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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**

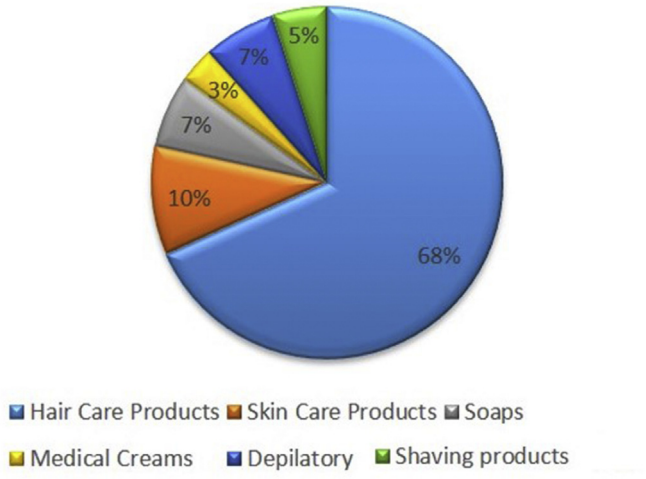

Fig. 1. Distribution of test materials.

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.

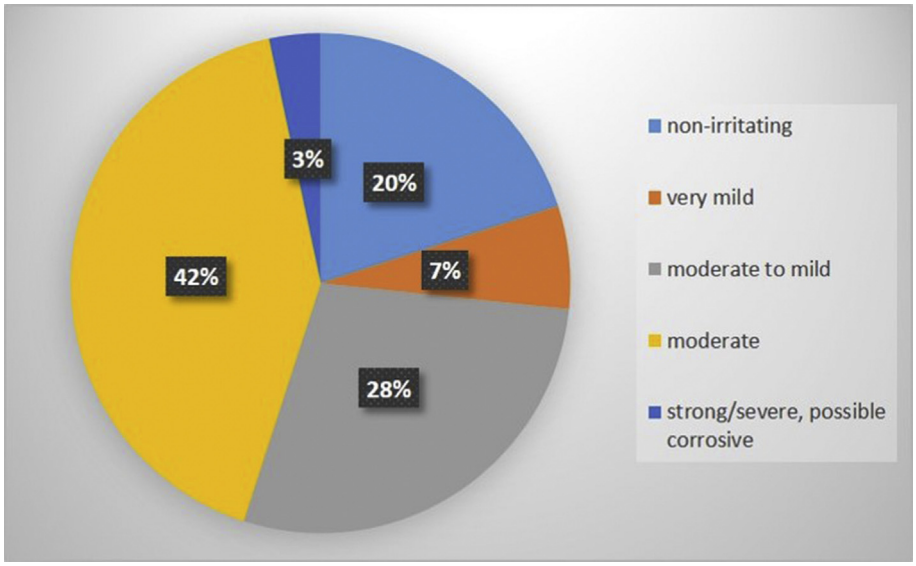

Fig. 2. Distribution of test materials according to their irritancy potentials.

2.5. MTT assay

Based on the "ET-50 Test" for use with EpiDerm™ Skin Model (EPI-200) assay protocol, the viability of the tissues was assessed by MTT assay. MTT was prepared in DPBS at 1 mg/ml concentration. Briefly, the tissue samples were taken out of the wells at the end of the different treatment periods. Each tissue insert was thoroughly rinsed with Ca^{2+}- and Mg^{2+}- free DPBS to remove any residual test material and were placed in a fresh 24 well plate pre-filled with 300 µL/well MTT solution. All plates were incubated for 3 h at 5 ± 1% CO_2, 37 ± 1 °C and 95% RH. After incubation time, each insert was removed carefully; the bottom of inserts was blotted with sterile tissue paper and the insert was placed in a fresh 24 well plate. In extraction step, 2.0 ml isopropanol was added each well and the inserts were immersed in this solution. The plates were sealed with parafilm to avoid any evaporation and placed onto the plate shaker for 2 h at room temperature.

After extraction period, two aliquots per tissue sample (200 µl each) were pipetted into the 96 well plate for reading. The optical density (OD) of the samples was determined at 570 nm. The % tissue viability was determined for each tissue using the equation below:

% tissue viability = 100 × [OD corrected (sample)/OD corrected (negative control)].

From the dose response curve, precise ET-50 values were determined. According to the manufacturers' guideline, the Benchmark ET-50 values and groupings in Table 2 were used to categorize the *in vivo* irritancy responses.

3. Results

In this study, 60 cosmetic end-products were evaluated with *in vitro* ET-50 test using EpiDerm™ Skin Models, according to the manufacturer's protocol. The cell viability was determined at the end of each exposure time and the ET-50 values were derived from the cytotoxicity curves of % MTT viability vs. exposure times. ~3% of products were detected as strong/severe irritant/possible corrosive (1 shampoo and 1 hair lotion), 42% of test materials were moderately skin irritating (22 shampoos, 1 hair cream, 1 cleanser, 1 depilatory), 28% of test materials were moderate to mild skin irritating (11 shampoos, 1 hair lotion, 1 hair serum, 1 skin creams, 2 depilatories, 1 shaving product), 7% were very mild skin irritating (1 hair cream, 2 masks, 1 medical cream) while 20% of the test products were non-irritating (2 herbal hair oils, 2 skin creams, 4 soaps, 1 medical cream, 1 depilatory, 2 shaving products). According to the results (Table 3), approximately half of total products were found as moderate skin irritant, as also shown in Fig. 2.

Table 2
Benchmark ET-50 values and correlation of *in vitro* and *in vivo* results.

ET-50 (hrs)	In vivo skin irritating potential
< 0.5	strong/severe irritant, possible corrosive
0.5–4	moderate irritating
4–12	moderate to mild irritating
12–24	very mild irritating
24	non-irritating

ET-50: Effective time 50.

The skin irritation potential of 34 shampoos, which were the majority of the hair care products, were evaluated in this study. 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. One hair cream was very mild skin irritant while the other one was found as moderate skin irritant. Among three herbal hair oils, one was found to be strong/severe skin irritant/possible corrosive while two were found to be non-irritating. One hair serum and one hair lotion were found to be moderate to mild skin irritant.

Among six skin care products; three were skin creams. Two of these creams were determined as non-irritant while one of them was found to be moderate to mild skin irritant. Among other skin care products, two skin masks were found to be very mild skin irritant while one cleanser was found to be moderate skin irritant. On the other hand, four soaps were found to be non-irritant. Among two medical creams, one (a baby care cream) was found to be non-irritant and the other cream was detected as very mild skin irritant.

In this study, four depilatories were tested. One was found to be non-irritating while two were found as moderate to mild skin irritant and one was moderate skin irritant. Among shaving products, two of them were detected as non-irritating while one was moderately to mildly irritating. Skin irritating potentials of all of the tested products are shown in Fig. 3.

4. Discussion

The human skin is a very good barrier that is protective against environmental factors and different chemicals present in

Table 3
Skin irritation categories of test materials.

In vivo Irritancy/ET-50 (hrs)	Total Quantity	Non-irritating/24 h	Very mild/ 12–24 h	Moderate to mild/ 4–12 h	Moderate/0.5–4 h	Strong/severe irritant, possible corrosive < 0.5 h
Product Category						
Hair Care Products						
Shampoos	**34**			**11**	**22**	**1**
Hair Cream	**2**		**1**		**1**	
Herbal Hair Oils	**3**	**2**				**1**
Hair Serum	**1**			**1**		
Hair Lotion	**1**			**1**		
Skin Care Products						
Creams	**3**	**2**		**1**		
Mask	**2**		**2**			
Cleanser	**1**				**1**	
Soaps	**4**	**4**				
Medical Creams	**2**	**1**	**1**			
Depilatories	**4**	**1**		**2**	**1**	
Shaving products	**3**	**2**		**1**		
TOTAL	**60**	**12**	**4**	**17**	**25**	**2**

pharmaceutical formulations and cosmetic products. Evaluation of the skin irritation and/or corrosion potential of the ingredients of a cosmetic product is necessary in order to assess its toxic effects and safety (Macfarlane et al., 2009). Skin irritation can be seen after dermal use of cosmetic and personal care products (Steinhoff et al., 2001; Berardesca and Distante, 1994; OECD, 2002). Other than skin irritation, allergic reactions and acne can also be seen after the use of cosmetics (Andersen, 1986). Evaluating the skin irritant and/or corrosive properties of the end product can also be helpful in describing the potential hazard.

As indicated above, before March 11, 2013, the skin irritant and corrosive potentials of the cosmetic products were evaluated by animal experiments in the both European Union and Turkey. However, on this date, the commercial sale of any type of cosmetics and personal care products that were tested on animals was banned in the European Union as well as in Turkey. Since then, the safety evaluation of all cosmetic products and personal care products is being performed by using alternative *in vitro* toxicity tests (EC, 2009, 2013; TMMDA, 2005). Turkey is now trying to apply European Union regulations for cosmetics and personal care products. Turkish Medicines and Medical Devices Agency (TMMDA) Cosmetic Control Laboratories are responsible for the safe use of cosmetic products throughout the country. TMMDA authorized laboratories conducts skin irritation/corrosion tests on the complaint products and due to the product's irritation potential (moderate irritating or strong/severe irritant, possible corrosive), TMMDA may take the products off the market (TMMDA, 2016). However, there are no strict rules to prepare a full dossier with the skin irritation, phototoxicity and cytotoxicity results of a local product that will be

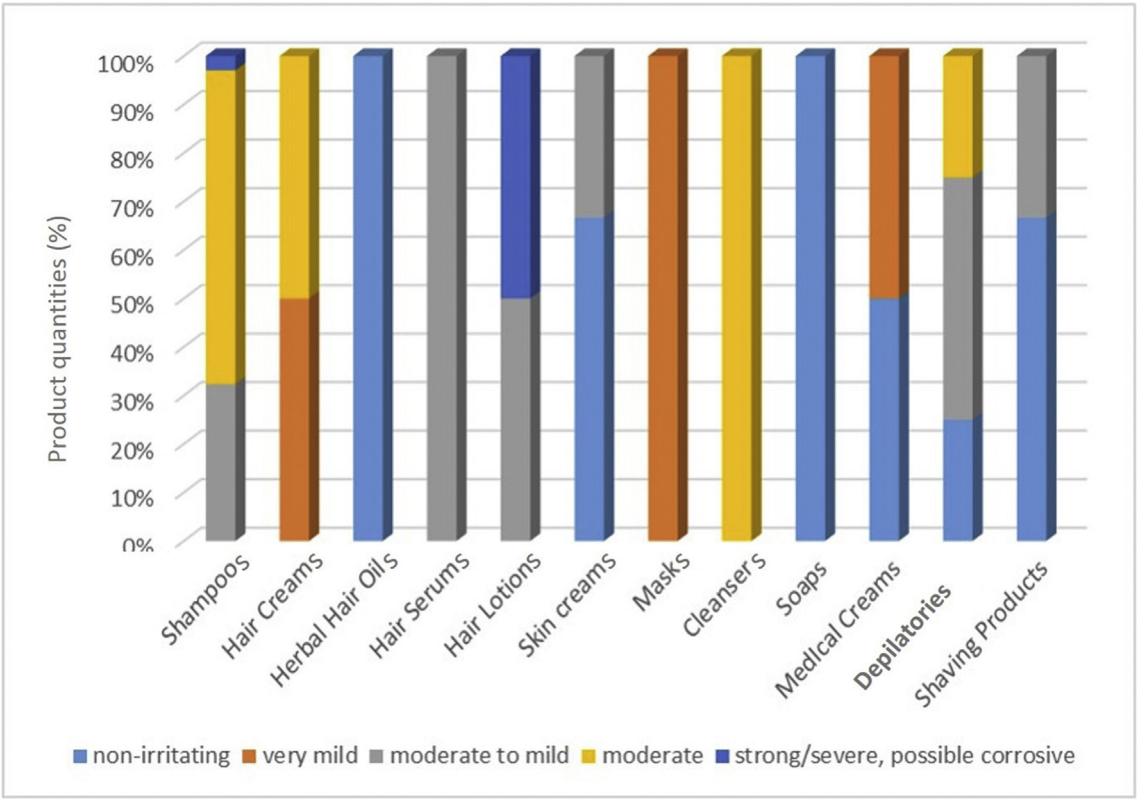

Fig. 3. The irritancy potentials of different categories of test materials.

released to the market. Therefore, Turkey still has a long way to go in order to implement strict rules for the cosmetics that are produced within the country. In addition, Turkey needs to implement a system in order to control the composition of the exported products from developing countries.

In the last ten years, the *in vitro* 3D RhE models are preferred as alternative methods because of their morphological and physiological similarities to the human skin. Among those, the EpiDerm Skin Irritation test (EpiDerm SIT) was developed and validated for *in vitro* skin irritation testing of chemicals, including cosmetic and pharmaceutical ingredients and medical device extracts. In this RhE model, liquid, semi-solid, waxy and solid test products can be used. MTT assay, which is both sensitive and non-time consuming, is used to evaluate the tissue viability and researchers can discriminate between irritants of GHS category 2 and non-irritants. This procedure can be used as full replacement of the *in vivo* rabbit skin irritation test for hazard identification and labeling of chemicals in line with EU regulations (Kandárová et al., 2009; De Jong et al., 2018; Coleman et al., 2018).

In Turkey, cosmetic market is growing rapidly, as well as by 10% every year. Only 10% of the products marketed in Turkey are produced within the country. The biggest market share of the cosmetic sector belongs to hair care products, with a market share of 59% belonging to shampoos. Other than hair care products, shaving products, depilatories, bath and shower products, hand soaps, lip and eye makeup products, deodorants, anti-perspirants, perfumes, colognes and baby care products are highly consumed in Turkey. Natural cosmetic products have a market share of 5% among all cosmetics (Turkish Government Ministry of Economy, 2016). Due to the economic conditions, most people in lower and middle-class use limited number of cosmetic and personal care products in Turkey and as shampoos are indispensable for personal care. Therefore, the product sampling in this study can be described as a good representative of cosmetic/personal care products consumed in Turkey.

To our concern, there is not any study in literature that evaluates the skin irritation potential of cosmetic and personal care products marketed in Turkey and in Middle East by using alternative test methods, particularly by Epiderm RhE model. In this study, we observed that only 20% of the cosmetic products are non-irritating. However, 70% of the cosmetic products marked in Turkey are either moderate to mild skin irritant or moderate skin irritant. These products can be problematic among individuals with very sensitive skin and also among babies and children as parents sometimes use adult skin care or hair care products for this susceptible population. The most remarkable finding of this study was %3 of products were detected as strong/severe irritant/possible corrosive. These products can even cause more striking effects insusceptible populations like children. Therefore, the importance of end-product testing for skin irritation and corrosion should once more be highlighted. However, specifically in our country, reporting the adverse effects related to cosmetic usage is extremely rare. The underlying reason is that the mild and moderate reactions are usually identified and cured by the user himself.

Post-marketing vigilance systems are important for both drugs and cosmetics. Cosmetovigilance is a recent concept that is a form of health surveillance and it mainly deals with the rate of adverse reactions caused by the use of cosmetic products. As the adverse reactions caused by cosmetics is usually underestimated, self-diagnosed and self-medicated, the users do not frequently report the adverse reactions, including mild to moderate skin irritation. Therefore, these types of toxic effects are usually underrated (Sautebin, 2008). Many countries in Europe (including Belgium, Estonia, Italy, Norway and Portugal) are trying to implant their cosmetovigilence systems while Germany and Sweden already have the system (Sautebin, 2008). Turkey is trying to implant her own cosmetovigilence system in the last years. However, there is still so much work to be done (TMMDA, 2016).

There are different surveys conducted in different parts of the world, concerning the adverse effects caused by cosmetic products. However, to our concern there is not any survey or study that mentions the adverse effects (including skin irritation) caused by the use of cosmetic or personal care products. In Italy, a survey was conducted on 4373 people; in which 845 (19.3%) of them later rejected to fill in the survey. The rest of the people admitted to be included in the survey. 81.9% of the females and 78.6% of the males accepted to participate ($p < 0.05$). The adverse effects were mostly caused by creams, emulsions and oils for the skin (23.6%), followed by toilet soaps and underarm cosmetics (22.2%) and make-up products and make-up removal products (12.1%). The mostly encountered adverse effects were divided into two groups: cutaneous (95.6%, seen in 1445 cases) and systemic. Mostly observed cutaneous effects were burning (36.3%), itching (31.5%) and eczema (23.8%). Mostly observed systemic effects were headache (40.3%), nausea (24.2%) and dizziness (14.5%). 59.5% of the participants did not seek for help while 28.7% asked for consultation to a medical specialist and 8.3% asked for consultation to a general practitioner. 44.6% of the participants changed the product they were using while 27.2% discontinued to use the product that caused adverse effect/s (Di Giovanni et al., 2006). A study conducted in Netherlands evaluated the reports obtained from customers (n = 1294), general practitioners (n = 153) and dermatologists (n = 163). Mostly reported adverse effects were on eyes/eyelashes, face, neck, scalp, armpit, arms and lips and the highly encountered undesired effects were erythema, itching, burning sensation, edema, scaling and pain. The reports highly mentioned that make-up products, moisturizers, deodorants, hair care products, cleansing products, sunscreen products, soaps (including bath and shower products), shaving products, childcare products and dental products could cause adverse effects. Dermatologists mostly reported adverse effects of soaps and hair care products whereas consumers highly reported adverse effects of using make-up products, moisturizers and deodorants. The ingredients that were suggested to cause the adverse effects were isothiazolinones (MI or MCI/MI mix), fragrance mix I, cocamidopropyl betaine, nickel sulfate (although prohibited in European Union), fragrance mix II, methyldibromo glutaronitrile, *p*-phenylenediamine, colophonium, cobalt chloride, hydroxyisohexyl 3-cyclohexene carboxaldehyde, quaternium-15 and formaldehyde (Salverda et al., 2013). The results of another study performed in Jigjiga Town (Eastern Ethiopia) among 559 residents (76% were female) suggested that shampoo, deodorant, face powder, lipstick, hair cosmetics, toothpaste, eye make-up products, skin colorants, nail polishes and soaps were highly used for the purposes of cleansing, beautification, protection, whitening, hair coloring and anti-aging. 44% of the participants reported they used herbal preparations among with cosmetics as well. The mostly encountered adverse effects were allergic reactions (~35%), appearance of acne (~15%), hirsutism (~13%), skin thinning (~10%), hair breakage (~9%) and skin soreness (~5%), discoloration of face (~5%). These studies provide information on the dermal irritating potentials of different cosmetic products. The results of such studies will be useful in evaluating the safety potentials of different ingredients present in cosmetic and personal care products (Bilal et al., 2016).

To our knowledge, this is the first study that evaluates the skin irritation potentials of final cosmetic products marketed in Turkey, with alternative *in vitro* methods. Such studies should be encouraged by TMMDA and health authorities in Turkey as they can shed a light on the adverse skin effects of marketed products. Authorities should consider asking for skin irritation, phototoxicity and cytotoxicity tests for the ingredients as well as for the end-products before a product is being marketed. The producers should be forced to prove the safety of their end-products by using validated *in vitro* alternative tests.

This study has some limitations: Products could have been tested with both ET-50 and with Epiderm SIT 200 protocols. On the other hand, due to the high amount of tax taken from the exportation of Epiderm kits as well as due to their high price, we have only worked with 60 samples. However, these samples were chosen among the highly consumed cosmetic products.

In conclusion, we can suggest that alternative *in vitro* tests should continuously be used to test both the ingredients and the final formulations. Although all of the ingredients should be tested for skin irritation before getting marketed by the producers, end-product tests are not usually performed by most of the cosmetic companies. Governments should encourage and perhaps entail cosmetic final formulation skin irritation/corrosion tests before getting license or permission for marketing. Moreover, marketing controls should be performed periodically on end-products and reference laboratories should be authorized to analyze both the ingredients and the final formulations by alternative skin irritation/corrosion methods, particularly in developing countries like Turkey. This type of testing beforehand will limit the adverse effects caused by the use of cosmetic end-products and personal care products.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

Andersen, K.E., 1986. Testing of cosmetics and toiletries. Acta Physiol. Scand. 554, 180–190.

Berardesca, E., Distante, F., 1994. The modulation of skin irritation. Contact Dermatitis 31 (5), 281–287.

Bilal, A.I., Tilahun, Z., Shimels, T., Gelan, Y.B., Osman, E.D., 2016. Cosmetics utilization practice in Jigjiga Town, eastern Ethiopia: a community based cross-sectional study. Cosmetics 40, 1–10.

Coleman, K.P., Grailer, T.P., McNamara, L.R., Rollins, B.L., Christiano, N.J., Kandárová, H., De Jong, W.H., 2018 Aug. Preparation of irritant polymer samples for an in vitro round robin study. Toxicol. In Vitro 50, 401–406.

De Jong, W.H., Hoffmann, S., Lee, M., Kandárová, H., Pellevoisin, C., Haishima, Y., Rollins, B., Zdawczyk, A., Willoughby, J., Bachelor, M., Schatz, T., Skoog, S., Parker, S., Sawyer, A., Pescio, P., Fant, K., Kim, K.M., Kwon, J.S., Gehrke, H., Hofman-Hüther, H., Meloni, M., Juliusi, C., Briotet, D., Letasiova, S., Kato, R., Miyajima, A., De La Fonteyne, L.J.J., Videau, C., Tornier, C., Turley, A.P., Christiano, N., Rollins, T.S., Coleman, K.P., 2018 Aug. Round robin study to evaluate the reconstructed human epidermis (RhE) model as an in vitro skin irritation test for detection of irritant activity in medical device extracts. Toxicol. In Vitro 50, 439–449.

Di Giovanni, C., Arcoraci, V., Gambardella, L., Sautebin, L., 2006. Cosmetovigilance survey: are cosmetics considered safe by consumers? Pharmacol. Res. 53 (1), 16–21.

Draize, J.H., Woodard, G., Calvery, H.O., 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Therapeut. 82 (3), 377–390.

EC (European Commission), 2010. Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union. 276, 33–79.

EC (European Commission), 2013. European Commission. Communication from the commission to the European Parliament and the council on the animal testing and marketing ban and on the state of play in relation to alternative methods in the field of cosmetics. http://ec.europa.eu/consumers/sectors/cosmetics/files/pdf/animal_testing/com_at_2013_en.pdf, Accessed date: 11 February 2018.

EC (European Commission), 2009. Regulation (EC) No 1223/2009 of the european parliament and of the council of 30 november 2009 on cosmetic products. Official Journal of the European Union. http://ec.europa.eu/health//sites/health/files/ndocrine_disruptors/docs/cosmetic_1223_2009_regulation_en.pdf, Accessed date: 11 February 2018.

Jírová, D., Basketter, D., Liebsch, M., Bendová, H., Kejlová, K., Marriott, M., Kandárová, H., 2010. Comparison of human skin irritation patch test data with in vitro skin irritation assays and animal data. Contact Dermatitis 62 (2), 109–116.

Kandárová, H., Letašiová, S., 2011. Alternative methods in toxicology: pre-validated and validated methods. İnterdisciplinary Toxicol 4 (3), 107–113.

Kandárová, H., Hayden, P., Klausner, M., Kubilus, J., Sheasgreen, J., 2009. An in vitro skin irritation test (SIT) using the EpiDerm reconstructed human epidermal (RHE) model. JoVE. https://doi.org/10.3791/1366. pii: 1366.

Macfarlane, M., Jones, P., Goebel, C., Dufour, E., Rowland, J., Araki, D., Costabel-Farkas, M., Hewitt, N.J., Hibatallah, J., Kirst, A., McNamee, P., Schellauf, F., Scheel, J., 2009. A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: skin irritation. Regul. Toxicol. Pharmacol. 54 (2), 188–196.

MatTek, 2010. Protocol for: MTT Effective Time-50 (ET-50) for Use with EpiDerm™ Skin Model (EPI-200), Ashland, MA, USA.

Monterio-Riviere, N., 2009. In: Riviere, J. (Ed.), Structure and Function of Skin. Dermal Absorption Models in Toxicology and Pharmacology. CRC Press, Taylor and Francis Group, New York, NY, pp. 1–19.

OECD (Organisation for Economic Co-operation and Development), 2002. Guideline for the Testing of Chemicals. OECD Test Guideline (TG) 404. Acute Dermal Irritation/Corrosion. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg404.pdf, Accessed date: 11 February 2018.

OECD (Organisation for Economic Co-operation and Development), 2013. Guideline for the testing of chemicals. In: Vitro Skin Irritation: Reconstructed Human Epidermis Test Method, . https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg439-2013-508.pdf, Accessed date: 11 February 2017.

Salverda, J.G.W., Bragt, P.J.C., de Wit-Bos, L., Rustemeyer, T., Coenraads, P.J., Tupker, R.A., Kunkeler, L.C., Laheij-de Boer, A.M., Stenveld, H.J., van Ginkel, C.J., Kooi, M.W., Bourgeois, F.C., van Gorcum, T.F., van Engelen, J.G., van Dijk, R., de Graaf, J., Donker, G.A., de Heer, C., Bruynzeel, D., 2013. Results of a cosmetovigilance survey in The Netherlands. Contact Dermatitis 68 (3), 139–148.

Sautebin, L., 2008. Understanding the adverse effects of cosmetics: a pilot project in cosmetovigilance. Drug Saf. 31 (5), 433–436.

SCCP (Scientific Committee on Consumer Products), 2006. THE SCCP'S Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation. 6th Revision. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp _o_03j.pdf, Accessed date: 11 February 2018.

Steinhoff, M., Brzoska, T., Luger, T.A., 2001. Keratinocytes in epidermal immune responses. Curr. Opin. Allergy Clin. Immunol. 1, 469–476.

TMMDA (Turkey Medicine and Medical Devices Agency), 2005. Guideline on Promotion Actions Cosmetic Products. https://www.titck.gov.tr/PortalAdmin/Uploads/UnitPageAttachment/TbYc1abr.pdf.

TMMDA (Turkey Medicine and Medical Devices Agency), 2016. Guideline on the notification of adverse effects/serious adverse effects of cosmetic products. Available from. https://www.titck.gov.tr/PortalAdmin/Uploads/UnitPageAttachment/U7mT8sjY.pdf.

Turkish Government Ministry of Economy, 2016. Cosmetic sector. Available from. http://www.ekonomi.gov.tr/portal/content/conn/UCM/uuid/dDocName:EK-051185, Accessed date: 11 February 2018.