Comparing the effects of eggshell membrane, olive oil, or castor oil as a skin anti-aging agent

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Abstract

Background and aims: Skin aging is a life-annoying problem especially for women that needs to act so that appearance of signs of aging can be delayed as long as possible. Here, the current work was intended to discover the effects of eggshell membrane (EM), olive oil (OO), or castor oil (CO) as a skin anti-aging agent (SAAA).

Materials and methods: The thirty-day study included the use of randomly-divided four groups of 20 rabbits, T1 (oral-administered with freshly egg-extracted EM 2gm/day), T2 (oral-administered with OO 5ml/day), T3 (oral-administered with CO 5ml/day), and T4 (nothing supplied). After the experiment was done, the parameters measured for detecting the effectiveness of the substances were component of skin amino acid (glycine, alanine, proline, and hydroxyproline). In addition, histopathological examination of the rabbit skin was conducted for 2 time points (one week and six weeks after starting of the experiment).

Results: The results of T1 showed significant (p<0.01) higher percentages of components of amino acid in the rabbit skin than those from the other groups. Moreover, the histopathological examination revealed that T1 demonstrated

the strongest proliferation of epidermal cells in the wrinkle of rabbit skin for the two time points when compared to the other groups.

Conclusion: The current study demonstrated the promising effects of the eggshell membrane as a skin anti-aging agent.

Keywords: Aging, alanine, anti-aging, glycine, hydroxyproline, proline.

Introduction

Like other outer-body organs of humans, skin is affected by various environmental factors. It is considered one of the main walls that protect the body organs from those detrimental factors such as pathogenic microorganisms. In addition to this major infection protecting role, skin hugely acts as an important regulator of water loss and body core temperature through sweating. With more than the above-mentioned functions, skin provides the body with beautiful cosmetic properties that enhance the positive behaviors of people in a community (1–5).

The overall body organs is prone to the aging processes from birth; however, when a person is getting older, skin can noticeably be visualized for this process leading to the appearance of various skin aging symptoms such as skin roughness, increase the incidence of benign tumor occurrence, slack skin, skin elasticity loss, elevated levels of skin transparency, increased skin fragility, high easiness of skin injury occurrence, etc. (6,7).

According to these effects of skin aging, people keep trying to stop or delay the skin aging processes by using different cosmetics purchased with huge amount of money(8). Many researchers are increasingly working on aging and skin aging in particular to find suitable anti-aging agents. Relying on those observations, the current work was intended to discover the effects of eggshell membrane (EM), olive oil (OO), or castor oil (CO) as a skin anti-aging agent (SAAA).

Materials and methods

Animals

The thirty-day study included the use of randomly-divided four groups of 20 rabbits. The White-New-Zeeland rabbits averagely weighted at 2650±25 gm hosted in the animal house (temperature control, ventilation, 12:12 at

light: dark hours, and controlled feeding), College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq. Spots of about two squared centimeters in the hair coat of the rabbits were clipped and hair-removed using a special ointment.

Preparation of eggshell membrane

From local stores in Al-Diwaniyah City (Iraq), 60 eggs were recruited in the current study. The eggs were first rinsed thoroughly three times by using distilled water (DW). Then, the opening of each egg was performed through the air-chamber area. After that, cleaning out of each egg was done by discarding the liquid contents. Later, 1% acetic acid was used to fill the empty eggs for manually separating the EMs. Finally, the membranes were stored at 4ºC until use.

Eggshell membrane extraction of collagen

Either 0.5M acetic acid or 0.5M citric acid was employed to extract collagen from EMs. Either acid and each EM were mixed thoroughly at weight: volume ratios of 1:4, 1:6, 1:8 and 1:10 using a 4°C water bath with a shaking property for 2hrs. Then, the mixtures were centrifuged at 6000rpmfor 4 min followed by another step of mixingat 4°C for 24hrs. After that, a homogenization processat 4°C/6000rpm for 2minswas performed which then centrifuged at 10°C/10000g for 20mins. The extraction of the precipitate was induced for three times. Gonçalves-Neto*et al*., 2002 (9)were followed to identify the amino acid (glycine, alanine, proline, and hydroxyproline) levels and the type of collagen in the supernatants, respectively. A dialyzing process of the acetic-acid-based extracted supernatant was performed in DW at 4°C for 24hrs before analysis.

Rabbit skin extraction of collagen type-1

After a DW-based skin soaking was initiated recovering 70% of the contents, fat and connective tissues were removed using a flushing step. Methods from Kittiphattanabawon et al. (2005) (10)were relied on to extract rabbit skin collagen with slight modifications. Each skin sample was cut-divided into one-Cm^2 measured squares, 0.5M-acetic-acid based dissolved at (1:10 w/v), shaking-mixed at 140rpm at room temperature for 24hrs, and filtered with filter papers N0.1.

Design of experiment

T1 (oral-administered with freshly egg-extracted EM 2gm/day), T2 (oral-administered with OO 5ml/day), T3 (oral-administered with CO 5ml/day), and T4 (nothing supplied). After the experiment was done, the parameters measured for detecting the effectiveness of the substances were skin amino acid (glycine, alanine, proline, and hydroxyproline) components detected in skin biopsies ($1Cm^2$). In addition, histopathological examination of the rabbit skin was conducted for 2 time points (one week and six weeks after starting of the experiment).

Tests

Glycine, alanine, proline, and hydroxyproline were measured by using amino acids auto analyzer. For skin biopsies, histological examination was performed, and the slides were visualized utilizing a light microscope at X40 &100.

Statistical analysis

Analysis of the observed data was processed using a Chi Square test. The null hypothesis was rejected if *p* value was equal or less than 1%.

Results

Eggshell levels of amino acids

The EM amino acid levels are shown in table 1.

Table 1: Eggshell membrane collagen type-1 levels of amino acids (%)

Amino acids	Eggshell membrane collagen type-1 levels (%)
Glycine	29.31
Alanine	10.11
Proline	10.78
Hydroxyproline	10.39

Rabbit skin content levels of amino acids

The results of T1 showed significant (p<0.01) higher percentages of amino acid components of the rabbit skin than those from the other groups, table 2.

Table 2: Amino acid percentage levels in one gram of rabbit skin of the experimental groups

Amino acids	Amino acid levels (%)			
	T1	T2	T3	T4
Glycine	6.01*	5.48	5.39	4.68
Alanine	7.20*	5.95	5.91	4.38
Proline	6.62*	5.18	5.03	4.18
Hydroxyproline	6.40*	5.10	5.01	3.71

*: Significant at $p \leq 0.01$.

Histopathological examination

For the histopathological examination at the week-1 time-point,the results revealed that T1 and T2 demonstrated the strongest proliferation of epidermal cells in the wrinkle of rabbit skin when compared to the other groups, figure 1 (T1, T2, T3, and T4).

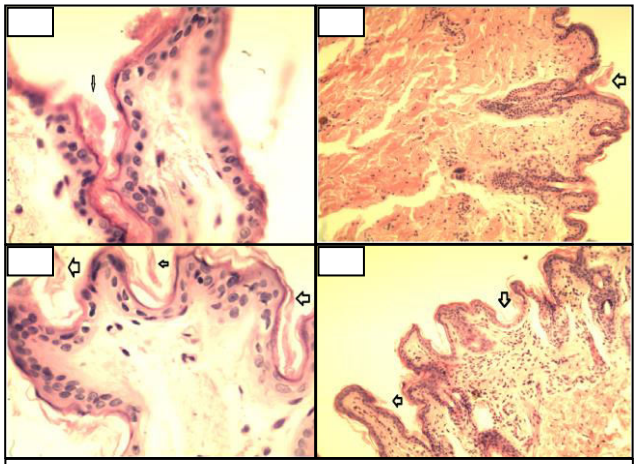

Figure 1: Histopathological changes at the time-point week-1. T1 and T2

For the histopathological examination at the week-6 time-point, the results revealed that T1 demonstrated the strongest proliferation of epidermal cells in the wrinkle of rabbit skin when compared to the other groups, figure 2 (T1, T2, T3, and T4).

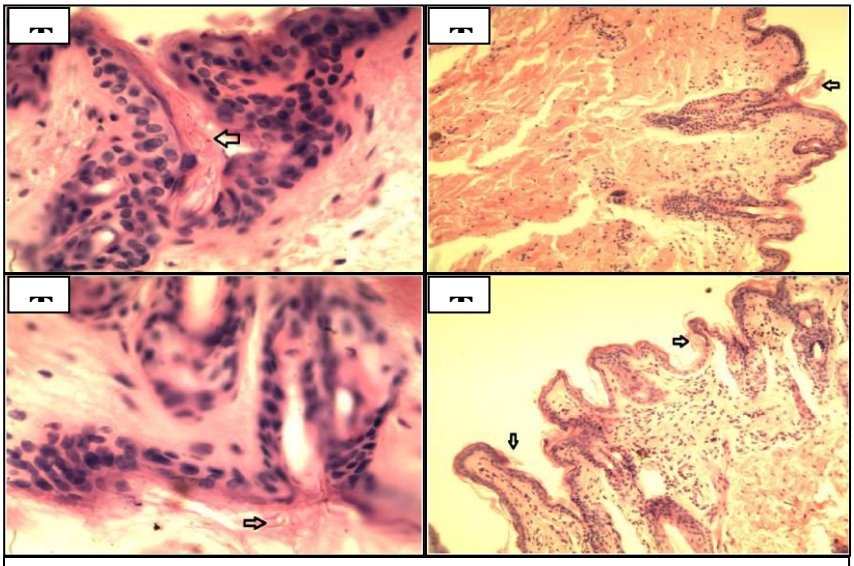

Figure 2: Histopathological changes at the time-point week-6. T1 demonstrated the strongest proliferation

Discussion

Like any other organ in the human body, skin starts facing aging due to getting older and/or due to exposure to various environmental factors such as infectious and chemical agents with skin showing roughness, benign tumors, slackness, loss of elasticity, high transparency, elevated fragility, high injury occurrence, etc. (6,7). Fighting the aging process is a hustle-inducing problem; however, the current work was intended to discover the effects of eggshell membrane (EM), olive oil (OO), or castor oil (CO) as a skin anti-aging agent (SAAA).

For the eggshell membrane collagen type-1 levels of amino acids, our results agree with (11) who identified the presence of a wide-range of amino acids in the EM contents.

For the rabbit skin content levels of amino acids, the results of T1 showed significant (p<0.01) higher percentages of amino acid components of the rabbit skin than those from the other groups. The use of eggshell membrane has been

found beneficial for inhibiting collagenase as this enzyme activity hydrolyzes collagen in the skin leading to the early appearance of skin wrinkles due to loss of elasticity and dryness of the skin (12,13). Yoo *et al*, (2014)(14) has performed an anti-collagenase activity using eggshell membrane hydrolysates and detected that EM had a strong effects against this enzyme which might lead to improving skin health. This anti-collagenase activity of the EM may be due to the action of certain amino acids such as leucine and valine which have the ability to block-bind to the collagenase active site and stop its work (12–14). Our results agree with the fact that indicates the presence of amino acids in the EMs may enhance the delay in the aging process of skin (12,13). Moreover, Yoo *et al* (2015)(15) have performed a study to measure the effects of EM hydrolysates as a cosmetic, UV-ray protecting, and anti-skin dryness agent, and observed that EM was successful in ensuring the fulfillment of those aims.

Conclusion

The current study demonstrates the promising effects of the eggshell membrane as a skin anti-aging cosmetic agent.

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