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Toxicity and Genotoxicity of Beauty Products on Human Skin Cells *In Vitro*

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Abstract

Background: We use beauty products in high quantities every day and in the process, we are exposed to a wide variety of chemicals used in these products. These chemicals are a particularly insidious form of body pollution because they enter the human body through multiple routes. The problem with commercial products and particularly beauty products is that millions of people apply beauty products to their skin daily for long time.

Objective: To determine the toxicity and genotoxicity effects of four facial beauty products on tow human skin cells. Also, to find out which product ingredients can induce the most toxicity and genotoxicity on human skin cells.

Methodology: The *in vitro* toxicity and genotoxicity of facial beauty products were determined using a human keratinocyte cell line (HaCaT) and a human fibroblast cell line (CCD-1064SK). The products were an Anti-aging face moisturiser with mixture of natural ingredients (Facial Moisturizer - Camellia & Geranium Blossom) and Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals with TiO_2 and Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals without TiO_2. Glycerol was the negative control. Toxicity was measured by Crystal violet assay and Methyl tetrazolium cytotoxicity (MTT) assay. Apoptosis/necrosis proportion, nuclear division index (NDI) and genotoxicity were detected by cytokinesis block micronucleus (CBMN) assay.

Results: Glycerol did not induce any toxicity or genotoxicity. Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals with and without TiO_2 showed significant toxicity in both assays. No toxicity observed with Facial Moisturizer - Camellia & Geranium Blossom but there was a significant necrosis. Populations of cells treated with diluted Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals with and without TiO_2 showed increased proportions of apoptosis/necrosis. The nuclear division index (NDI) was decreased by Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals with and without TiO_2 and Facial Moisturizer - Camellia & Geranium Blossom. Nivea Visage Q10plus Anti-Wrinkle which includes synthetic chemicals with and without TiO_2 showed increased frequency of micronuclei (MNi). Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 proved to induce significantly more micronuclei (MNi) than the product without TiO_2.

Conclusion: The study results indicate that facial beauty products can cause cytotoxicity and genotoxicity *in vitro* using dilutions of the commercial formulations.

Keywords: Toxicity; Genotoxicity; Safety assessment; Beauty products; Cosmetic ingredients; Cell culture; Chromosomal damage

Introduction

We use large quantities of beauty products every day and, in the process, are exposed to a wide variety of chemicals used in these products. These chemicals are a particularly insidious form of body pollution because they enter the human body through multiple routes. It is easy to swallow them, inhale them and absorb them through the mucous membrane of the eyes, mouth or nose. Our skin absorbs approximately 60% of the chemical ingredients and sends them into the bloodstream, from whence they can reach every organ in the body seconds after absorption [1]. Women using a lot of cosmetics are thought their skin absorb up to 2 kg of chemical cosmetic ingredients each year [1]. Government reports in the US and EU indicate that about 90% of the ingredients used in cosmetics are not safe for people in the long-term [1]. Most beauty products contain a mixture of chemicals that only make the problem worse [2]. Unfortunately, the companies that make them are self-regulating, and government agencies do not press the manufacturers to prove their products are safe [1,2]. In the US, cosmetic and personal care products are not regulated by the Food and Drug Administration (U.S. FDA) [3,4]. However, drugs do require extensive testing and approval by the FDA [3]. Also, one study has noted the results of studies screening blood sample from over the entire world, indicate that most people are carrying a huge amount of chemicals in their bodies [1,5]. These studies used biochemical methods for screening. Another study have shown that exposure to chemicals demonstrates that most American children and adults carry inside them nearly 100 substances or chemicals including pesticides and toxic compounds [5]. Many of these cause cancer, damage the immune system and affect human behaviour and the central nervous system. The sources of these chemicals include household exposure to pesticides and detergents, cosmetics, toiletries, paints and fabric treatments [1,2,6]. They can affect the body over the long term and accumulate in different organs and the bloodstream and then pass through the urine, semen and in the form of breast milk. After a while and the body becomes

overloaded and at risk of total breakdown [1]. Some cosmetics contain mercury which is used to lighten the skin and people who use products containing mercury are at a high risk of mercury poisoning [7]. In the United States mercury compounds are used as preservatives in small concentrations for eye area products and FDA regulations in the US have restricted cosmetics products that contain mercury [7]. Some moisturizers contain mineral oil which can slow down cell renewal and promote early skin ageing [1]. A study tested 88 brands of eye shadow and found that approximately 75% of these products contained at least one of the 5 elements: lead, nickel, chromium, arsenic or cobalt [8]. Lead can damage any part of the human body and in particular the nervous system [9]. Even the elements found in small doses in these products may cause hormone disruption [10]. Some sun blocks and moisturizers with sun blocks contain Titanium dioxide (TiO_2) which is a potential hazard and carcinogen [11,12]. Finally, most shampoos and other toiletries or liquid formulas contain nitrosamines that can cause cancer [13]. Some products are labelled as hypoallergenic but probably still contain potentially carcinogenic substances [14]. In this study, four different facial beauty products were examined to assess the effects on two human skin cells (Human keratinocytes HaCaT skin cells and human fibroblast CCD-1064SK cells). Products were Nivea Visage Q10 Plus Anti-Wrinkle face moisturizer which includes synthetic chemicals + TiO_2, Nivea Visage Q10 Plus Anti-Wrinkle face moisturizer which includes synthetic chemicals (Improved formula, without TiO_2), Facial Moisturizer - Camellia & Geranium Blossom which includes a mixture of natural ingredients and Glycerol B.P.

Ingredients	Toxic effects
Octocrylene	Skin allergen. Restricted for use in cosmetics in Japan. Produces excess ROS that can interfere with cellular signalling, cause mutations, lead to cell death and may be implicated in cardiovascular disease. Measured to accumulate in people.
Ethylhexyl Salicylate	Low allergies and immunotoxicity, ecotoxicology
Methylpropanediol	Not expected to be potentially toxic or harmful
Glyceryl Stearate	Suspected to be an environmental toxin
Butyl Methoxydibenzoylmethane	Toxin in mice
C12-15 Alkyl Benzoate	Suspected to be an environmental toxin
Tocopheryl Acetate	Human skin toxin or allergen—strong evidence. Has caused tumours in animals.
Chondrus Crispus	Organ system toxin (non-reproductive)
Dimethicone	Organ system toxin (non-reproductive)
Trisodium EDTA	Penetration enhancer
Caprylic/Capric Triglyceride	Ecotoxin
Limonene and Parfum	Irritant. Possible human immune system toxin or allergen. Restricted in cosmetics
Ingredients	Toxic effects
Methylparaben	Human endocrine disruptor-strong evidence
Phenoxyethanol	Irritant (skin, eyes or lungs), occupational hazard, organ system toxin (non-reproductive)
Cera Microcristallina	Organ system toxin (non-reproductive)
Paraffinum Liquidum	Human immune and respiratory toxin or allergen—strong evidence
Benzyl Alcohol	Occupational hazard, organ system toxin (non-reproductive)
TiO_2	Carcinogen
Thylhexylglycerin	Irritant (skin, eyes or lungs); organ system toxin (non-reproductive)
Carbomer	No carcinogenicity data available, but it is found to be irritating to the respiratory tract.
Sodium Phenylbenzimidazole Sulfonat	May cause skin irritation, if swallowed will cause vomiting.
Trimethoxycaprylylsilane	Not expected to be potentially toxic or harmful

Table 1: The ingredients and toxic effects of Nivea Visage Q10Plus Anti-Wrinkle face moisturizer + TiO_2. The toxic effects of the ingredients were classified by [39-41].

Materials and Methods

Materials

RPMI 1640 media and foetal bovine serum (FBS) were purchased from Gibco® Cell Culture Media - Life Technologies (Australia). Cytochalasin B (Cyt-B) solution, Sodium dodecyl sulphate (SDS, approximately 99%), Phosphate buffered saline (PBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). Spectrophotometer plate reader (BIO-TEK Instruments Inc., USA). Diff-Quik stains were purchased from Lab Aids (Australia). Cytospin centrifuge (Shandon, England). TrypLE™ was purchased from Life Technologies (Australia). All other reagents were obtained from sigma, unless otherwise stated.

Products to be examined

Nivea visage Q10Plus Anti-wrinkle face moisturiser with titanium dioxide (TiO_2)

Nivea visage Q10plus Anti-Wrinkle day moisturizer cream plus extra UVA protection (SPF 15) is produced by Nivea which is a worldwide company. Nivea Visage Q10plus Anti-Wrinkle purchased from a local pharmacy in Adelaide, South Australia. It suits all skin types. The product aims to increase the natural Q10 level and prevent wrinkles. Also, it is protecting from UVA+UVB. The ingredients of Nivea Visage Q10plus are mixture of chemicals (Table 1).

This formula of the product contains Titanium Dioxide (TiO_2) which is a nanoparticle that is used widely in pigments, cosmetics, and skin care products because it the benefit of protecting the skin from UV light, particularly in Nano sized particles less than 100 nm [15]. Titanium Dioxide (TiO_2) has been classified as carcinogen [16]. Some studies have shown that Titanium Dioxide TiO_2 can damage DNA directly or indirectly via inflammatory response or oxidative stress [15].

Nivea Visage Q10Plus Anti-Wrinkle face moisturizer (Improved formula, without titanium dioxide (TiO_2)

This product is an improved formula of Nivea Visage Q10plus Anti-Wrinkle day moisturizer. It is released into the market after removed the original product which contains Titanium Dioxide TiO_2. It has almost the same ingredients as the original one except for the absence of TiO_2. The Package is labelled with 'skin compatibility dermatologically approved'. This product aims to UVA protection. The product is suitable for sensitive skin.

Grown facial moisturizer - camellia & geranium blossom

Facial Moisturizer - Camellia & Geranium Blossom is a natural moisturizer made from bioactive ingredients. It is made by extractions from Camellia and Rose Hip Seed Oil which consists of vital phytosterols that rehydrate and nourish the skin. Cane sugar is also present, and it releases bio saccharides that soothe the skin and combats the effects of UV and pollution while Mayblossom releases flavonoids which normalize sebum production and reduces pore size. The product was purchased from a local chemist.

Glycerol British Pharmacopoeia B.P.

Pharmaceuticals Pty Ltd produces glycerol B.P. It was purchased from a local pharmacy. It is 90-100% Glycerol (Glycerine). It can be prescribed to be taken internally as a mild laxative and externally to soften and moisturize the skin. Glycerol may reduce food intake in diabetic rats [17]. Therefore, there is a label on the package warning the diabetic patient. Glycerol is a common basic ingredient in many moisturizers. Therefore, it used as a negative control for the beauty products experiments.

Cell lines and cell culture

A human non-cancer keratinocytes cell line HaCaT were a gift from the Department of Haematology and Genetic Pathology-Flinders Medical Centre, School of Medicine at Flinders University, Adelaide. Skin fibroblast cell line CCD-1064Sk (A human normal skin cells) was obtained from ATCC, US (ATCC® CRL-2076™). Keratinocytes cell line HaCaT was maintained in RPMI 1640 medium, with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Thermo Scientific, Australia). A human normal skin fibroblast cell line CCD-1064Sk was maintained in Iscove's Modified Dulbecco's medium (IMDM medium) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were seeded in tissue culture flasks and incubated at 37°C in a 5% CO_2 fully humidified incubator. HaCaT cells were subcultured when they reached 60–80% confluence.

Cell treatment

The 96_well flat bottom was seeded with 104 cells/well and incubated for 19 h to allow the adherence of cells at 37°C in 5% CO_2. The media were aspirated and replaced with 100 µL of the treatment solution per well and were treated for 1 h prior to bioassays or genetic assays. The negative or untreated control (0 dose) was the media.

Crystal violet assay

Crystal violet stains the DNA of the live cells that adheres the plate after the dead cells are washed away [18]. The relative number of viable cells was determined using crystal violet assay (CV) as described in [19]. Briefly, 50 µL of crystal violet stain (0.5% of crystal violet in 50% methanol) was added to each well and incubated for 10 minutes at ambient temperature. After 10 minutes, the plate was gently washed with distilled water then air dried. 50 µL of 33% acetic acid was added to de-stain the cells. The absorbance (ODs) was measured on a spectrophotometric plate reader using a test wavelength of 570 nm with a reference wavelength of 630.

Methyl tetrazolium cytotoxicity assay (MTT Assay)

The tetrazolium salt 3-4,5 dimethlthiazol-2,5 diphenyl tetrazolium bromide (MTT) assay is based on a colorimetric assay for mammalian cell growth and survival, and it depends on the ability of viable cells to metabolize the yellow and water-soluble tetrazolium salt [20]. Cells were seeded at 104 in a volume of 100 µl into each well of a 96-well flat bottom plate. MTT solution with a final concentration 0.5 mg/ml was added and then incubated for 4 h at 37°C. After incubation, 80 µl 20% SDS in 0.02 M HCl was added. The plates were incubated overnight in the dark at room temperature. Thee absorbance (ODs) was measured on a spectrophotometric plate reader using a reference wavelength of 630 nm and a test wavelength of 570 nm.

Cytokinesis block micronucleus assay (CBMN Assay)

The mechanism of cell killing and genotoxicity of beauty products was carried out using Cytokinesis-Block Micronucleus Assay (CBMN) assay as described [21,22]. Briefly, after treatment Cyt-B (4.5 µg/ml)

was added to the media and the cultures were incubated at 37°C for 23 h. Cells were trypsinized (TrypLE™ Express Enzyme (1X), phenol red) and collected onto slides by a cytospin centrifuging for 5 minutes at 47 ×g (@6000 rpm). Slides were air-dried, fixed by DiffQuick Fixative for 10 min, and then double stained with stain 1 (red DiffQuick Stain) and then Stain 2 (blue DiffQuick Stain). Slides were scored as described in [23]. The chromosomal damage induced by treatment and total number of micronuclei (MNi) in binucleated (BN) cells totalled 1000. Slides were scored at a magnification of 250X or 40 X. Criteria for scoring micronuclei MNi, nucleoplasmic bridge (NPB) or nuclear buds (NBUDs) were as described [21]. Cytotoxicity determination induced by treatment, and the percentage of apoptosis/necrosis were evaluated in 500 cells and calculated according to published formulae [23,24].

Statically analysis

Data were presented as the mean ± S.E.M. of the standard error. The experiments were replicated at least three independent times. Statistical analysis of the data was carried out using ANOVA, followed by Tukey's HSD post hoc test. These tests were performed using SPSS software (Version 22). Differences were considered significant when the p-value was less than 0.05. Responses to treatment were compared to the untreated control (0 doses) which is represented as 100% survival.

Results

Cytotoxicity effects of beauty products on human skin cells

The toxicity of four beauty products on Keratinocytes human skin cells (HaCaT) and human normal skin fibroblast (CCD-1064Sk) *in vitro* was determined by incubating cells with treatments for 1h. Two cytotoxicity assays were carried out to indicate the toxicity of beauty products. The MTT cell survival assay was used to determine the relative survival cells when yellow MTT is reduced to purple formazan in the mitochondria of living cells. The Crystal Violet (CV) assay was used to determine the relative cell number when Crystal violet stains the DNA of the live cells that adhere to the plate after the dead cells are washed away. There was significant toxicity with doses of 3% w/v and 0.3% w/v for Nivea Visage Q10plus Anti-Wrinkle face moisturizer (with TiO_2) after treated HaCaT cells for 1h (Figure 1). Also, significant toxicity was induced by the highest dose (3% w/v) of Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) on human fibroblast CCD-1064SK cells determined by MTT and Crystal Violet (Figure 2). However, no significant toxicity emerged on HaCaT human skin cells or human fibroblast CCD-1064SK cells when using treatments of Glycerol B.P or Facial Moisturizer - Camellia & Geranium Blossom.

The Nuclear division index (NDI) is a method employed to measure the proliferative status of viable cells that can be used to assess general toxicity [21,25]. Table 2 shows the value of NDI for all beauty products examined and which had a significantly lower NDI value in the highest dose (3% w/v; 1.4 ($P<0.05$) of Nivea Visage with or without TiO_2 and Facial Moisturizer - Camellia & Geranium Blossom treatment at dose 5% w/v; 1.4 $P<0.05$ on HaCaT cells.

Mechanism of cell killing

The CBMN results (Figure 3 B) detected a significant increase in late apoptosis and early necrosis induced by the highest dose of Nivea visage with TiO_2 3% w/v and significantly induced in 0.3% w/v and 0.03% w/v doses of Nivea visage without TiO_2 after treated HaCaT cells for 1 h. Otherwise, no significantly apoptosis or necrosis induction was observed in the treatments of Facial natural treatment (Facial Moisturizer - Camellia & Geranium Blossom) and Glycerol B.P on HaCaT cells as shown in Figure 3A.

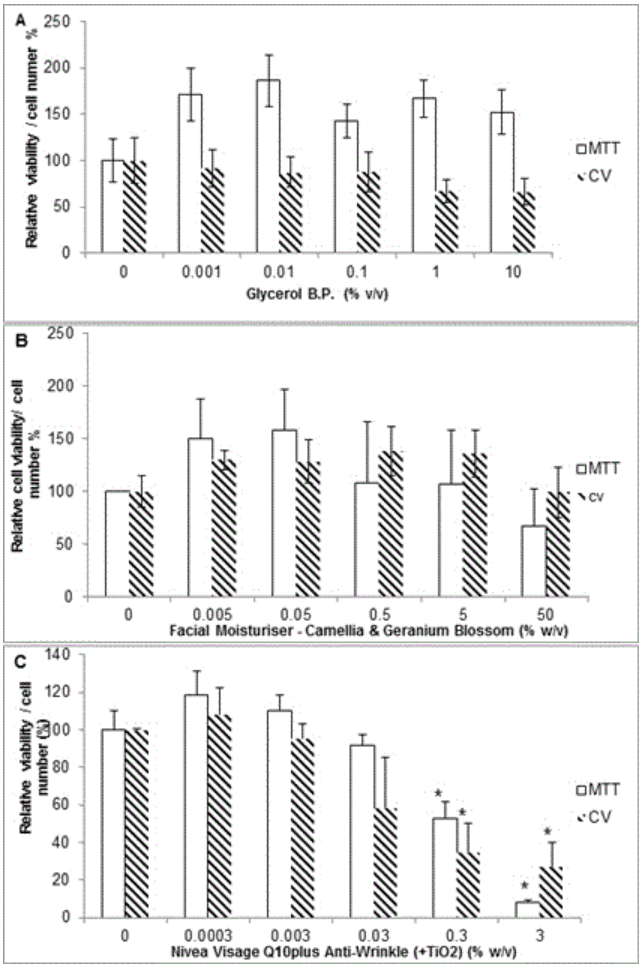

Figure 1: Relative cell viability and cell number (%) after treatment of HaCaT human skin cells with (A) Glycerol B.P, (B) Facial Moisturizer - Camellia & Geranium Blossom and (C) Nivea Visage Q10plus Anti-Wrinkle face moisturizer (with TiO_2) for 1 hour. Relative survival was measured by the MTT assay. Relative cell number was measured by the crystal violet assay. Data are shown as a percentage compared to untreated control and are mean of three replicates ± standard error of the mean (S.E.M). Treatments significantly different from untreated control at $P<0.05$ are presented as '*'.

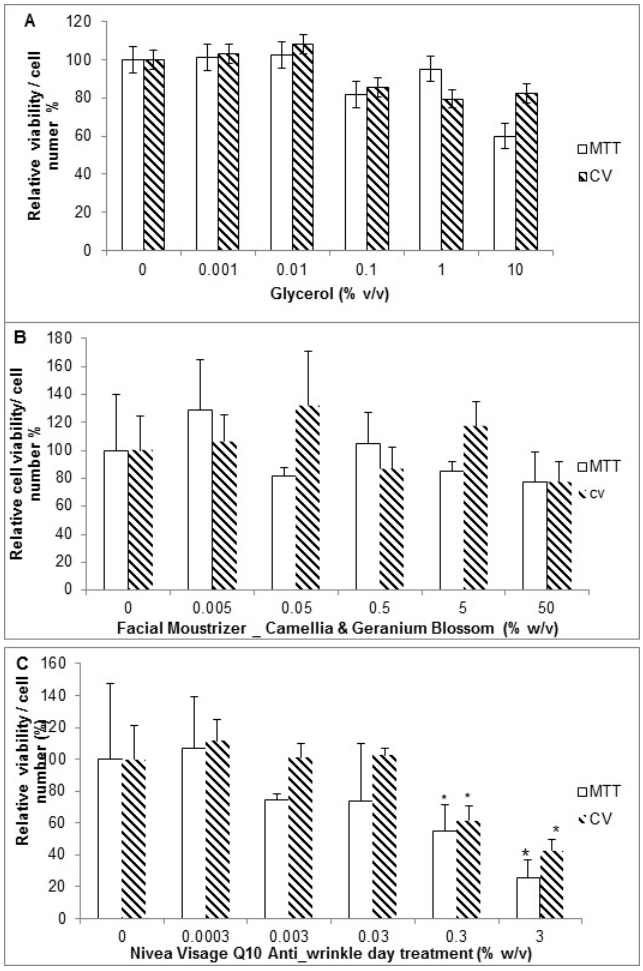

Figure 2: Relative cell viability and cell number (%) after treatment of CCD-1064Sk normal fibroblast human skin cells with (A) Glycerol B.P, (B) Facial Moisturizer - Camellia & Geranium Blossom and (C) Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) for 1 hour. Relative survival was measured by the MTT assay. Relative cell number was measured by the crystal violet assay. Data are shown as a percentage compared to untreated control and are mean of three replicates ± standard error of the mean (S.E.M). Treatments significantly different from untreated control at P<0.05 are presented as '*'.

Nivea visage without TiO_2 induced significant late apoptosis and early necrosis on fibroblast cells (CCD-1064SK) at dose 0.03% w/v and significant early necrosis induced by Facial Moisturizer - Camellia & Geranium Blossom at dose 5% w/v on CCD 1064SKas shown in figure 4. However, no significant induction of apoptosis or necrosis was observed on the the tretment of Glycerol B.P on CCD-1064SK cells.

Finally, this result was consistent with the previous result of Nivea Visage at dose 3% w/v induced necrosis detected by apoptosis assay followed flow cytometry (data not shown).

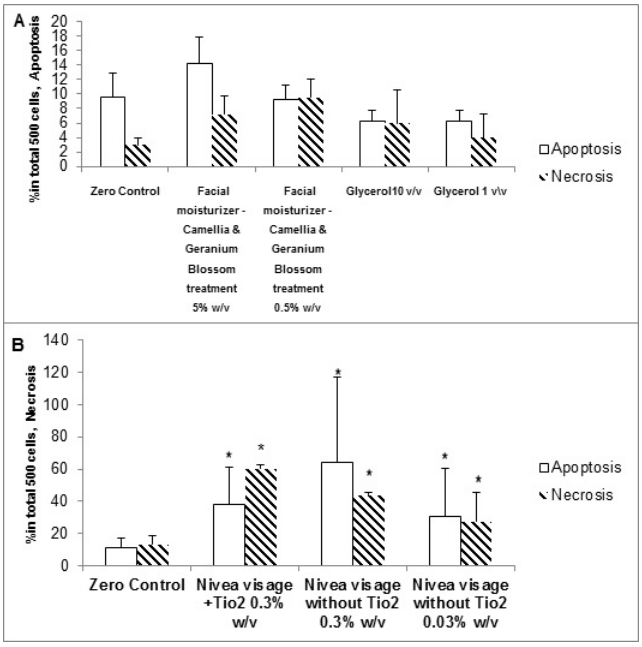

Figure 3: Apoptosis and necrosis induction detected by the CBMN assay for HaCaT cells followed by 1 h treatment using (A) Facial natural treatment (Facial Moisturizer - Camellia & Geranium Blossom) and Glycerol B.P and (B) using Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 and Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, free of TiO_2). Treatment Data are shown as the mean of three observations ± SEM. Treatments significantly different from untreated control at P<0.05 are presented as '*'.

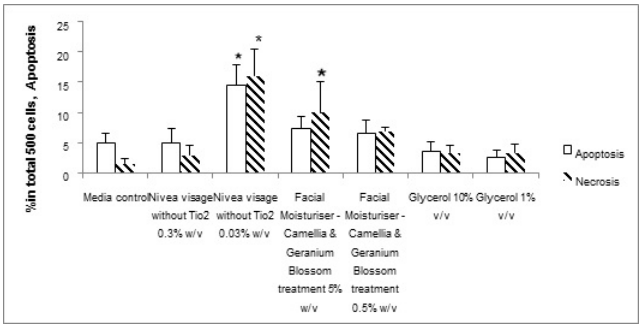

Figure 4: Apoptosis and necrosis induction detected by the CBMN assay for CCD_1064SK cells followed by 1 h treatment using Nivea Visage Q10plus Anti-Wrinkle face moisturizer (improved formula, without TiO_2 0.3% w/v and 0.03% w/v and Facial Moisturizer - Camellia & Geranium Blossom 5% w/v and 0.5% w/v and Glycerol B.P 10% v/v and 1% v/v. Data are shown as the mean of three observations ± SEM. Treatments significantly different from untreated control at P<0.05 are presented as '*'.

Genotoxicity of beauty products on human skin cells

The genotoxicity of beauty products on HaCaT human skin cells and CCD_ 1064SK was carried out using Cytokinesis-Block Micronucleus Assay (CBMN) assay. The following measures of genotoxicity chromosome breakage and chromosome loss (micronucleus MNi), chromosome rearrangement (nucleoplasmic bridges) and gene amplification (nuclear buds) [26]. The frequency of the induced micronuclei (MNi) indicates the extent of chromosomal changes induced by beauty products. The result of CBMN assay showed the genotoxicity effects of beauty products on HaCaT cells as shown in Figures 5. No significant increase in MNi observed in the results of treated HaCaT cells with Glycerol B.P and Facial Moisturizer - Camellia & Geranium Blossom (natural product) for 1 h (Figure 5 A).

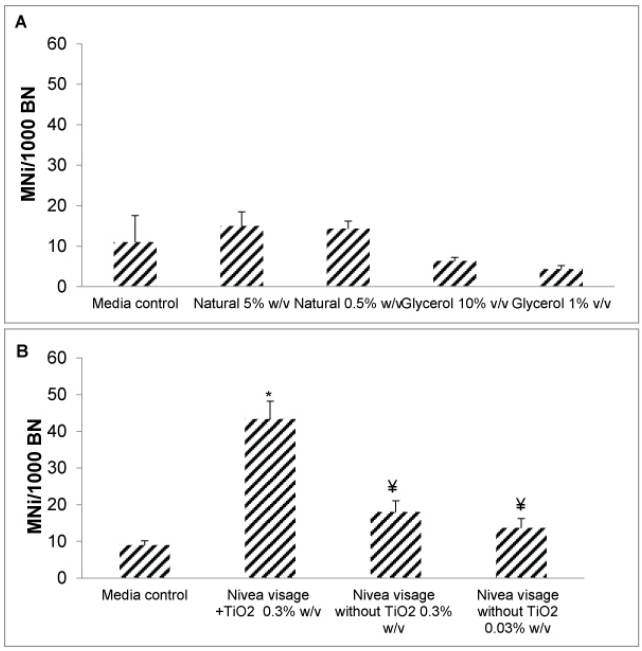

Figure 5: Frequency of micronucleated binucleate (MNBNCs) as measured by the CBMN assay following exposure of HaCaT cells to (A) Glycerol B.P and Facial Moisturizer - Camellia & Geranium Blossom (natural product) and (B) Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 and Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) treatment. The data are the mean ± S.E.M. from three separate experiments. Treatments significantly different from the untreated control at P<0.05 are presented as '*' or '¥' Treatments differed significantly from Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2.

However, there was significant increase in the number of MNi at the dose 0.3% w/v of Nivea Visage Q10plus Anti-Wrinkle face moisturizer + TiO_2. Also, Figure 5B showed the result of HaCaT cells after treated with two different formulas of Nivea visage product (Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 and Nivea Visage Q10plus Anti-Wrinkle face moisturizer without TiO_2. There was significant difference between the two formulas of Nivea Visage products on HaCaT cells. The product of Nivea Visage which contains TiO_2 was significantly difference from the product of Nivea Visage without TiO_2 and from untreated control. This result means that the product of Nivea Visage which contains TiO_2 cause significant gentoxicicity on HaCaT cells compare to the product of Nivea Visage removed TiO_2 showed less genotoxic on HaCaT cells. Nucleoplasmic bridge (NPB) and Nucleoplasmic buds (NBUDs) were also observed in the products of Nivea Visage Q10plus Anti-Wrinkle face moisturizer + TiO_2 and Facial Moisturizer - Camellia & Geranium Blossom (natural product) but they did not reach a significant level. A significant increase in micronucleus (MNi) was observed at 0.3% w/v and 0.03% w/v doses of Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) after treated CCD_1065SK cells (Figure 6). Other treatments demonstrated an increase in the number of MNi but they did not reach a significant level (Figure 6). Nucleoplasmic bridge (NPB) and Nucleoplasmic buds (NBUDs) were not observed in all products treatments on CCD_1065SK cells.

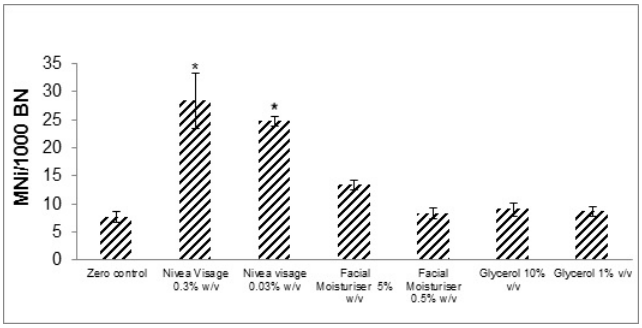

Figure 6: The frequency of micronuclei (MNi) per 1000 binucleated cells was determined using the Cytokinesis Block Micronucleus (CBMN) assay following exposure of CCD_1065SK cells to Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) treatment, Glycerol B P, Facial Moisturizer - Camellia & Geranium Blossom (natural product). The data are the mean ± S.E.M. from three separate experiments. Treatments significantly different from untreated control at P<0.05 are presented as '*'.

Discussion

In this study, two human normal skin cell lines were used to examine the toxicity and genotoxic effects of beauty products *in vitro*. Human keratinocyte cell line (HaCaT) which is derived from full epidermal differentiation capacity, functions as the outermost layer of the skin [27-29]. Human dermal fibroblast cells CCD-1046 within the dermis layer of skin are responsible for generating connective tissue [27,30]. Glycerol B.P served as a negative control in this study because it is a common basic ingredient in many moisturizers. There was no cytotoxic effect of exposure 1h to Glycerol B.P on HaCaT or CCD 1064SK cells. The Nuclear division index (NDI) obtained from the CBMN assay provides a measure of cell division [31]. There was no significant change in the NDI value which reflects the fact that Glycerol B.P did not affect the cell cycling in both cell lines. Furthermore, the evaluation of apoptosis and necrosis of Glycerol B.P on human skin cells detected by CBMN assay showed no significant difference from the untreated control. Also, the frequency of micro nucleated binucleate (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were not observed in 1000 binucleated (BN) cells which indicated that no genetic damage occurred after treatment

with Glycerol. Therefore, Glycerol is a safe ingredient used in several cosmetics products. The Facial Moisturizer - Camellia & Geranium Blossom treatment used in this study is a product of mixture of natural ingredients listed on the product. It showed no significant toxicity compared to the untreated control on CCD_1064SK cells. Consistent with this result is the fact that there was no cytotoxicity observed on treated HaCaT cells with Facial Moisturizer - Camellia & Geranium Blossom treatment for 1h (Figure 1). The Nuclear division index (NDI), based on the result of the CBMN assay, revealed a significant decrease in dose 5% w/v lower NDI value 1.4 ($P<0.05$) in HaCaT cells. This means a change occurred in the rate of cell cycling - they took longer to divide, or the viable cells failed to divide during the cytokinesis-block [21]. No apoptosis or necrosis induction was observed after HaCaT cells were treated with Facial Moisturizer - Camellia & Geranium Blossom treatment for 1h at both doses. However, a small but statistically significant necrosis was observed in CCD-1064Sk cells at the higher (5% w/v) dose of the Facial Moisturizer - Camellia & Geranium Blossom treatment after treatment for 1h.

Furthermore, the CBMN result indicated that no chromosomal damage causes by Grown Facial Moisturizer - Camellia & Geranium Blossom after human skin cells were treated for 1h.

Grown Facial Moisturizer - Camellia & Geranium Blossom treatment did not demonstrate significant toxicity or genotoxicity in human skin cell lines, but this does not mean it is a safe product to use. The change in NDI value indicates the decrease in the cell cycle was 1.4 ($P<0.05$) on the HaCaT cell line. Also, significant necrosis observed after treated fibroblast cell lines with Facial Moisturizer - Camellia & Geranium Blossom for 1h at dose (5% w/v).

Green or botanical products are not well regulated by government agencies. There is advice to avoid products that use essential oils such as lavender oil or tea tree oil that are classified as hormone disruptors [32,33].

	NDI value	
Treatments	HaCaT cell line	CCD_1064SK cell line
Media control	1.8	1.6
NVAW + TiO_2 dose 0.3% w/v	*1.4	-
NVAW + TiO_2 dose 0.03% w/v	1.7	-
NVAW without TiO_2 dose 0.3% w/v	*1.4	1.5
NVAW without TiO_2 dose 0.03% w/v	1.6	1.5
FMCGB treatment dose 5% w/v	*1.4	1.5
FMCGB treatment dose 0.5% w/v	1.6	1.6
Glycerol dose 10% v/v	1.7	1.6
Glycerol dose 1% v/v	1.8	1.6

Table 2: Nuclear Division Index (NDI) comparison between untreated control (0 doses) and beauty products. Cells were plated and described in the materials and methods section. Cells exposed to beauty treatments for 1h. NDI was determined by the cytokinesis-block micronucleus (CBMN) assay. Data are shown as percentages compared to untreated control and are mean of 3 replicates, standard error of the mean ± (S.E.M). A significant difference from untreated control at *$P<0.05$.

Titanium dioxide (TiO_2) which is a nanoparticle that is classified as a carcinogen [15]. Titanium dioxide (TiO_2) plays a role in the induction of apoptosis as well as oxidative stress. Moreover, studies have shown that Titanium dioxide (TiO_2) causes genetic damage linked to DNA-adduct formation in human lung cells [34-36]. The metabolic effects of Titanium dioxide (TiO_2) on keratinocytes HaCaT cells have also being investigated. One study indicated that Titanium dioxide affect the mitochondria [11]. Another study demonstrated a significant uptake of TiO_2 in keratinocytes in human skin cells (HaCaT); this was performed using transmission electron microscopy (TEM) and flow cytometry [37].

In our studies Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 was compared to an identical product without the TiO_2 allowing for an evaluation of the effect of the TiO_2. The result of MTT and Crystal Violet showed significant toxicity with the two doses of Nivea visage +TiO_2 was noted- up to 76% and 92% cells were killed after a 1 h exposure to a 0.3% and 3% (w/v) dose, respectively (Figure 1).

The mechanism of cell death was elucidated using the CBMN assay. There was a significant induction of late apoptosis and early necrosis at (0.3% w/v) on HaCaT human skin cells (Figure 3B). Also, a significant low NDI value (1.4 ($P<0.05$)) was observed at the 3% w/v dose (Table 2).

Also, nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were observed. These outcomes illustrate some of the mechanisms of chromosome damage when using Nivea Visage Q10plus Anti-Wrinkle day moisturizer. The frequency of chromosome rearrangement is indicated by NPB and NBUDs. NPB may arise from dicentric chromosomes and NBUDs from gene amplification [21]. A dicentric chromosome and an acentric chromosome fragment are formed as a result, and they manifest themselves in the formation of an NPB and MN [21]. The formation of NPBs could lead to misrepair of DNA strand breaks which could also lead to a dicentric chromosome and concatenated ring chromosome. One dicentric chromosome mechanism could result in telomere end fusion which is caused via shortening or loss of the telomere capping protein [21]. This study is consistent with that conducted by [38] which demonstrated that sunscreens containing Titanium dioxide can catalyse oxidative damage to DNA *in vitro* and in human cell culture.

On the other hand, Nivea Visage Q10 plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) showed significant toxicity on CCD-1064SK at (3% and 0.3% w/v) measured by MTT and Crystal Violet assays. The mechanism of cell death scored by the CBMN assay (Figure 4) showed there was significant induction of apoptosis and necrosis at (0.03% w/v) on CCD-1064.

Genetic damage effects detected by the CBMN assay showed a significant increase in MNi (28.3 MNi/1000 binucleated cells, n=3) ($P<0.05$) in CCD_1064SK cell lines. However, there was no significant increase in MNi with HaCaT cells as (Figure 5B). Also, a significant low NDI value (1.4 ($P<0.05$)) was observed only in 3% w/v dose on HaCaT cells (Table 2). This means that the cells took a longer time to divide in HaCaT cells after being treated with Nivea Visage Q10plus Anti-Wrinkle face moisturizer (Improved formula, without TiO_2) for

1h. Nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were not observed on CCD_1064SK.

This product consists of a mixture of chemicals ingredients that are the same as those reported in Table 1, except Titanium dioxide which was removed from this improved formula that released into the market by Nivea Company. Therefore, the even though none of these chemicals individually are known to be carcinogenic it is apparent that the mixture has shown carcinogenicity [2]. It is hypothesized that chemicals in mixtures could interact with each other and become carcinogenic. A brain cancer cluster study concluded that different mixtures of chemicals can induce the same cancer types despite using different mechanisms. None of the chemicals are known to individually cause brain cancer [2].

Interestingly, Nivea Visage Q10plus Anti-Wrinkle face moisturizer with TiO_2 proved to induce significantly more micronuclei than the product without TiO_2. As mentioned earlier, the difference is compatible with findings that TiO_2 enters the nucleus and cytoplasm of keratinocytes causing oxidative stress damage to DNA [37].

Consumers who are using Nivea Visage Q10plus Anti-Wrinkle day moisturizer are in fact exposed to an undiluted product (100%) with the potential to create long-term damage. Carcinogens in cosmetics and personal care products are potentially greater cancer risks than food contaminated with industrial carcinogens or pesticides because chemicals ingested into the body by mouth are absorbed by the intestines and pass into the venous blood. These chemicals are then transported to the liver which exists to detoxify the substances to varying degrees by enzymes before they can reach the rest of the body [33]. However, carcinogens absorbed by the skin can bypass the liver and circulate through the blood stream, thus reaching every organ in the body [1,33].

In conclusion, the current study has shown the possible harmful effects of several beauty products on normal human skin cells *in vitro*. In particular, the anti-aging face moisturizer which has a synthetic chemical product (Nivea Visage Q10plus Anti-Wrinkle day moisturizer +TiO_2) induced the highest toxicity and genotoxicity of the beauty products tested. Also, Nivea Visage Q10plus Anti-Wrinkle day moisturizer without TiO_2 induced significant toxicity and genotoxicity on human fibroblast CCD_1064Sk cells. On the other hand, the face moisturizer containing natural ingredients (Facial Moisturizer - Camellia & Geranium Blossom) was a relatively less toxic product compared to other beauty products, and Glycerol B.P. (the negative control) showed no toxic effect in either human cell line. Finally, further investigation could be done to study specific chromosome damage occurred by Nivea visage using fluorescent probes by fluorescence in situ hybridization (FISH). Also further work could be done to understand the underlying mechanism of action of the effects of Facial Moisturizer - Camellia & Geranium Blossom on the nuclear division index (NDI).

Conflict of Interest

The author declares that there is no conflict of interest.

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