

Evaluation of the clinical efficacy and safety of an eye counter pad containing caffeine and vitamin K in emulsified Emu oil base

Fatemeh Ahmadraji, Mohammad Ali Shatalebi

Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: People strive to maintain the best look and have a youthful appearance. The skin around the eye is an area without many oil glands and collagen in comparison to the body rest, so it is more prone to fine lines and dehydration, which makes it look dull. The aim of this research is to develop and evaluate *in vivo* efficacy of an anti-wrinkle and dark circle eye pad consisting of 3% w/w caffeine and 1% vitamin K in a convenient, healthy, and cosmeceutical base.

Materials and Methods: Pre-emulsions containing selected ingredients in a specified effective concentration were prepared. The selected formulation, based upon pharmaceutical parameters, was coated on a nonwoven fabric in an eye counter shape, and stored in a jar. Then *in vivo* trial was carried out for investigation of its anti-wrinkling efficacy on 11 healthy women. In a single blind trial, the sample pad was applied under the right eye and the placebo pad, consisting of water, was placed under the left eye simultaneously. The efficacy and safety of the formulation was evaluated visually and with a multi skin test MC 900 instrument.

Result: After four weeks, the skin around the right eye of all the subjects experienced a reduction in the depth of wrinkles and dark circles, and from a subjective point of view, the appearance and elasticity of the skin were improved.

Conclusions: The caffeine pad containing vitamin K in an Emu oil base shows an improvement in eye counter appearance with anti-dark circle capacity and as an emollient.

Key Words: Caffeine, emu oil, vitamin K, *in vivo* test method, eye counter pad

Address for correspondence:

Dr. M. A. Shatalebi, Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran. E-mail: shatalebi@pharm.mui.ac.ir

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INTRODUCTION

One of the first places where visible signs of aging begin to emerge is around the eyes. The delicate skin in this area is extremely thin^[1] and contains little subcutaneous fat tissue.^[2]

During aging, the skin cells divide more slowly, and the inner layer, called the dermis, begins to thin.

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Networks of the elastin and collagen fibers loosen and unravel, causing depressions on the surface.^[3] The skin also loses its elasticity, is less able to retain moisture, oil-secreting glands are less efficient, and the skin is slower to heal. All of these contribute to the development of wrinkles.^[4]

Dark circles under the lower eyelids are another problem that can have several causes like aging, genetics, lack of sleep, and stress,^[5] but two of the most important ones are:

- **Vessel fragility:** Dark under-eye circles begin in the capillaries. If the blood vessels become fragile, they can be broken easily and hemoglobin leaks out into the surrounding skin. As the hemoglobin breaks down, pigmented degradation products form and accumulate in the dermis and epidermis. This may cause a dark discoloration around the eye^[6]
- **Poor circulation under eyes:** Poor circulation results in less oxygenated blood, and hence, causes a bluish appearance.

Nowadays, there are a wide range of procedures and products that can effectively treat the skin around the eyes. Fine wrinkles can be treated with dermal therapies that result in the contraction of collagen or by therapies that plump up the epidermis and dermis, such as retinoic acid^[7] trichloroacetic acid (TCA) or α -hydroxy acids (AHA)^[8]

Studies show that skin care products containing antioxidants can have benefits that improve skin health and help prevent aging and dark circles. Vitamins E, C, and caffeine are some important antioxidants that are used in cosmetic products.^[9,10] The SkinMedica company has produced a product consisting of 15% vitamin C plus a vitamin E complex to help reduce wrinkles and fine lines around the eye.

The objective of this research work is to verify the effect of the anti-wrinkle and dark circle eye pads, consisting of 3% caffeine and vitamin K in Emu oil bases, in a single blind, randomized, placebo- controlled study.

Caffeine as a crystalline compound has vasoconstriction properties.^[11] It stimulates the blood circulation around the eyes and also accelerates the flow of blood in the capillaries around the eyes.^[12] This automatically makes the skin around the eye appear fresh and rejuvenated. Caffeine has antioxidant properties^[13] that may help reduce the risk of wrinkles and protect the skin against free radical damage.

Vitamin K is a fat-soluble vitamin.^[14] The relation of Vitamin K to the circulation system (increases

blood coagulation and improves circulation) has been the reason for its use as an ingredient, to reduce the vascular condition that causes skin problems such as dark circles. A study by Elson in 1999, which evaluated the use of 1% vitamin K combined with retinol 0.15% for the treatment of periorbital hyperpigmentation, demonstrated that this preparation was effective in the treatment of under-eye circles in 93% of the patients studied.^[15]

Studies have shown that Emu oil penetrates through the layer of the dermis and prevents cells from dehydration because it contains high levels of unsaturated fatty acids like linolenic and oleic acids.^[16] It improves the structure and function of the cell membranes, as also the skin barrier function. Improving the skin barrier function reduces the transepidermal water loss, leaving the skin more hydrated. This keeps the skin cells strong and decreases the appearance of fine lines and wrinkles.^[17] The anti-aging effect of Emu oil was proven in a double-blind study at the Boston University School of Medicine, in 1996, by Dr. Michael Holick. According to this research, Emu oil was topically applied to the skin of mice for a two-week-long period. Finally, it was seen that the Emu oil produced a 20% increase in the growth activity of the skin of these animals.

MATERIALS AND METHODS

Caffeine was supplied by Merk KGaA Company. Vitamin K was kindly donated by the Osveh Pharmaceutical Company, Tehran Iran. Emu oil was obtained from the Abyaneh Cosmetic Company, Isfahan Iran.

The Glycerin, Tween 20, glycerin monostearate, and sodium benzoate, used in this study were of analytical grade.

Preparation of formulation

The emulsion was prepared by weighing the ingredients of the oil and water phases as shown in Table 1 and heating the oil phase until all the components had melted, and heating the water phase to the same temperature under gentle shaking, to ensure homogeneity, and obtaining the emulsion by adding the oil phase to the water phase. The system was

Table 1: Formulation of anti-wrinkle eye pad

Formula	Composition (100 g)
Oily phase	Emu oil (10 g)
	Vitamin K (1 g)
	Glycerin monostearate (2.6 g)
Water phase	Caffeine (3 g), sodium benzoate (2 g), glycerin (6 g), tween 20 (1.3 g), ethanol (7.8 g), water (65.11 g)

stabilized by gentle shaking while the formulation cooled to room temperature.

This stable emulsion was loaded on the cotton pad, and then, the different investigations of the physicochemical properties like content uniformity, weight variation, and *in vitro* release study were carried out. The pharmaceutical finding of this research study is going to be submitted in advance in the Biomedical Research Journal.

In vivo trial

Sensitivity test (Four-hour human skin patch test)^[18]

The pads were tested for their potential to cause skin irritation in 25 healthy women aged between 19 and 26 years. The pads were applied to the skin of the upper outer arm of the volunteers for up to four hours. The placebo pads (pad consisted of water) were applied to the other 25 volunteers. The sites are assessed for the presence of erythema, irritation, or itching, 24 hours, 48 hours, and 72 hours after pad removal.

The erythema was scored on a scale of 0 - 3, according to the Table 2.^[19]

Hydrating, pigmentation, and elasticity efficacy

For the study of hydrating, pigmentation, and elasticity efficacy, 11 healthy female subjects were enrolled randomly.

Inclusion criteria

- Female healthy subjects
- Subjects involved in the test who had wrinkles and dark circles
- Subjects who had not been involved in any other similar study for at least the last two months

Exclusion criteria

Patients with concomitant severe skin infection, history of hypersensitivity to cosmetic products, individuals below 18 years of age, patients with pre-existing severe systemic disease necessitating long-term medication, patients with genetic and endocrinal and hematological disorders, and those patients who refused to give informed written consent were excluded from the study. Pregnant and lactating women were also excluded from the test.

Table 2: Guidelines for the score of erythema after the application of the product

Score	Grade	Guideline
0	None	No redness present
1	Mild	Faintly detectable erythema; very light pink
2	Moderate	Dull red, clearly distinguishable
3	Severe	Deep, dark red

The measurement apparatus was a multi skin center MC 900, (Courage + Khazaka electronic company with Power supply: 100 - 240 V, Dimensions: Approximately 42.5 × 22.2 × 7.5 cm) which made it possible to determine these standards. It had three flat probes, with a surface area of 1.5 cm², which was applied at constant pressure on the skin. These probes measured the content of water in the surface layer of the skin, the content of pigmentation, and the percent of elasticity of the skin.

At first, the purpose of the study was explained to the volunteers, and informed written consent was obtained from each participant. To reduce the possible experimental errors, throughout the trial period, the volunteers were advised not to use any other type of preparation except that under the study, every night, and the patients were allowed to voluntarily withdraw from the study if they experienced serious discomfort during the study or if they sustained clinical events requiring specific treatment. Also, one week prior to the start of the study, the subjects refrained from using any moisturizer, anti-dark circle or wrinkle cream, or lotion foundation.

The prepared anti-wrinkle pad was put in a jar labeled A and the placebo pad was placed in jar B. Eleven volunteers were chosen randomly and in a single blind manner; they were advised to apply the pad in jar A around the right eye and B around the left eye for one hour every night, for a period of four weeks.

Prior to the day of application all women were assessed for elasticity, hydration, and pigmentation value. They were followed up for a period of four weeks, and at each weekly follow-up visit, the improvement in skin elasticity and hydration, and the decrease in skin pigmentation value were measured.

At the end of the fourth week, the overall performance of the anti-wrinkle and dark circle eye pad was evaluated.

RESULTS

The skin sensitivity studies were performed on the pad, to observe any visual skin erythema, irritation, or itching after the application of the pad to the humans. The pad did not cause any noticeable irritation or sensitivity on the skin and was well-tolerated at the site of topical application, throughout the study.

Three criteria, as mentioned earlier, were carried out on each volunteer. The average and the standard deviation were calculated for each one of the trials. All the results underwent an Analysis of

Variance (ANOVA) statistical treatment to give a confidence level of 95%, to check whether there were any significant differences between the averages compared in the case and control groups.

Moisturization test

Differences in the moisturization percent before and after the application of the pad are shown in Figure 1. In comparison with the values found before treatment in the skin around the right eye of volunteers, there was an increase in hydration after one month of treatment.

Elasticity test

Figure 2 shows the evolution in the elasticity percent of the skin before and after application of the pad in women. In the skin around the right eye, 100% of the subjects' measured elasticity improved, ranging from 61 to 75%.

Pigmentation test

Figure 3 shows the improvement in the appearance of the under eye circles in the right eye of 100% of the subjects by day 28. Reduction in dark circle around the eye (%) was significant, with a response rate of 16% (from the baseline).

The effect of the active formulation and the placebo pad on the clinical parameters in the management of moisturization, elasticity, and dark circles is indicated herewith in Tables 3 and 4.

DISCUSSION

To the best of my knowledge, a pad formulation that consists of three important components, caffeine, vitamin K, and Emu oil, for the treatment of wrinkles and dark circles around the eyes has not been studied. The benefit of this formulation, in comparison with other formulations, is the synergic effect of its ingredients. Emu oil is oil-compatible with the human skin lipid and can be used as an enhancer and drug carrier, to help in the permeation of caffeine and vitamin K through the skin. There are many carriers to apply over the skin such as ointments, creams, and gels, but the pad carrier is a single dose and a healthy way to apply over the eye-sensitive area.

Clinical assessment of the patients during this study revealed that there was a progressive and regular increase in the moisturization, elasticity percentage, and pigmentation content of the skin around the eyes after the application of active formulation versus before treatment. According to Figures 1 - 3, the effect of the pad on elasticity was more than its effect on hydration and pigmentation improvement. Analysis of the mean and standard deviation showed that active formulation had a consequential effect when

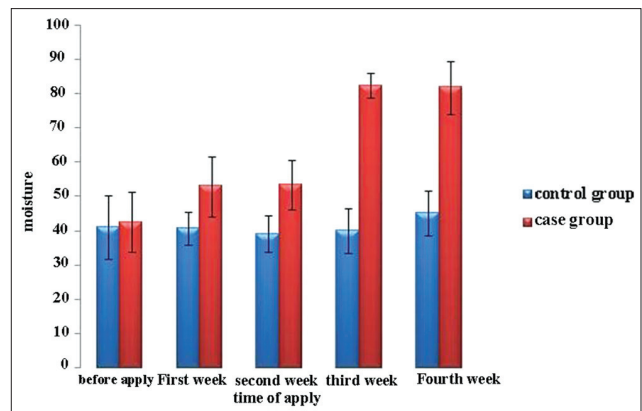


Figure 1: The mean improvement in skin moisturization after application of placebo and active formulations

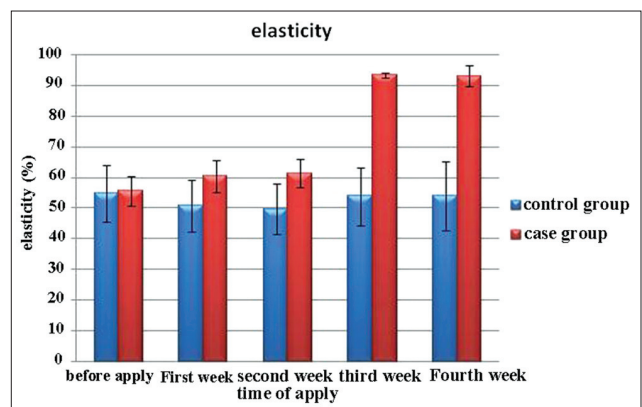


Figure 2: The mean improvement in skin elasticity in the control and case groups

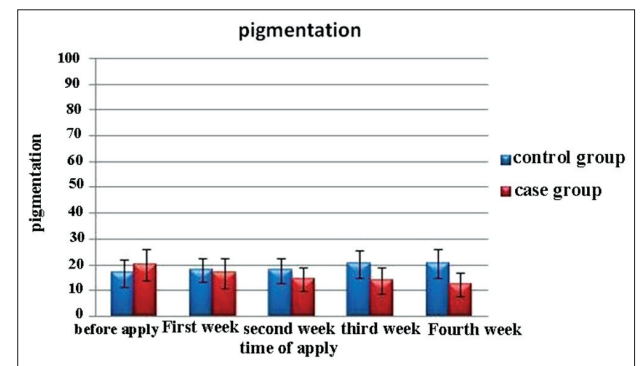


Figure 3: The mean reduction in skin pigmentation in the control and case groups

compared with the placebo pad ($P < 0.05$). This ruled out any variables' effect on the experiment and proved the efficacy of the active formulation.

Statistical analysis carried out by using the ANOVA and Tukey's test showed that the changes in these three criteria were not significant in seven and fourteen days after applying the active pad compared with before treatment. Also these results showed that

Table 3: Mean and standard deviation of moisturization, elasticity, and dark circle content in the skin around the right eye of volunteers

Time of application	Parameters	Mean (Arbitrary unit)	Standard deviation
Before application	Elasticity (%)	55.4545	4.84487
	Hydration	42.5455	8.80186
	Pigmentation	19.7273	5.990069
First week	Elasticity (%)	60.4545	5.20315
	Hydration	53.0909	8.80289
	Pigmentation	16.6364	5.80125
Second week	Elasticity (%)	61.2727	4.60632
	Hydration	53.4545	7.31250
	Pigmentation	14.1818	4.70783
Third week	Elasticity (%)	93.9091	0.83121
	Hydration	82.4545	3.58786
	Pigmentation	13.9091	5.08831
Fourth week	Elasticity (%)	91.0909	3.44832
	Hydration	81.6364	7.6364
	Pigmentation	12.1818	4.66515

Table 4: Mean and standard deviation of moisturization, elasticity, and dark circle content in the skin around the left eye of volunteers

Time of application	Parameters	Mean (arbitrary unit)	Standard deviation
Before application	Elasticity (%)	54.72	9.30689
	Hydration	41	6.52687
	Pigmentation	16.6364	5.39023
First week	Elasticity (%)	50.8182	8.29238
	Hydration	40.8182	4.95617
	Pigmentation	17.7273	4.64954
Second week	Elasticity (%)	49.6364	8.22524
	Hydration	39.1818	5.32575
	Pigmentation	17.7273	4.83923
Third week	Elasticity (%)	53.8182	9.38955
	Hydration	40.0909	6.39460
	Pigmentation	20.1818	5.21187
Fourth week	Elasticity (%)	54	11.13553
	Hydration	45.2727	6.40454
	Pigmentation	20.3636	5.60844

the maximum efficacy of the pads appeared in the third week ($P < 0.05$), but there were no significant changes between the third and fourth weeks. Accordingly this result revealed that this formulation had an accumulative moisturizing, elasticity, and pigmentation effect during three weeks of its use and then a plateau situation was observed.

Improving skin hydration and elasticity was due to the Emu oil and caffeine that was used in this formulation. Emu oil penetrated the epidermis to truly hydrate and nourish the skin without clogging the pores. This helped in reducing the appearance of fine lines and wrinkles, by promoting skin renewal and improving skin elasticity.

Caffeine as a powerful antioxidant helps rejuvenate the skin and improve elasticity.^[20] It helps to neutralize the skin damaging free radical, which can cause a breakdown of the collagen and cause wrinkles.

One study by Joseph RK and his team, in 2009, showed that caffeine cream significantly improved crow's feet wrinkles around the eyes after four weeks,^[21,22] but the prepared formulation was able to treat periorbital wrinkles in three weeks. This finding proved the synergic effect of the material that was used in the formulation, especially Emu oil.

The improvement in dark circles around the eye after the application of the formulation was related to the vitamin K used in this product. Vitamin K, by strengthening the capillary walls and reducing the visibility of blood vessels through the thin skin under the eyes, can help dark circles to improve prosperity.

A gradual increase in the values of the hydration and elasticity parameters indicate that the formulation possesses anti-aging properties and improves the pigmentation value, which proves the anti-dark circle quality of this product.

CONCLUSION

This study shows that the anti-wrinkle pad is effective and safe to use for the management of under eye skin wrinkles and the efficacy of this pad can be seen in three weeks.

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