Cytotoxic Effects of Ceteareth-20 and Paraffinium Liquidum

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Abstract

Aim: The aim of this study is to investigate the effects of ceteareth-20 and paraffinium liquidum on cell viability and cytotoxicity in human lymphocyte in vitro.

Methods: We studied the cytotoxic and inhibitory effects of ceteareth-20 and paraffinium liquidum on cell proliferation using lactate dehydrogenase (LDH) assay and cell proliferation (WST-1) assay.

Results: The cytotoxicity was enhanced when cells were treated with 1%, 5%, 25% and 50% paraffinium liquidum dilutions (p < 0.05). Moreover, cell number significantly reduced after 24 hours when they were treated with the same concentrations of paraffinium liquidum (p < 0.05). On the other hand, ceteareth-20 at concentrations at 1%, 5%, 25% and 50% showed no significant cytotoxic effect. These results showed that paraffinium liquidum dilutions have cytotoxic and proliferative effect otherwise ceteareth-20 dilutions have only proliferative effects on cultured human lymphocytes.

Conclusion: It can be said that paraffinium liquidum and ceteareth-20 is harmful to use in beauty and cosmetic products. Future studies might allow alternative agents to be used instead of paraffinium liquidum and ceteareth-20.

Keywords
Paraffinium liquidum, Ceteareth-20, Cytotoxicity

Introduction

The perception of beauty is being impacted by the culture, society and geography, changing and evolving continuously. There are a lot of beauty and personal care products available, but these products are mainly used by people without paying much attention to the chemicals listed in the ingredients. Currently, a lot of cosmetic products include chemical ingredients to increase their cosmetic properties, conserve their effectiveness, and generate a more viable product. Unconscious use of these chemical ingredients can increase the risk for sensitization [1,2]. Previous studies showed that chemical ingredients in cosmetic products have a potential to cause numerous health problems including irritation [3], inflammation [4], allergic contact dermatitis [5]. Some of the chemical additives used in beauty and personal care products were determined as carcinogenic in animals and in vitro [6-8]. Although the cosmetic product is applied in very low amount or the chemical ingredient present at low concentrations within the product, the product can cause unwanted skin reactions or even more serious health problems [9]. However, many chemical additives are not investigated in terms of their genotoxicity and cytotoxicity.

Paraffinum liquidum is a widely used chemical ingredient in cosmetic and personal care products. It has many common names used in the industry such as heavy mineral oil, light mineral oil, liquid paraffin, liquid petrolatum, mineral oil mist, paraffin oil, mineral oil, petrolatum liquid, petroleum oil, white mineral oil and white oil [10]. Paraffinum liquidum has a complex combination of highly refined, saturated branched-chain and napthenic hydrocarbons that are used in medicinal cosmetics, food and pharmaceuticals [11]. Molecular weight of paraffinum liquidum is determined as 423 g/mol and there is no genotoxicity and cytotoxicity studies in the literature. Another substance of unknown
genotoxicity and cytotoxicity is ceteareth-20, which is the polyethylene glycol ether of cetearyl alcohol. It is known as PEG-20 cetostearyl alcohol, peg-20 cetyl/stearyl ether, polyethylene glycol 1000 cetyl/stearyl ether, polyoxyethylene (20) cetyl/stearyl ether ceteareth. Ceteareth-20 has a molecular weight of 70.49 g/mol and it is dissolved in water and alcohol to form a colloid solution. Commercial Ceteareth-20 might contain toxic impurities such as 1,4-dioxane [12] and it is used by cosmetic products such as hair dyes [13], shampoos [14], body creams [15]. The aim of this study is to examine the effects of ceteareth-20 and paraffinium liquidum which are content of water based hair dyes, on cell viability and cytotoxicity in human lymphocyte in vitro.

Material and Methods

RPMI, FBS, Ficoll, L-glutamine, Penicillin, Streptomycin were obtained from Biochrom AG (Mannheim, Germany) and Biological Industries (Kibbutz Beit Haemek, Israel). WST-1 and LDH kits were purchased from Roche (Mannheim, Germany). Ceteareth-20 and paraffinium liquidum were provided by Doga Pharmacy Company, Istanbul.

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from heparinized blood samples using Ficoll solution by density gradient centrifugation. RPMI1640 medium with 2 mmol/L L-glutamine, 10% fetal bovine serum and antibiotics (penicillin and streptomycin) were added into PBMCs. Cells were incubated in the presence of 0.5% CO₂ at 37 °C at 1.0 × 10⁶ cells per mL with Ceteareth-20 and Paraffinium liquidum concentrations of 1%, 5%, 12.5%, 25% and 50% for 24 and 48 hours, respectively [16].

Cytotoxicity assay (LDH Assay)

Cytotoxic effects of Ceteareth-20 and Paraffinium liquidum extracts were shown by the activity of released lactate dehydrogenase (LDH) from damaged cells. Cells were added as 1 × 10⁶ cell/mL in 96-well plate. Cells were incubated with different concentrations of ceteareth-20 and paraffinium liquidum at 37 °C and 5% CO₂ for 24 and 48 hours, respectively. The procedure of LDH kit (LDH; Roche) was implemented and the reaction mixture was put into all wells (100 μL). Then, they were incubated for 30 minutes at room temperature. The 96-well plate was incubated in the darkness 24th and 48th hours and absorbance was measured at 490 nm with ELISA reader (BioTek-PowerWaveS, USA). Assays were duplicated. Cytotoxicity was analyzed according to the following formula: Cytotoxicity (%) = (experimental value - negative control)/(positive control - negative control) × 100 [17].

Proliferation assay (WST-1)

The cell viability was calculated by using WST-1 assay and cells were added as 1 × 10⁶ cell/mL equality in 96- well plate. They were incubated with different concentrations of Ceteareth-20 and Paraffinium liquidum at 37 °C and 5% CO₂ for 24 h. WST-1 was put in a 10 μL/well volume. All procedures were implemented according to commercial company procedure. Then the cells were incubated for 4h in a humidified atmosphere (37 °C, 5% CO₂). Absorbance of samples was calculated at 420 nm using ELISA reader (BioTek-PowerWaveXS, USA). Means ± SD values were shown in Figure 1 and Figure 2 [18,19].

Statistical Analysis

LDH activity and WST-1 assay results were analysed...
analysis showed a significant difference in cell viability between the Paraffinium liquidum treated group and control group after 24 hours of incubation. WST-1 results show that the cell proliferation was decreased when cells were treated with both Paraffinium liquidum and ceteareth-20 concentrations after 24 hours (P < 0.05).

**Cytotoxicity effect (LDH)**
- ap < 0.05 compare with control group,
- bp < 0.05 compare with PD-1-treated group,
- cp < 0.05 compare with PD-2-treated group,
- dp < 0.05 compare with PD-3-treated group,
- ep < 0.05 compare with PD-4-treated group.

**Cell viability (WST-1)**
- ap < 0.05 compare with control group,
- bp < 0.05 compare with PD-1-treated group,
- cp < 0.05 compare with PD-2-treated group,
- dp < 0.05 compare with PD-3-treated group,
- ep < 0.05 compare with PD-4-treated group.

**Discussion**

Beauty and personal care products are directly contacted to the skin and exposure is the most significant topic [20]. People are used to a lot of beauty and personal care products.
care products in daily life and these products contain many chemical additives. Many researchers have indicated that cosmetic products can contain potential toxic agents [21-23]. Approximately 10,000 ingredients are found in beauty and personal care products and can be directly associated with too many diseases such as cancer, genetics disorders and birth defects. US FDA (Food and Drug Administration) forbid some chemical additives like glycol, lead, mercury, formaldehyde [24].

Cytotoxicity and genotoxicity of cosmetic products can be described, distinguished and evaluated both in vivo and in vitro methods [25-27]. Thus, we studied the cytotoxic and inhibitory effects of ceteareth-20 and paraffinum liquidum on cell proliferation, which were evaluated using LDH assay and WST-1 assay in this study. Ceteareth-20 and paraffinum liquidum are found in many beauty and personal care products, but there is no previous investigation of their inhibitory effects on cell proliferation. Baby oils, body and face creams, hair dyes, shampoos contain paraffinum liquidum and ceteareth-20. Many famous brands use them, because they are not soluble in the water. Some researchers have thought that paraffinum liquid is a probable carcinogen or cancer-causing agent which it contains 1,4 dioxane [28]. Ceterareth-20 is used in many application that increases the viscosity [29]. In our study; paraffinum liquidum dilutions had cytotoxic and proliferative effect otherwise ceteareth-20 dilutions have only proliferative effects on human cultured lymphocytes. On the other hand, ceteareth-20 at concentrations 1%, 5%, 25% and 50% have no significant cytotoxic effect but it has only proliferative effects on cultured human lymphocytes. Because of its molecular formula and it can be bind cell membrane on human cultured lymphocytes.

Conclusion

As a consequence we could say that paraffinum liquidum and ceteareth-20 can be harmful to use in water based beauty and cosmetics cosmetic products. So instead of paraffinum liquidum and ceteareth-20, alternative agents can be used. Next research on paraffinum liquidum and ceteareth-20 will be study by confirming our results with using genotoxicity assays.

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Conflict of Interest

There are no conflicts of interest.

References


