Ectoine, A Safeguard against Irritation Induced by Cleansing Cosmetics

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Introduction

The use of cleansing cosmetics may elicit adverse effects when they contact the skin, which serves as the first barrier against external hazards. Surfactants in cleansing cosmetics are one of the primary causes of skin irritation [1]. They interact with the stratum corneum and even deeper skin layers, leading to dryness, itchiness, desquamation, and other skin barrier disorders [2; 3]. A reduced irritation effect of cosmetic products can be achieved by an appropriate combination of product components. Besides the application of surfactant mixture, many other materials, such as polymers and refatting substances, are developed to decrease skin irritancy [4].

Ectoine (2-methyl-1,4,5,6-tetrahydropyrimidine-6-carboxylic acid) is a rare amino acid produced by extremophile and enable them to survive under various types of harsh environments [5]. Enrichment of ectoine within the cell allows the organisms to shield their biopolymers from extreme exposure to temperature, dryness, and salinity. Previous studies have demonstrated that ectoine can prevent protein folding, protect lipid layer, stabilize cell membrane, and recover skin viability[6–9].

In the present study, we investigated the effect of ectoine on the skin and eye irritation potential of phototype cleansing cosmetics (shower gel, shampoo, and hand wash). Furthermore, we explore that usage of ectoine before cleansing cosmetics can provide the skin a protective shield during the cleansing process.

Materials & Methods

Phototype Cleansing Cosmetics Ingredients Composition

The composition of shower gel, shampoo, and hand wash was following listed in Table 1. All three formulas contain the anionic type, which shows a strong tendency for skin and eye irritation. Shower gel and shampoo are also made up of amphoteric and non-ionic surfactants.

Zein Test

The zein test performed is a modification of the method developed by Gotte. The fundamental principles underlying the binding of protein and surfactants could apply to zein protein and surfactants in cleansing cosmetics. Based on that, we can determine the remaining quantity of zein protein in a cleansing cosmetics solution. The amount of zein protein dissolved can be reflected by the absorption at 280nm, which is also contrarily relevant to the irritation potency of cleansing cosmetics.

Corneosurfametry Test

Cyanoacrylate skin surface strippings (CSSS) were harvest from the inner forearms, which were incubated with 1% wt. ectoine and 10% wt. testing solution in a time order. The color of toluidine blue dyed tapes were qualified by reflectance colorimetry (Chroma Meter CR400, Minolta, Osaka, Japan). The values of a*, b*, and luminance L* were recorded with reflectance colorimetry, and Chroma C * can be calculated through the equation: $C^*=(a^{*2}+ b^{*2})^{1/2}$. Based on these indicators, the colorimetric index of mildness (CIM) and Index of irritation (IOI) is obtained according to the following formula:

$CIM = L^*-C^*$

$IOI = 1- (CIM_{\text{test product}} / CIM_{\text{water}})$

BALB/c 3T3 Neutral Red Uptake Test

The 3T3 NRU Test is executed according to ECVAM DB-ALM Protocol n° 46, which is also modified from OECD 129. The capacity of cleansing cosmetics to cause injury to structures and functions was evaluated on the BALB/c 3T3 mouse fibroblast cell line. IC50, the concentration of test substance expressed as mg/ml that induces 50% inhibition of neutral red uptake, provides an indication of toxicity. The optical density (OD) of the resulting-colored solution, which was gently shaken for 15 mins to be homogeneous, were read at 540 nm in a microtiter plate reader (Spectrophotometer Multiskan Sky, ThermoScientific, Singapore). The Hill function analysis is accomplished using GraphPad Prism 8.0 and calculated IC50 values for each test substance.

Table 1. Ingredients composition of cleansing cosmetics

	Ingredient	Concentration (100%)
Shower gel	Aqua	Ad. 100
	Sodium lauryl sulfate	13
	Cocamidopropyl betaine	8
	Sodium cocoyl glutamate	7
	Glycerin	3
	Alkyl polyglycoside	2
	Sodium hyaluronate	1
	Preservative	1
	Peg-120 methyl glucose trioleate	0.8
	Sodium chloride	0.7
	Cocamide methyl mea	0.5
	Ph regulator	0.3
	Edta	0.1
	Dipotassium glycyrrhizate	0.02
Shampoo	Aqua	Ad. 100
	Sodium c14-17 alkyl sec sulfonate	12
	Cocamidopropyl betaine	10
	Sodium methyl cocoyl taurate	5
	Glycerin	3
	Preservative	0.9
	Sodium hyaluronate	0.5
	Sodium chloride	0.5
	Guar hydroxypropyltrimonium chloride	0.3
	Peg-120 methyl glucose trioleate	0.3
	pH regulator	0.2
	Edta	0.1
Hand wash	Aqua	Ad. 100
	Sodium laureth-12 sulfate	6
	Acrylates copolymer	6
	Glycerin	3
	Sodium dodecylbenzenesulfonate	2
	Preservative	0.3

Results & Discussion

Table 2. Reduced Rate of Irritant Potential for Two Application Usage of 1% wt. Ectoine

		Reduced Rate of Irritant Potential (100%)	
		Mixed-Treated Ectoine	Pre-Treated Ectoine
Zein Test	Shower Gel	6.50	21.54
	Shampoo	1.94	-1.42
	Hand Wash	-1.77	5.54
Corneosurfametry Test	Shower gel	-2.83	5.64
	Shampoo	20.45	28.36
	Hand Wash	7.63	24.37
Cytotoxicity Test	Shower Gel	-15.46	40.15
	Shampoo	8.66	64.60
	Hand Wash	10.75	23.99

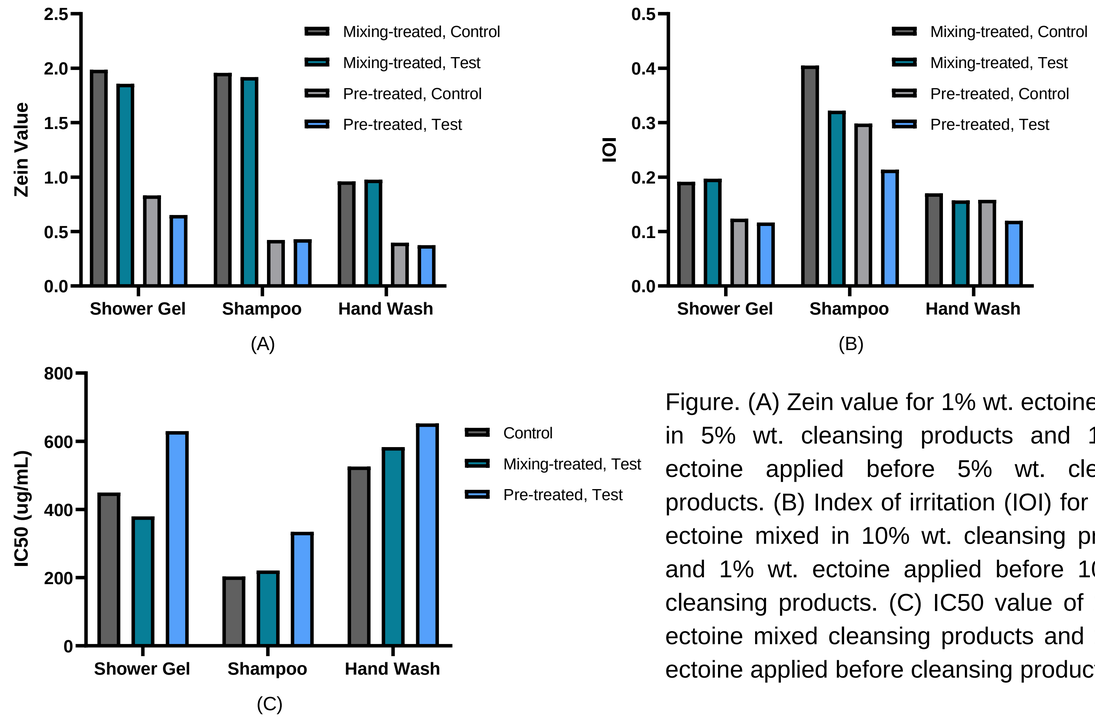

Figure. (A) Zein value for 1% wt. ectoine mixed in 5% wt. cleansing products and 1% wt. ectoine applied before 5% wt. cleansing products. (B) Index of irritation (IOI) for 1% wt. ectoine mixed in 10% wt. cleansing products and 1% wt. ectoine applied before 10% wt. cleansing products. (C) IC50 value of 1% wt. ectoine mixed cleansing products and 1% wt. ectoine applied before cleansing products

Protection against Protein Degradation

As showed by Figure (A), for shower gel and hand wash, the pre-treated ectoine group present a better resistance against irritation than solution mixing ectoine in the Zein test. The Zein Test is especially suitable to compare the skin irritation potential of anionic surfactants since they are the strong solubilizers to zein protein, so the effect of ectoine on anionic surfactant should respond greatly for the reduced rate of irritant potential. In contrast, the irritancy reduced rate is proportional to the overall surfactant in the cleansing cosmetics instead of the anionic surfactants in shower gel and hand wash. That is to say, ectoine can provide broad protection against kinds of surfactant.

Lipid Stabilization

A noticeable decrease in the index of irritation was identified in all three products applied with ectoine pre-treatment. Moreover, the pre-treated ectoine group shows a consistently lower IOI value compared with ectoine mixed in test solutions. The ability of solubilization of lipid components is one of the mechanisms potentially leading to skin irritation. Lipid removal and structural change increase the amount of water removed from the surface layers of the epidermis (TEWL), which results in a disorder of the skin's barrier function.

Cells Membrane-Improving Effects

From Figure (C), ectoine pre-treatment reduced irritation potential for all three products. Furthermore, reduced rate illustrated that pre-treatment of ectoine can provide a better cover on cell membrane than mixed-treatment.

Conclusions

These in vitro results identified ectoine as a promising protector against irritation, decreasing the potential of irritancy on both eyes and skin. Ectoine maintains a water shield on the skin with its strong water-binding activity, providing the ability to prevent protein denaturation and sebum loss, and boost fibroblast cell activity.

Moreover, we compared the irritation reduced rate of two ectoine application ways in the tests based on surfactants-skin interaction, which demonstrates that using ectoine and cleansing cosmetics step by step gives more effective protection to cleansing cosmetics mixing with ectoine.

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