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Evaluation of skin irritation potentials of different cosmetic products in Turkish market by reconstructed human epidermis model

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ABSTRACT

Human skin is a protective barrier against the toxic effects of cosmetics. Marketing of cosmetic products with ingredients tested on animals was prohibited in 2013. Since then, safety evaluation of cosmetic products is performed by using alternative *in vitro* toxicity tests. *In vitro* 3-D reconstructed human epidermis (RhE) tissue models are now used to define skin irritation/corrosion potentials of cosmetic ingredients and end-products. The main aim of this study was to evaluate skin irritation potentials of topically used cosmetic end-products which were marketed in Turkey during 2015–2017, by using the EpiDerm *in vitro* 3D-human skin model. Sixty widely used cosmetic products were collected from different markets/cosmetic shops. Among hair care products, only one shampoo was found to be strong/severe skin irritant/possible corrosive while 22 shampoos were moderate skin irritant and 11 shampoos were moderate to mild skin irritant. Among 6 skin care products, one was found to be moderate to mild skin irritant. We can suggest that alternative *in vitro* tests should continuously be used to test both the ingredients and the final cosmetic formulations.

1. Introduction

Human skin protects the organism against environmental factors and chemicals in the pharmaceutical formulations and cosmetic products (Monterio-Riviere, 2009). The potential of chemical ingredients in the cosmetics as well as the end-products to cause acute skin irritation must be evaluated in order to protect the general population and particularly the susceptible populations (like children). Skin irritation is the most common local toxic effect after exposure to dermally applied cosmetic products and it can be described as “the reversible damage of the skin following the application of a test substance for up to 4 h” (SCCP, 2006) whereas skin corrosion can be defined as “irreversible damage to the skin, namely visible necrosis through the epidermis and into the dermis, following the application of a test substance for the duration period of 3 min up to 4 h” (OECD, 2002).

In order to evaluate of the potential hazard of a chemical ingredient or a cosmetic end-product, skin irritation was carried out using the Draize skin irritation test in rabbits historically (Draize et al., 1944; OECD, 2002). In ethical terms, Draize test had the potential to cause significant suffering and pain in animals. On 11 March 2009, European Union banned animal testing to assess the safety of cosmetic ingredients. In addition, the sale of cosmetic products containing ingredients tested on animals was prohibited on March 11, 2013 (EC, 2010, 2013). Ever since, the safety evaluation of the cosmetic products is performed by using alternative *in vitro* toxicity tests (Kandárová and Letašiová, 2011).

After these two bans, the *in vitro* reconstructed human epidermis (RhE) tissue models are now preferred as alternative methods for defining the skin irritation and skin corrosion potentials of cosmetic ingredients and end-products as their morphology is similar to human skin (Kandárová and Letašiová, 2011). Episkin™, Prediskin™ and Epiderm™ skin models are the most commonly used 3D reconstructed RhE models. In a study by Jírová et al. (2010), the researchers compared Episkin and Epiderm methods to the *in vivo* 4-h human patch test (HPT). The Episkin model showed 76% accuracy with 4-h HPT; however it only showed 56% accuracy with the Draize test. The Epiderm model showed 70% accuracy with the 4-h HPT, but exhibits 56% accuracy with the Draize test. The researchers concluded that the sensitivity and accuracy of the *in vitro* alternative methods surpassed their expectations and Epiderm model even had higher accuracy when compared to Episkin (Jírová et al., 2010).

EpiDerm skin model is validated and is present in OECD Test Guideline 439: In Vitro Skin Irritation (OECD, 2013). By using EpiDerm™ skin model, it is possible to evaluate the skin irritation/

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corrosion potentials of dermally applied compounds, chemicals, cosmetic/personal care product chemical ingredients and final formulations, namely end-products (MatTek, 2010). EpiDerm™ has human epidermal tissue structure and cellular morphology and it shows greater uniformity. In addition, the results obtained by using this model give high reproducibility, accuracy, specificity and the experiments are relatively less time-consuming (MatTek, 2010). Moreover, the model enables the researchers to conduct two different protocols: One is "Epiderm SIT200" protocol, in which chemical ingredients or end-products are classified as "irritant" or "non-irritant. The other is "Effective Time-50 (ET-50)" protocol which provides a classification for chemical ingredients or end products "strong/severe, possible corrosive", "moderate", "moderate to mild", "very mild" or "non-irritating". The main end point is evaluation of "tissue viability" by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (MatTek, 2010).

Today, the use of cosmetics and personal care products among both men and women is increasing due to providing good skin texture, boosting the attractiveness and emotional and self-esteem issues. As the use of cosmetic products is increasing day by day, the incidence of their unwanted effects is growing. The mostly encountered effects are mild and moderate skin irritation reactions. On the other hand, susceptible persons and susceptible populations, like children, can develop severe skin irritation reactions against cosmetics. Although the cosmetic ingredients should be tested for skin irritation before the product is marketed, there are no obligations for the cosmetic producers to test their final formulations for skin irritation. Therefore, throughout the world, most of the studies were conducted on the skin irritation potentials of chemical ingredients within the cosmetic products and there are very few studies that show the skin irritation potentials of cosmetic end-products. These studies were usually conducted with low number of cosmetic samples and *in vitro* alternative methods were not the method of choice mostly.

The main aim of this study was to evaluate the skin irritation potentials of topically used cosmetic end-products which were marketed in Turkey during 2015–2017, by using the EpiDerm *in vitro* 3D-human skin model as an alternative test of skin irritation. To our concern, this is the first study that evaluates the skin irritation potentials of final cosmetic products marketed in Turkey, with alternative *in vitro* methods.

2. Materials an methods

2.1. Reagents and kits

EpiDerm™ Skin Model kits (EPI-200) were purchased from MatTek Corporation (Ashland, MA, USA) (MatTek, 2010). The kit includes an assay medium (EPI-200-ASY), 1% Triton X-100 solution (TC-TRI-1.0%) calcium/magnesium-free Dulbecco's phosphate-buffered saline (DPBS) (MatTek, Ashland, MA). MTT was obtained from Sigma-Aldrich (Saint Louis, MO).

2.2. Test materials

Sixty widely used cosmetic products were collected from different markets or cosmetic shops randomly for testing. These include hair care products, skin care products, shaving products, depilatories, soaps and medical creams. Types of test products are given in Table 1 and their distributions (%) are shown in Fig. 1.

2.3. Reconstructed EpiDerm™ human epidermis model

"Effective Time-50 (ET-50)" protocol was used throughout the experiments. The experiments were performed according to OECD Test Guideline 439 (OECD, 2013) and the supplier's protocol (MatTek, 2010). Standard EpiDerm™ kit consists of individual tissues in which

Table 1
Types of test products.

Product Category	Quantity
Hair care products	
Shampoo	34
Hair cream	2
Herbal hair oil	2
Hair lotion	2
Hair serum	1
Skin care products	
Cream	3
Mask	2
Cleanser	1
Soap	4
Medical cream	2
Depilatory	4
Shaving products	3
TOTAL	**60**

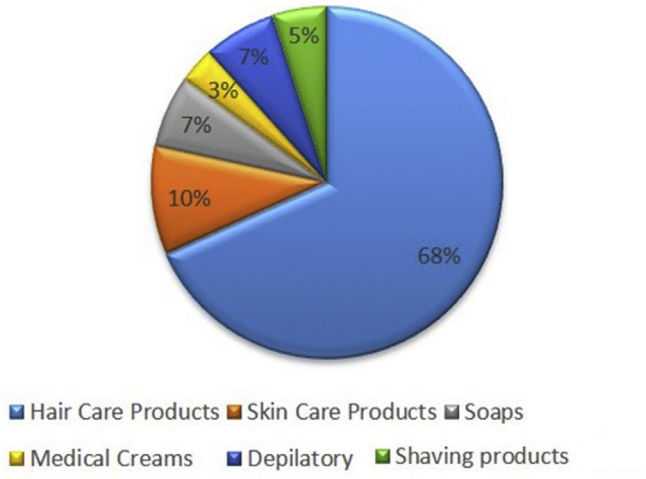

Fig. 1. Distribution of test materials.

are grown at air–liquid interface and cultured on collagen-coated, cell culture inserts and the cell insert sit just on the surface of the medium and the apical surface of the tissue is exposed to the atmosphere. The 3D structure of EpiDerm consists of highly organized and proliferative basal cells, spinous and granular layers and the cornified epidermal layers are mitotically and metabolically active. The tissues were shipped from the supplier at 4 °C on agar-supplemented in 24 well plates and usually arrived our laboratory within 2 days.

The cells were transferred into the 6-well plates containing 0.9 ml the pre-warmed assay medium and were maintained at 5 ± 1% CO_2, 37 ± 1 °C and 95% relative humidity (RH) overnight before the experiment. Following the overnight pre-incubation, the medium was discarded and replaced with 0.9 ml (per well) of pre-warmed, fresh assay medium.

2.4. Treatment conditions

Tissues were treated with test materials 4, 8, and 12 h 1.0% Triton X-100 (provided with the kit) was used as a positive control and DPBS was used as the negative control in all the experiments. For one baby care product, additional treatment period (24 h) was also used. For liquid test materials, 100 µL was added on the EpiDerm™ sample (Fig. 2). For solid materials, before application, the tissue surface moistened with 25 µL DPBS to improve the contact of the tissue surface with the test chemical and later test materials were applied as 100 mg. Test materials were applied onto the tissue surface without dilution. All experiments were performed in duplicate.