A RANDOMIZED, SPLIT-FACE, PLACEBO-CONTROLLED TRIAL ON THE EFFECTIVENESS OF 0.1% ADENOSINE COMBINED WITH 5% MAGNESIUM ASCORBYL PHOSPHATE CREAM FOR PERIORBITAL WRINKLES

Puthyro THAO[1] and Thamthiwat NARARATWANCHAI [1]
1 School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University
Bangkok, Thailand; 6752001009@lamduan.mfu.ac.th; thamthiwat.nar@mfu.ac.th

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ABSTRACT
This study examined the short-term effects and tolerability of a topical cream containing 0.1% adenosine combined with 5% magnesium ascorbyl phosphate (MAP) for the treatment of periorbital wrinkles compared with placebo. Fourteen healthy participants aged 30–50 years with visible periorbital wrinkles completed a randomized, double-blind, split-face, placebo-controlled trial. The active cream was applied nightly to one periorbital side and the placebo cream to the contralateral side. The primary endpoint was the between-side difference in change from baseline in Visioscan wrinkle score at week 12 and the secondary endpoint was the between-side difference in Cutometer R2 skin elasticity at week 12.The active-treatment side showed greater improvement than the placebo side in Visioscan wrinkle scores and Cutometer R2 measurements during the study period. At week 12, the mean between-side difference in change from baseline was 19.04 point for Visioscan wrinkle score and 0.0568 for Cutometer R2 ($p = 0.009$ and $p < 0.001$, respectively). Only one participant reported transient mild dryness, and no serious adverse events were observed. In conclusion, the fixed combination of 0.1% adenosine and 5% magnesium ascorbyl phosphate was associated with short-term improvements in objective skin measurements and was generally well tolerated in this small sample. These findings support further investigation of the formulation but do not establish long-term effectiveness or ingredient-specific effects.
Keywords: Periorbital Wrinkles, Adenosine, Magnesium Ascorbyl Phosphate, Split-Face Trial, Skin Elasticity

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INTRODUCTION.

Our skin naturally ages throughout the course of our lives. The aging process is due to intrinsic and extrinsic factors. Natural or intrinsic aging is a slow process, determined by our genes. It causes thinning of the skin, decreased production of collagen and loss of firmness. While extrinsic factors such as sun exposure, smoking and pollution accelerate the aging process by causing oxidative stress and inflammation(Zhang & Duan, 2018). Together, these processes result in common signs of facial aging including wrinkles, uneven skin tone and dryness, remarkably in areas frequently exposed to stressful environmental factors.

As concerns regarding the manifestation of aging intensify, there has been a corresponding increase in public interest in skincare and anti-aging products. This encouraged the researchers to explore and discover several cosmetic treatments including topical products that aim at improving the firmness of the skin, reducing the wrinkles appearances, and decelerating the aging process. Numerous options are available, however, non-invasive treatments are among the popular choices due to their favorable safety profile, convenience and lower associated risks.

Crow's feet, also referred to as periorbital wrinkles, are one of the most noticeable signs of aging on the face. These wrinkle lines are a big esthetic concern for many, making them look older, stressed, or tired. Wrinkles are mainly caused by the natural aging process, prolonged sun exposure, repetitive muscle contractions and degradation of collagen and elastin which is the combination of both intrinsic and extrinsic factors(Rittie & Fisher, 2015).

Age is associated with a significant increase in the incidence and severity of skin wrinkling. For example, a research in Australia reported 72% of males and 47% of women under 30 years old had moderate to severe photodamage including wrinkles; and severity increased with age(Manriquez et al., 2014). In a major population-based study in China, the prevalence of facial wrinkling was 33% in young adults, 87.8% in middle-aged people and 100% in the elderly, with both the prevalence and severity of wrinkles rising proportionally with age(Wang et al., 2009). The findings suggest that wrinkling is prevalent and progressing in Western and Asian populations and that proper treatments are needed.

There are several options to treat periorbital wrinkles to date. These include topical agents such as retinoids, peptides and antioxidants that stimulate collagen synthesis and reduce damage from free radicals. But those treatments can cause skin irritation and may not be as effective on the deep wrinkles. Botulinum toxin injections, dermal fillers, and laser resurfacing are more invasive options that can give better results especially for deeper wrinkles but tend to be more expensive and have more risk of unwanted effects. Thus, the non-invasive options to manage wrinkles have been in increasing demand as they are safe, economical and effective. Recent studies have shown the important functions of adenosine and vitamin C in the skin aging process. Adenosine is a naturally occurring nucleoside inside a human body. It is involved in a range of physiological activities such as energy metabolism, nucleic acid synthesis, cell signaling and neurotransmission. It is adenine attached to a ribose sugar and is one of the four major nucleotides of RNA. Deoxyadenosine is the DNA form of deoxy. Adenosine exerts its actions through activation of the adenosine receptors (ARs) or P1 receptors, which are G-protein coupled receptors. These receptors are classified into four subtypes A1, A2A, A2B and A3, all of which are activated by extracellular adenosine. In the context of skin aging, adenosine enhances skin structure by activating A2A receptors which are located on dermal fibroblasts. This activation led to the synthesis of collagen, especially type I and type III collagen, which is critical in skin firmness and elasticity. These mechanisms support dermal remodeling and may contribute to the reduction of the wrinkle's appearance(Shaikh et al., 2016).

Adenosine and magnesium ascorbyl phosphate (MAP) may work in synergy to improve wrinkles. Adenosine plays a role in collagen synthesis. MAP is an antioxidant which can also help mature and stabilize collagen. Together, these two chemicals may be more effective than each alone in repairing skin damage, enhancing skin firmness and minimizing fine lines and wrinkles than each agent alone. This combination offers a possibly safe and non-invasive treatment for wrinkles,

particularly in sensitive locations like the peri orbital area. Adenosine and vitamin C have both been extensively studied for their own anti-aging benefits, but little is known of their combined effects especially with combination of magnesium ascorbyl phosphate in the treatment of periorbital wrinkles. Thus, the purpose of the study was to evaluate the efficacy and safety of topical cream containing 0.1% adenosine and 5% magnesium ascorbyl phosphate in the improvement of periorbital wrinkles.

LITERATURE REVIEWS

Adenosine

Adenosine is a naturally occurring purine nucleoside consisting of an adenine base linked to a ribose sugar moiety via a β-N9-glycosidic bond, working as an endogenous autacoid that regulates many physiological processes in the human system.

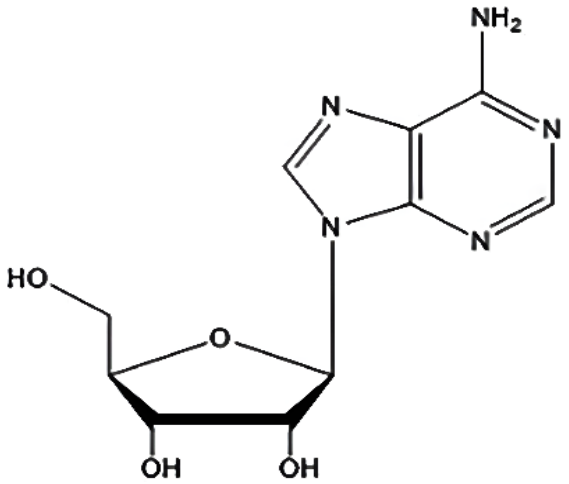


Figure 1 Adenosine structure

Adenosine synthesis occurs through both extracellular and intracellular mechanisms. The extracellular production of adenosine results from the breakdown of adenine nucleotides, whereas intracellular formation follows two distinct biochemical pathways. In normal physiological states, adenosine is predominantly produced intracellularly through the dephosphorylation of adenosine 5′-monophosphate (AMP) catalyzed by cytosolic 5′-nucleotidase, or alternatively through the enzymatic hydrolysis of S-adenosyl homocysteine (SAH) by SAH hydrolase .Adenosine, in contrast to classical neurotransmitters, is considered as metabolite that can be generated by any cell and subsequently interact with specific adenosine receptors (ARs), making it an ubiquitous signaling molecule with various biological activities. It exerts its biological effects through interaction with four distinct G-protein-coupled adenosine receptor subtypes: A_1, A_2A, A_2B, and A_3, each characterized by unique tissue distribution patterns, pharmacological profiles, and coupling to specific secondary messenger systems (Marucci et al., 2022).

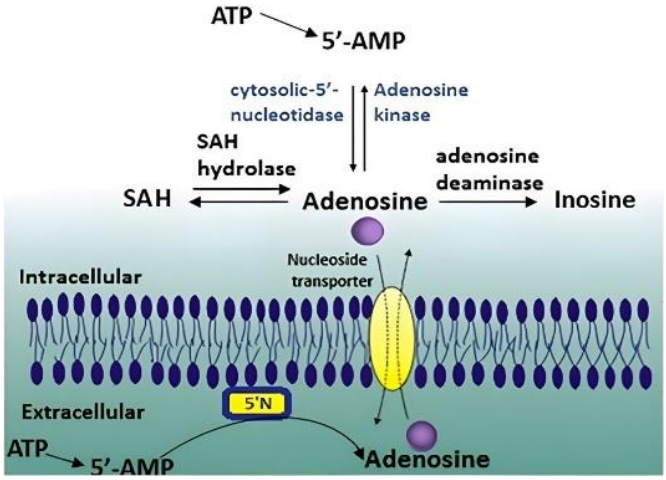


Figure 2 Adenosine production and metabolism. 5′-Nucleotidase (5′N)

The main adenosine receptors found in the skin are A2A and A1A receptors. A2A receptors are located on the cell membranes of fibroblasts, while A1 receptors are located on the cell membranes of adipocytes. The initial scientific report demonstrating the presence of A2A adenosine receptors on human dermal fibroblasts was published in 1997 (Montesinos et al., 1997). Subsequent research by the same investigators showed that activation of these A2A receptors with the selective agonist CGS-21680 modulates collagen synthesis in human dermal fibroblasts (Marucci et al., 2022).The collagen-promoting effects of adenosine are mediated by the activation of the A2A receptor, which initiates intricate intracellular signaling pathways characterized by an increase in cyclic adenosine monophosphate (cAMP) levels and the subsequent activation of downstream molecules. This signaling cascade facilitates enhanced production of both type I collagen (Col1) and type III collagen (Col3), each regulated through different sensitivity levels and specific signaling mechanisms (Perez-Aso et al., 2014). One study believes that adenosine promotes the production of collagen type I mainly by activating the adenosine A2A receptor (A2AAR) on skin fibroblasts. When adenosine binds to A2AAR, it triggers a chain of signals starting with an increase in the molecule cyclic AMP (cAMP) inside the cell. This activates other proteins like PKA and ERK1/2, which then increase the activity of genes that produce collagen type I (Marucci et al., 2022). In addition, activating A2AAR lowers the level of a protein called Fli1, which normally slows down collagen production. With less Fli1 present, another protein called CTGF increases, further encouraging collagen type I production and helping rebuild the skin's structure (Chan et al., 2013).Other recent studies have demonstrated that the synthesis of collagen type III via the adenosine A_2A receptor (A2AAR) occurs through activation of β-catenin signaling pathways. Upon stimulation of A2AAR, both the canonical and non-canonical Wnt/β-catenin cascades are triggered. Specifically, this activation leads to increased intracellular levels of β-catenin, including its dephosphorylated form associated with the canonical pathway, and the phosphorylated form linked to the non-canonical pathway. These activated β-catenin proteins then translocate to the nucleus where they stimulate the transcription of genes encoding collagen type III, thereby promoting its production. Furthermore, this mechanism involves interactions with additional signaling pathways, including the cyclic AMP-dependent protein kinase A (cAMP-PKA) and the p38 mitogen-activated protein kinase (MAPK) pathways. Interestingly, a lower intensity of A2A receptor activation tends to promote the synthesis of collagen type I, while higher levels of stimulation predominantly drive collagen type III production through the activation of β-catenin and p38 MAPK pathways(Shaikh et al., 2016).

Beyond collagen regulation, adenosine also plays a pivotal role in hypodermis fat tissue homeostasis through its interaction with A1 adenosine receptors (A1ARs), which are abundantly

expressed in adipocytes. When activated, these receptors suppress lipolysis by lowering intracellular cyclic AMP (cAMP) levels and inhibiting the activity of lipolytic enzymes such as hormone-sensitive lipase and triglyceride lipase. This mechanism favors the storage of triglycerides, leading to adipocyte enlargement and an associated rise in leptin secretion. Over time, the reduced capacity for lipid breakdown and the progressive accumulation of fat within subcutaneous compartments contribute to structural remodeling of adipose tissue. Such alterations are closely linked to age-related skin changes, including diminished elasticity, altered facial contours, and the redistribution of volume across different fat pads (Marucci et al., 2022).

Vitamin C

In humans, our skin contains very high amounts of Vitamin C. It is transported from vessels to cells and is stored in the intracellular compartment. The absorption of vitamin C from the bloodstream and its distribution throughout the skin layers relies on sodium-dependent vitamin C transporters (SVCTs), which are integral to vitamin C transport in multiple tissues. Remarkably, epidermal keratinocytes express both SVCT1 and SVCT2 while the dermis and sub dermis express only SVCT2 (shown in Figure 3), enabling efficient uptake and regulation of vitamin C availability within the skin (Steiling et al., 2007). Its concentration is 6-64mg/100g wet weight in the epidermis and 3-13mg/100g wet weight in the dermis (Rhie et al., 2001). In situations where systemic vitamin C levels are insufficient, topical formulations can supplement epidermal vitamin C delivery, though this process depends heavily on product formulation variables (Nusgens et al., 2001). The hydrophilic nature and ionic charge of vitamin C create resistance against penetration through the hydrophobic stratum corneum barrier. Successful dermal penetration occurs when formulations maintain a pH below 4, converting vitamin C to its non-ionized ascorbic acid form (Pinnell, 2003).

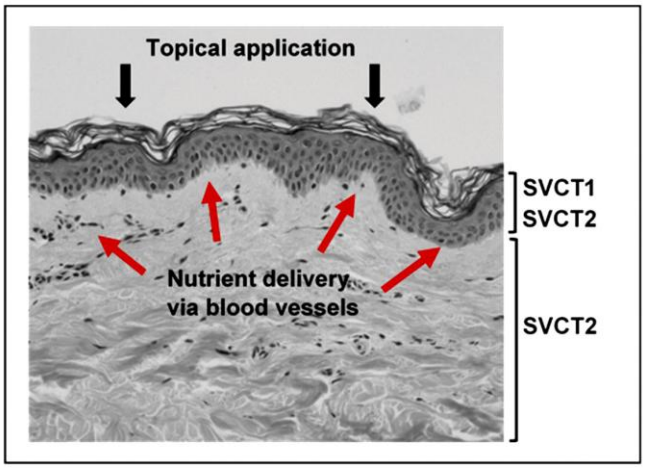


Figure 3 Location of SVCT1 and SVCT 2 in the skin (Rhie et al., 2001).

Vitamin C is essential for collagen production as it's utilized as a co factor for enzymes called prolyl and lysyl hydroxylases, which stabilize the collagen structure (Hinek et al., 2014). It also promotes collagen gene expression by stimulating mRNA production in skin cells. Studies with fibroblast cells in the lab show that without enough vitamin C, collagen synthesis decreases, and less crosslinking occurs, highlighting its role in maintaining collagen strength and skin health (Phillips et al., 1994).

Vitamin C also serves as an essential antioxidant within the skin, primarily functioning to neutralize free radicals generated by environmental exposures such as ultraviolet radiation and pollution. This scavenging activity protects cellular components from oxidative stress, which otherwise leads to degradation of proteins like collagen and elastin that maintain skin structure. Additionally, vitamin C's antioxidant properties help reduce inflammation and promote skin repair

mechanisms, contributing to improved skin texture, elasticity, and overall dermal health. As a result, vitamin C is widely recognized for its pivotal role in combatting skin aging and maintaining skin vitality (Pullar et al., 2017).

Beyond its antioxidant and collagen synthesis functions, vitamin C also plays an important role in skin pigmentation control. Studies show that vitamin C inhibits melanogenesis through multiple pathways, particularly by interfering with tyrosinase activity, the rate-limiting enzyme in melanin synthesis. This inhibition occurs through both direct enzyme suppression and indirect effects via vitamin C's antioxidant capacity. Furthermore, vitamin C reduces the oxidation of dopaquinone, a direct precursor in the melanin synthesis pathway. These anti-melanogenic properties contribute to vitamin C's effectiveness in reducing hyperpigmentation and promoting a more even skin complexion(Wang et al., 2018).

Adenosine and Vitamin C in periorbital wrinkle

The effectiveness of adenosine and vitamin C in reducing periorbital wrinkles is supported by their complementary mechanisms of action. Adenosine works by stimulating collagen production through activation of specific receptors on dermal fibroblasts, which supports the formation and remodeling of collagen essential for skin elasticity and firmness (Perez-Aso et al., 2014; Shaikh et al., 2016). Vitamin C acts as a strong antioxidant, defending skin cells from oxidative stress and promoting collagen stability by aiding enzymes responsible for collagen maturation, namely prolyl and lysyl hydroxylases. Moreover, vitamin C suppresses melanin production, helping to even out the skin tone and reduce pigmentation issue (Pullar et al., 2017).Adenosine is generally incorporated in topical formulations at concentrations up to 1% to ensure both efficacy and safety, as supported by its regulatory safety assessment in cosmetics, while Magnesium Ascorbyl Phosphate has been reported to be effective at the concentration 1 to 10% (Leny & Harahap, 2016).

But the combination of adenosine and vitamin C presents notable formulation challenges, particularly regarding pH compatibility requirements for optimal stability and activity of both active ingredients. While L-ascorbic acid requires an acidic environment (pH <3.5) for stability and effective skin penetration, adenosine typically functions well at neutral pH ranges, creating a formulation dilemma that necessitates the use of magnesium ascorbyl phosphate (MAP) as the vitamin C derivative of choice. MAP offers the advantage of maintaining stability at pH 7, which aligns better with adenosine's stability at pH 4.5-7.5 (Information, 2021). Also, it can reduce potential skin irritation compared to the low pH conditions required for ascorbic acid. This pH compatibility makes MAP an essential compromise in adenosine-vitamin C combination formulations, allowing both active ingredients to remain stable and effective.

RESEARCH METHODOLOGY

Study design and participants

This randomized, double-blind, split-face, placebo-controlled clinical trial evaluated the effectiveness and safety of a topical cream containing 0.1% adenosine and 5% magnesium ascorbyl phosphate (MAP) for reducing periorbital wrinkles. The split-face design allowed each participant to serve as their own control, thereby reducing interparticipant variability related to age, skin characteristics, and environmental exposure. 16 participants were enrolled and the study was conducted at Mae Fah Luang University Hospital.

Sample size calculation

The sample size for the present study is determined based on data from a comparable double-blind, randomized, controlled split-face clinical trial titled "The efficacy of 3% Emblica officinalis extract cream on the reduction of periorbital wrinkles "(Aye, 2018).

The findings from the research are as follows:

- Results of Cutometer for Skin Elasticity at crow’s feet area showed a mean increase from 0.83 at week 0 to 0.92 at the 12-week mark.

- In the controlled group, the results indicated a mean of 0.83 at week 0, which increased a little bit to 0.87 by week 12.
- Changes from the baseline of the Emblica officinalis extract group (μ_1) =0.92 – 0.83 = 0.09
- Changes from the baseline of the placebo-controlled group (μ_2) =0.87 – 0.83 = 0.04
- Mean difference (μd) = μ_1 - μ_2 = 0.09 – 0.04 = 0.05
- In the Emblica officinalis extract group, standard deviation (S_1) = 0.06
- In the control group, standard deviation (S_2) = 0.07
- Total number of participants for the Emblica officinalis extract group and the control group (n_1=n_2) = 20

α = 0.05 (two-tailed); $Z_{0.025}$ = 1.96
β = 0.20; $Z_{0.20}$ = 0.84

$$S_p^2 = \frac{[S_1^2(n_1 1 - 1) + S_2^2(n_2 - 1)]}{n_1 + n_2 - 2}$$
$$= \frac{[0.06^2(20 - 1) + (0.07)^2(20 - 1)]}{20 + 20 - 2}$$
$$= 0.00425$$
$$n = \frac{(Z_{\alpha/2} + Z_\beta)^2 S_p^2}{\mu_d^2}$$
$$= \frac{(1.96 + 0.84)^2 0.00425}{0.05^2}$$
$$= 13.32 \approx 13$$

In which:
n = sample size
S_p^2 = pooled variance
μd = mean change between two groups
To account for the dropout rate, 20% of the calculated sample size was added. Therefore, the final sample size (n) is 16.

Eligibility criteria

Inclusion criteria were healthy male and female volunteers aged 30 to 50 years with periorbital wrinkles graded 2 to 4 on the Rao-Goldman five-point visual scale, regular use of a sunscreen with a minimum sun protection factor (SPF) of 50, and willingness to comply with all study requirements and attend every scheduled follow-up visit.

Exclusion criteria included known allergy or hypersensitivity to adenosine, MAP, or any other component of the study creams; active skin infection, dermatitis, eczema, or any other dermatological condition of the periorbital area; current smoking or a history of smoking, excessive alcohol consumption, or substance abuse within the previous 6 months; thyroid disease or photosensitivity; occupation or hobbies involving strong sun exposure; botulinum toxin in the forehead or periorbital area within 6 months, periorbital filler within 12 months, mesotherapy within 3 months, facial laser rejuvenation within 6 months, radiofrequency within 12 months, or intense pulsed light or microdermabrasion within 3 months and a positive patch-test reaction (+, ++, or +++) at 48 hours or later.

After enrollment, participants were withdrawn from the study if they chose to leave for any reason, or if they developed a severe allergic reaction or skin irritation after applying the creams. They were also withdrawn if they received any other facial treatment during the trial, such as botulinum toxin, filler, laser, radiofrequency, mesotherapy, or other topical products around the eyes, or if they failed to cooperate or attend follow-up visits. Enrollment in another study that could interfere with the results of the present trial was also a reason for withdrawal.

Consent and screening

Ethical approval was obtained from by the Mae Fah Luang University Ethics Committee on Human Research in compliance with international guidelines such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice (ICH - GCP) with approval 106/2026 and the protocol no. 26007-20. Participants who satisfied the preliminary eligibility criteria received an explanation of the study objectives, procedures, expected benefits, and potential adverse effects. Written informed consent was obtained before any study-specific procedures were performed. Eligible participants then underwent a 48-hour patch test, with reactions interpreted according to the International Contact Dermatitis Research Group criteria, to screen for hypersensitivity to the study products.

Ethical safeguard

Digital photographs and study data were stored on encrypted, password-protected computers, with any printed records kept in locked cabinets within the research unit, and access to identifiable data was limited to the principal investigator and co-investigators, while anonymized data were used for analysis, presentation, and publication; photographs were used exclusively for this research, no identifiable images were published or presented, additional written consent was to be obtained should any photograph be required for publication or educational purposes, and all research data, including photographs, will be retained for five years after the study, after which identifiable data will be permanently deleted or securely destroyed.

Active and Placebo cream

The active cream contained 0.1% adenosine and 5.0% magnesium ascorbyl phosphate (MAP) in a cream-based vehicle comprising distilled water, glycerin, propylene glycol, cetearyl alcohol, sorbitan stearate, glyceryl stearate, mineral oil, shea butter oil, ceteareth-20, polysorbate 20, and phenoxyethanol. The placebo cream contained the same ingredients but without adenosine or MAP. A color additive iron oxides (CI77492) was incorporated into the placebo cream to match the yellow color of the active cream produced by MAP. Both creams were prepared as topical cream formulations with a pH of 7.0 and were placed in identical opaque black containers labeled only as Cream A and Cream B. The formulations were matched in color, texture, consistency, and fragrance free. The active and placebo creams were prepared, labeled, and packaged by the manufacturer. The manufacturer then provided the products together with a sealed document identifying the active and placebo formulations, and both the products and the sealed identity document were delivered to an independent physician, who retained the document until completion of the study.

Randomization and blinding

An independent physician who was not involved in the study generated a random allocation sequence using the computer-based random sequence generator at Randomizer.org. The sequence contained the numbers 1 to16 arranged in a computer-generated random order rather than in sequential numerical order. As participants enrolled, each participant was assigned the next available number in the pre-generated random sequence. Participants assigned an odd-numbered sequence value were allocated to Group A, whereas those assigned an even-numbered sequence value were allocated to Group B, resulting in eight participants in each group. In Group A, Cream A was applied to the right crow’s-feet area and Cream B to the left, whereas in Group B, Cream B was applied to the right crow’s-feet area and Cream A to the left. The independent physician retained the randomization sequence and the identities of Cream A and Cream B in sealed, opaque envelopes. The principal investigator and participants remained blinded to treatment allocation throughout the study period.

Intervention procedure and Outcome assessment

Participants were instructed to apply the assigned creams nightly for eight weeks, with the active cream on the crow's feet area of one side and the placebo on the contralateral side, by first cleansing the face gently with a mild cleanser and patting it dry with a clean towel or tissue, then dispensing pea size amount of cream for each side onto the corresponding fingertip, dabbing it gently over the lateral periorbital region at the crow's feet area while avoiding the eyelid and the lash line,

massaging it in circular motions and sweeping it outward for even absorption, and finally tapping the area softly until the cream was fully absorbed.

Assessments were performed at baseline, weeks 4, week 8 and week 12 using the Cutometer® MPA and the Visioscan® VC 98, with all measurements obtained at a standardized crow's feet site 1.5 cm lateral to the lateral canthus of each eye in every participant. The primary endpoint was the between-side difference in the change from baseline in the Visioscan® wrinkle score at week 12. Secondary endpoints included the between-side difference in the change from baseline in Cutometer skin-elasticity measurements at week 12. The treatment-related adverse reactions were recorded by the principal investigator at each follow-up visit. Adverse reactions, including pruritus, erythema, burning sensation, and dryness, were graded using a four-point severity scale: absent, mild, moderate, or severe.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20. Continuous variables are presented as mean ± standard deviation. Analyses were conducted separately for the Cutometer R2 elasticity measurement and the Visioscan wrinkle score. For each participant and follow-up visit, change from baseline was calculated separately for the active-treatment and placebo sides. For the Cutometer R2 measurement, higher values indicate greater skin elasticity; therefore, change from baseline was calculated as the follow-up value minus the baseline value. For the Visioscan wrinkle score, lower values indicate fewer wrinkles; therefore, change from baseline was calculated as the baseline value minus the follow-up value. At each follow-up visit, the treatment effect was defined as the between-side difference in change from baseline, calculated as active-treatment-side change minus placebo-side change. A positive effect estimate therefore indicated greater improvement on the active-treatment side than on the placebo side for both outcomes. The effect estimate at each follow-up visit was summarized using its mean and 95% confidence interval. Visit-specific effect estimates were tested against zero using two-sided one-sample t-tests, with a p-value of less than 0.05 considered statistically significant. To assess whether the between-side effect estimate differed among weeks 4, 8, and 12, a one-factor repeated-measures analysis of variance was performed using the participant-level effect estimates at the three follow-up visits. Mauchly's test was used to assess the assumption of sphericity. When sphericity was violated, the Greenhouse–Geisser correction was applied; when the assumption was met, the sphericity-assumed results were reported. The repeated-measures analysis was reported using the F statistic, degrees of freedom, p-value, and partial η^2 as the effect-size measure. All statistical tests were two-sided, and statistical significance was defined as $p < 0.05$.

RESEARCH RESULTS

General Characteristics of the Sample

A total of 16 participants were initially enrolled in this randomized, split-face, placebo-controlled trial. Unfortunately, two participants dropped out from this study before completion due to loss of follow-up leaving the final sample size of 14 participants. The mean age of the participants was 34.36 ± 4.56 years, with a range of 30 to 44 years. Occupation wise, 9 participants are students, 3 are doctors and another 2 are employees. 2 participants reported history of food allergies, but none reported underlying diseases or personal use of medication and supplements. Twelve participants reported no previous treatment before the study, whereas two participants reported a history of other non-periorbital rejuvenation treatments which were not expected to directly interfere with the assessment of periorbital wrinkles during the study. All 14 participants reported exposure to sunlight between 10:00 AM and 4:00 PM with the mean duration of 8.50 ± 5.65 minutes and range of 3 to 20 minutes.

Cutometer results

According to Table 1 and Fig.4, Cutometer R2 measurements at the crow's-feet area showed a gradual increase in mean R2 elasticity values on the active-treatment side during the 12-week

follow-up period. The mean value increased from 0.6115 ± 0.0847 at baseline to 0.6399 ± 0.0689 at week 4, 0.6627 ± 0.0590 at week 8, and 0.6816 ± 0.0450 at week 12. On the placebo side, the mean value increased slightly from 0.6108 ± 0.0839 at baseline to 0.6233 ± 0.0721 at week 4, remained similar at week 8 at 0.6250 ± 0.0707, and was 0.6241 ± 0.0660 at week 12.

The active-side changes from baseline were 0.0284±0.0256 at week 4, 0.0512±0.0383 at week 8, and 0.0701±0.0527 at week 12. The corresponding placebo-side changes were smaller, at 0.0125±0.0250, 0.0142±0.0277, and 0.0133±0.0309, respectively. The effect estimate, defined as the between-side difference in change from baseline, was calculated as active-treatment-side change minus placebo-side change. It was 0.0159 at week 4, 0.0370 at week 8, and 0.0568 at week 12. These positive values indicate that the increase in Cutometer R2 values was greater on the active-treatment side than on the placebo side at each follow-up visit.

The 95% confidence intervals for the effect estimates were 0.0018 to 0.0298 at week 4, 0.0184 to 0.0556 at week 8, and 0.0334 to 0.0802 at week 12. The between-side differences were statistically significant at week 4 ($p=0.030$), week 8 ($p<0.001$), and week 12 ($p<0.001$). These findings support a greater improvement in measured skin elasticity on the active-treatment side at each follow-up assessment. The effect was relatively small at week 4 and was numerically larger at weeks 8 and 12.

For the overall repeated-measures analysis, Mauchly's test indicated that the assumption of sphericity was met ($W=0.764$, $p=0.199$). Therefore, the sphericity-assumed result was used. The between-side difference in Cutometer change from baseline differed significantly among weeks 4, 8, and 12, $F(2,26)$, $p= 0.007$, partial $\eta^2 = 0.315$. This repeated-measures result demonstrates an overall difference in the effect estimate among the three follow-up visits.

Table 1 Cutometer R2 skin elasticity measurements and between-side differences in change from baseline at the crow's-feet area

Timepoint	Active side, mean ± SD	Placebo side, mean ± SD	Active-side change from baseline	Placebo-side change from baseline	Effect estimate	95% CI	p-value
Baseline	0.6115±0.0847	0.6108±0.0839	-	-	-	-	-
Week 4	0.6399±0.0689	0.6233±0.0721	0.0284 ±0.0256	0.0125 ±0.0250	0.0159	0.0018 to 0.0298	0.03
Week 8	0.6627±0.0590	0.6250±0.0707	0.0512 ±0.0383	0.0142 ±0.0277	0.0370	0.0184 to 0.0556	0.001
Week 12	0.6816±0.0450	0.6241±0.0660	0.0701 ±0.0527	0.0133 ±0.0309	0.0568	0.0334 to 0.0802	<0.001

Note: For each participant and follow-up visit, change from baseline was calculated separately for the active-treatment and placebo sides. The effect estimate, defined as the between-side difference in change from baseline, was calculated as active-treatment-side change minus placebo-side change. The reported effect estimate represents the mean of these participant-level between-side differences. The 95% confidence interval and p-value were calculated using a two sided one-sample t-test of the participant-level difference-in-change scores against zero.

Table 2 Overall repeated-measures analysis of the between-side difference in Cutometer R2 change from baseline

Outcome	Mauchly's test	Correction used	F statistic	p-value	Partial η^2
Between-side difference in Cutometer R2 change	W=0.764 p=0.199	Sphericity assumed	F(2,26)=5.986	0.007	0.315

Note: The analyzed outcome was the between-side difference in change from baseline, defined as active-treatment-side change minus placebo-side change. For each participant, this effect estimate was calculated at weeks 4, 8, and 12. Repeated measures ANOVA was used to assess whether the mean effect estimate differed among the three follow-up visits. Mauchly's test indicated that the assumption of sphericity was met; therefore, the sphericity-assumed results were reported. Partial eta squared represents the effect size for the time effect.

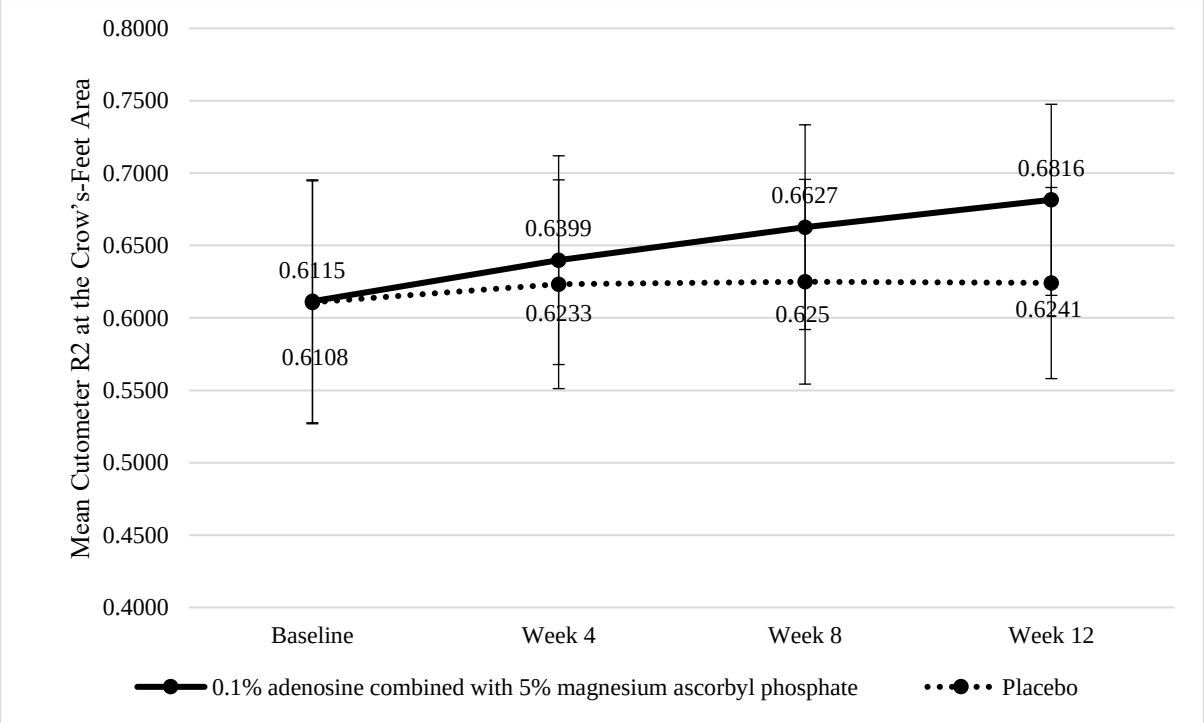


Figure 4 Linear graph comparing mean Cutometer R2 at the crow's-feet area at each visit between the side treated with 0.1% adenosine combined with 5% MAP and the placebo side.

Visioscan results

According to Table 3 and Figure 5, Visioscan wrinkle scores decreased on the active-treatment side during the 12-week follow-up period, from 57.40 ± 22.42 at baseline to 51.23 ± 17.44 at week 4, 47.77 ± 14.88 at week 8, and 41.79 ± 14.66 at week 12. On the placebo side, the scores were 52.64 ± 20.23 at baseline, 57.20 ± 18.11 at week 4, 56.53 ± 18.28 at week 8, and 56.07 ± 18.75 at week 12.

Because lower Visioscan scores indicate fewer wrinkles, change from baseline was calculated as baseline score minus follow-up score. The active-side changes were 6.17±7.28 points at week 4, 9.63±10.57 points at week 8, and 15.61±19.62 points at week 12. The corresponding placebo-side changes were −4.56±10.00, −3.89±9.81, and −3.43±9.08 points, respectively.

The effect estimate, defined as the between-side difference in change from baseline and calculated as active-side change minus placebo-side change, was 10.73 points at week 4 (95% CI, 2.50 to

18.94), 13.52 points at week 8 (95% CI, 4.07 to 22.95), and 19.04 points at week 12 (95% CI, 5.72 to 32.36). The between-side differences were statistically significant at week 4 (p=0.014), week 8 (p=0.009), and week 12 (p=0.009).

Repeated measures ANOVA was used to assess whether the between-side effect estimate differed among the three follow-up visits. Mauchly's test indicated that the assumption of sphericity was violated (W=0.410, p=0.005); therefore, the Greenhouse–Geisser correction was applied ($\epsilon = 0.629$). The effect estimate differed significantly among weeks 4, 8, and 12, F(1.258, 21.034) =4.593, p=0.040, partial $\eta^2 = 0.261$.

These results indicate that the active-treatment side had a greater reduction in Visioscan wrinkle scores than the placebo side at each follow-up visit.

Table 3 Visioscan wrinkle score measurements and between-side differences in change from baseline at the crow's-feet area

Timepoint	Active side, mean ± SD	Placebo side, mean ± SD	Active-side changes from baseline	Placebo-side changes from baseline	Effect estimate	95% CI	p-value
Baseline	57.40±22.42	52.64±20.23	-	-	-	-	-
Week 4	51.23±17.44	57.20±18.11	6.17±7.28	-4.56±10.00	10.73	2.50 to 18.94	0.014
Week 8	47.77±14.88	56.53±18.28	9.63±10.57	-3.89±9.81	13.52	4.07 to 22.95	0.009
Week 12	41.79±14.66	56.07±18.75	15.61±19.62	-3.43±9.08	19.04	5.72 to 32.36	0.009

Note: Because lower Visioscan wrinkle scores indicate fewer wrinkles, change from baseline was calculated as baseline score minus follow-up score. The effect estimate was defined as the between-side difference in change and was calculated as active-side change minus placebo-side change. The 95% confidence intervals and p-values were calculated from participant-level paired differences using two-sided one-sample t-tests against zero. The p-values represent visit-specific tests of the effect estimate.

Table 4 Overall repeated-measures analysis of the between-side difference in Visioscan winkle score change from baseline

Outcome	Mauchly's test	Correction used	F statistic	p-value	Partial η^2
Between-side difference in Visioscan change	W=0.410 p=0.005	Greenhouse-Geisser, e=0.629	F(1.258, 16.351) = 4.593	0.040	0.261

Note: The repeated-measure ANOVA evaluated whether the between-side difference in change from baseline differed across weeks 4,8 and 12. Mauchly's test indicated violation of the sphericity

assumption; therefore, Greenhouse-Geisser-corrected degrees of freedom and p-value were reported.

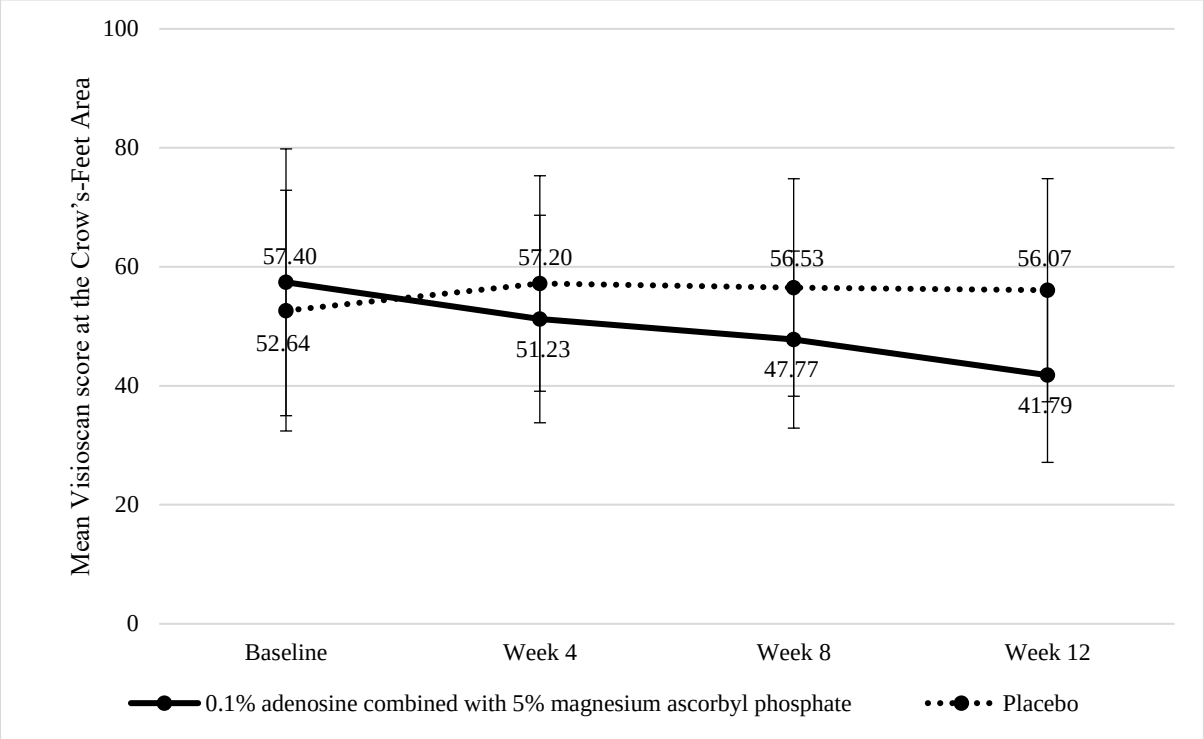


Figure 5 Linear graph comparing mean Visioscan winkle score at the crow's feet area at each visit between the side treated with 0.1% adenosine combined with 5% MAP and the placebo side.

Side Effects and Safety Assessment

During the 12-week period, only one participant reported transient mild dryness after starting the creams; however, the symptom was transient and resolved without any intervention. No other adverse reactions, including pruritus, erythema, or burning sensation, were reported throughout the follow-up period.

DISCUSSION AND CONCLUSION

In this study, the fixed combination of 0.1% adenosine and 5% magnesium ascorbyl phosphate was associated with improvements in the measured skin parameters over the 12-week follow-up period. Compared with the placebo side, the active-treatment side showed greater reductions in Visioscan wrinkle scores and greater increases in Cutometer R2 values. The small number of participants who completed the study limits the precision of the treatment-effect estimates. Therefore, these findings should be considered preliminary and interpreted cautiously.

In this study, the primary endpoint, which was the between-side difference in change from baseline in Visioscan wrinkle score at week 12, favored the active-treatment side. The active-treatment side showed a progressive reduction in wrinkle scores over the 12-week period, whereas scores on the placebo side remained relatively stable or increased from baseline. The between-side difference in change was positive at each follow-up visit, and the overall repeated-measures analysis indicated that the effect estimate differed among weeks 4, 8, and 12. This pattern is compatible with a greater observed treatment effect over time, although the findings should be interpreted within the limits of the study design.

The secondary endpoint, which was the between-side difference in change from baseline in Cutometer R2 skin elasticity at week 12, also favored the active-treatment side. Cutometer R2 values increased progressively on the active-treatment side, whereas values on the placebo side changed only modestly. The positive between-side differences at weeks 4, 8, and 12 indicate greater

improvement in the measured elasticity parameter on the active-treatment side, and the repeated-measures analysis demonstrated an overall difference in the effect estimate among the three follow-up visits. Taken together, the Visioscan and Cutometer findings showed a consistent direction of between-side difference across the two instrumental outcomes.

The observed effects may reflect the combined activity of the ingredients in the tested formulation. Adenosine and magnesium ascorbyl phosphate have been discussed in relation to biological processes relevant to skin aging, including cellular signaling, antioxidant activity, and collagen-related pathways. However, the present study evaluated clinical and instrumental outcomes rather than skin penetration, ingredient conversion, collagen synthesis, collagen maturation, or other biological markers. These mechanisms should therefore be considered possible explanations for the observed findings rather than the mechanisms demonstrated in this study.

This study also has several strengths. The randomized, split-face, placebo-controlled design helps reduce the influence of between-participant differences in skin characteristics, baseline wrinkle severity, and environmental exposure. Comparing the active and placebo sides within the same participant may be particularly useful for evaluating subtle changes in the periorbital area. In addition, the use of two complementary instrumental assessments, Visioscan® for wrinkle scoring and Cutometer® for skin elasticity, allowed the formulation to be evaluated across more than one skin parameter. The direction of the findings is also broadly consistent with previous reports describing improvements in skin-aging measures following adenosine-based interventions, although differences in formulation, treatment delivery, study design, and outcome assessment should be taken into account when making comparisons with other previous studies.

The safety findings were generally favorable during the study period. Only one case of mild dryness was reported, and no serious adverse events occurred. These observations support the short-term tolerability of the tested formulation in this study; however, the small sample size and limited follow-up prevent firm conclusions about its safety in larger populations or during prolonged use.

Several limitations should also be considered. The current trial evaluated a fixed combination of 0.1% adenosine and 5% magnesium ascorbyl phosphate, making it difficult to determine whether the observed benefits were primarily attributable to adenosine, magnesium ascorbyl phosphate, or an interaction between the two components. The 12-week follow-up period also limits conclusions about the durability of the observed changes. Although longer-term biological effects of vitamin C derivatives have been proposed, the present study did not directly evaluate the persistence of the treatment effect or the biological behavior of magnesium ascorbyl phosphate over longer periods.

Future studies with longer follow-up and separate active-ingredient arms, including adenosine alone, magnesium ascorbyl phosphate alone, and the combined formulation, would be needed to assess the durability of the observed effects and clarify the contribution of each component. Such designs could also help determine whether the components have an interaction beyond their individual effects. Finally, although the split-face design improves internal comparability, the single-center setting and modest sample size limit the generalizability of the findings. Larger, adequately powered, multicenter studies are needed to confirm these results in broader populations.

Overall, the findings indicate that the tested fixed combination was associated with greater short-term improvement in Visioscan wrinkle scores and Cutometer R2 measurements than placebo in this small study. These preliminary findings support further investigation of the formulation but do not establish long-term effectiveness, ingredient-specific effects, synergy, or a confirmed biological mechanism.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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