]. Zinc sulfate, present at 1%, functioned as a softening and antioxidant agent, alleviating inflammation, and promoting skin regeneration, supported by numerous studies highlighting its benefits [41-44]. Lastly, argireline, a natural peptide component, predominated in this lotion as the primary ingredient, offering a bio-safe cosmetic solution for diminishing facial lines and wrinkles [45]. Despite the limited literature on the structural-biological activity of argireline, its established impact on skin condition, particularly through muscle tone modulation, remains evident [46, 47]. Argireline, derived from the N-terminal amino acid sequence (residues 1-12) of the 25 kDa synaptosome-associated protein (SNAP 25), functions as a neurotransmitter inhibitor by hindering acetylcholine release. Through competitive binding with SNAP-25 within the SNARE complex, necessary for acetylcholine-containing vesicle release, argireline disrupts this complex, halting neurotransmitter release and Ca^{2+}-induced exocytosis. Consequently, muscle contraction weakens, promoting muscle relaxation. With repeated application, this process effectively prevents the formation of facial lines and wrinkles [48-50]. In the investigation, following a four-week regimen of once-daily application of the formulated lotion containing 5% argireline on forehead wrinkles, no adverse effects were reported among the female participants. The efficacy of the anti-wrinkle treatment was prominently observed, with significant reductions noted in wrinkle parameters, including volume, area, and depth, in the group utilizing the argireline-infused lotion. These findings collectively support the documented anti-wrinkle properties of argireline, aligning with previous reports on its effectiveness. Blanes-Mira et al. study revealed that ten female participants using an emulsion (O/W) containing 10% argireline twice daily over a span of 30 days, resulting in a noteworthy 30% reduction in wrinkle depth attributable to the applied product [49]. Research conducted in Spain in 2006 revealed that the consistent application of a cream containing 5% argireline twice daily led to a substantial 27% reduction in wrinkles after thirty days [51]. Furthermore, another study found that a 2% argireline solution yielded improvements in wrinkle reduction, as well as diminished roughness and depth of wrinkles, within one week of use [45]. Ruiz et al. found that among a group of 20 participants using an emulsion containing argireline, wrinkles experienced notable reductions, ranging from 41.83% to 78.25% [52]. Additionally, Yuan Wang et al. (2013) study revealed that using an O/W emulsion containing 10% argireline twice daily for one month led to a notable decrease in eye wrinkles among 45 Chinese individuals aged 25 to 60 [53]. To the best of authors knowledge, this study was the first to utilize accessible and cost-effective materials for a argireline lotion formulation specifically for Iranian women. Furthermore, a comprehensive review of the literature indicates a lack of more recent studies on argireline's anti-wrinkle effects.

Although this study demonstrated a significant anti-wrinkle effect of the formulated lotion, it faced some limitations. Primarily, the study had a small participant pool as it was hard to recruit additional volunteers, potentially due to individuals' reluctance to participate in skin lotion research, possibly stemming from concerns about their skin's aesthetics. Furthermore, some participants in the control group may have resigned due to perceived lack of efficacy. Additionally, the ideal method for assessing skin changes involves before-and-after biopsies for histological comparison, which serves as the gold standard for evaluating wrinkle improvement. Unfortunately, none of the volunteers were willing to undergo biopsies due to concerns about invasiveness and potential scarring post-operation.

CONCLUSIONS

According to the results obtained, the lotion, which was prepared according to the correct concentration of every ingredient component and 5% argireline, was well-tolerated and did not show any allergic reaction on skin of human subjects. Topical application of this lab-prepared lotion for thirty days consecutively, once a day, was found to be effective in decreasing the depth of wrinkles on the forehead of Iranian females.

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CONFLICT OF INTEREST

Authors declere no conflict of interest.

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