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RESEARCH ARTICLE

INVESTIGATION OF MERCURY AND TITANIUM CONTENTS IN SKIN WHITENING CREAMS COMMONLY USED IN YEMEN BY ICP-MS

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Abstract

In this study, ten (10) samples of skin-whitening creams were analyzed for determination of mercury and titanium. The samples were collected from various retail shops, pharmacies and beauty aid stores in the local market of Yemen. Levels of mercury and titanium in creams were determined using Inductive Coupled Plasma with Mass Spectrometry (ICP-MS). The concentration of mercury in the creams ranged from below 0.0167 to 47151 µg/g and that of titanium ranged from below 0.0083 to 59.442 µg/g. Fifty percent (50%) of creams samples for mercury had concentrations more than Maximum Permissible Limits by specifications of the US Food and Drug Administration's, (USFDA), German and Canada (Maximum Acceptable Limit of 1µg/g). The use of such creams may lead to health hazards. Therefore, it is recommended that all skin whitening creams should be checked for mercury levels and other toxic metals before marketing. Further research to better understand the sources of mercury and other toxic metals in whitening creams and other cosmetic products is recommended.

Keywords: Mercury, Titanium, Whitening Cream, ICP-MS, Yemen.

1. Introduction

Cosmetics are products applied to the body for the purpose of cleansing, beautifying or improving appearance and enhancing attractive features [1]. A cosmetic product is any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or applied to the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly for the purpose of cleaning, perfuming, protection, changing their appearance, correcting body odors' and keeping the surfaces in good condition [2, 3].

Titanium dioxide (TiO_2) is widely used in a variety of products including paints, cosmetics, orthodontic composites and food. TiO_2 in its nanoparticle form (nano-TiO_2) is now the only form used as an ultraviolet (UV) filter in sunscreens, but also in some day creams, foundations and lip balms. While its efficacy as a UV filter is proven in the prevention of skin cancers and sunburns, some concerns have been raised about its safety. The Scientific Committee on Consumer Safety (SCCS) concluded in 2014 that 'based on the currently available scientific evidence which shows an overall lack of dermal absorption of TiO_2 nanoparticles', the use of nano-TiO_2 at a concentration up to 25% as a UV filter in sunscreens could be 'considered to not pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin' [4].The International Agency for Research on Cancer (IARC) has classified TiO_2, in the bulk form, as a possible carcinogen for humans when inhaled, based on evidence in experimental animals. In addition, in their last opinion published in 2018, the Scientific Committee on Consumer Safety (SCCS) has concluded that the information was insufficient to allow assessment of the safety of use of nano-TiO_2 in spray applications that could lead to exposure of the lungs [4,5].

Mercury is used in cosmetic products such as skin whitening creams. Mercury is a neurotoxin. Mercury has been used by many names such as "mercurous chloride", calomel, mercuric, mercurio or mercury. Skin whitening creams commonly use inorganic mercury in the form of ammoniated mercuric chloride and mercuric iodide. The prolonged use of products containing mercury can lead to inflammation of the liver, kidneys and urinary tract.

Presence of mercury in skin creams has become a global public health problem. Mercury compounds are readily absorbed through the skin on topical application and have the tendency to accumulate in the body. They may cause allergic reactions, skin irritation or neurotoxic manifestations [6, 7].

Regarding the potential adverse effects of heavy metal (Pb, Cd, Hg) contamination, the widespread availability and use of cosmetic products has attracted the attention of researchers and clinicians [8-10]. The fact that, when heavy metal ions come into contact with the human body, they are absorbed and form complexes with the carboxylic acids (-COOH), amines ($-NH_2$), and thiols (-SH) of proteins, results in cell damage, death and/or leads to a variety of diseases. Treatment for metal intoxication usually involves a chelating agent that binds with the metal ions to form complexes that are then removed from the body [11, 12].

Mercury intoxication from cosmetics has been featured in numerous news stories in recent years. There is evidence suggesting children who had been exposed in-utero from their mothers experienced developmental issues. These children were affected with a range of symptoms including motor difficulties, sensory problems and mental retardation [7, 13]. Other metals such as aluminum, chromium also causes many adverse effects whereas titanium dioxide and zinc oxide can be used in their natural form instead of non-naonized form [14, 15].

The USFDA lays the responsibility of checking the safety of their products and ingredients before introducing them to the market on the cosmetic firms. Most developing countries lack any safety regulations for cosmetics and other products that comply with the USFDA's requirement such as labeling violations, the illegal use of color additives, and the presence of poisonous or deleterious substances such as pathogenic microorganisms [16].

Although the use of these products is harmful, their production and use continue, and it has become a global public health problem [17]. Due to this uncontrolled exposure, cosmetic products should be thoroughly evaluated for safety before marketing. In most countries, it is legally prohibited to use lead, arsenic, and mercury in skin cosmetic products for example; lead is prohibited as part of cosmetic compositions in Korea, the European Union, and China. Manufacturers and importers of cosmetics products should be required to generate a safety evaluation for each product including composition, specifications and final product evaluation [18-20].

Mercury-containing skin-lightening cosmetics tested in a variety of countries, for example, Saudi Arabia [12, 16, 21, 22], Nigeria [23], Mexico [24], Canada [25], Germany [26], Ghana [27], Hong Kong [28], India [4], and USA [29]. In Yemen published a few studies on cosmetics, including a study to evaluate the levels of hydroquinone in some skin whitening creams [30], and another study on assessing the levels of some heavy metals such as arsenic, nickel, cobalt, and copper in skin cosmetics [31]. However and according to our best knowledge there are no studies conducted on the analysis of Hg and toxic metals in Skin whitening cosmetics products of Yemen. The objective of this research work is to determine mercury and titanium contents in skin whitening creams commonly used in Yemen using ICP-MS.

2. Material and Methods

2.1 Instrument

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-MS) (7500cx, Agilent, Japan) was used for determination of metal content in samples. A closed-vessel microwave digestion system (Start D, Milestone, Italy) equipped with fiber optic temperature and pressure sensors were used for sample digestion.

2.2 Reagents and standard solution

All solutions were prepared in Millipore-purified water (conductivity > 18.0 $M\Omega.cm^{-1}$). All chemicals used were of analytical reagent grade. The reagents used for sample digestion were Nitric acid from Riedel-déHaën (65%, Puriss, Germany) Peroxide Hydrogen (35%, analysis grade), and hydrogen peroxide (30% w/v, analysis grade). The reagents used for metal analysis by ICP-MS were Nitric acid from Riedel-déHaën (65%, Puriss, Germany), L-cysteine from BDH (>98%, England). Mercury standard solution from High-Purity Standards (20 µg mL-1, Charleston, USA), ICP-MS multi- element standards from Agilent (10 µg mL^{-1}, Nos. 2A and 3, USA), respectively. All the plastic and glass-ware used were soaked overnight in 10% nitric acid, rinsed with distilled water, and finally with Millipore water before use.

2.3 Samples preparation

A total of 10 skin-whitening creams were analyzed for determination of mercury and titanium. The samples were collected from various retail shops, pharmacies and beauty aid stores in the local market of Yemen in 2014. None of them indicated mercury content or had mercury indicated as an ingredient. The skin whitening creams were imported from different sources such as Egypt, England, India, Jordan, Taiwan, Saudi Arabia, USA, 6A cream of manufactured unknown and 5A cream of locally produced. Three replicates of each sample were prepared and analyzed.

2.4 Application to microwave digested samples

All the samples were digested using the same method ALqadamy *et al.*, [21]. 0.3 gram of each samples were transferred to a 120 mL Teflon digestion vessel avoiding contact with the side of the vessel. Conc. nitric acid (5.0 mL) was added, followed by hydrogen peroxide (35%, 2.0 mL) and hydrofluoric acid (40%, 1.0 mL) added to the vessel using a graduated pipette. The vessel was sealed and left for about 15 minutes to ensure complete reaction. The sample was digested in a microwave (Milestone Start D) following the heating program shown in Table 1. After cooling to room temperature, the vessel was unsealed and the inner wall and lid were thoroughly rinsed with deionized water and deionized water (20 mL) was added to the digested solution. The solution was filtered through Whatman paper No.41 into a 25 mL polypropylene volumetric flask and diluted to volume with deionized water. Then, the sample was transferred to polypropylene bottles.

Table 1: Microwave heating program.

Step	Time.(Min)	Temp. (°C)	Power (W)
1	15	195	450
2	2.0	195	0
3	10	195	300
4	15	195	350

2.5 Preparation of standard solutions

Standard solutions of mercury and titanium were prepared from 1000 ppm Standard Stock solution from High-Purity Standards (Charleston, USA).

These stock solutions were serially diluted to give concentrations of 0, 0.5, 1, 1.5 and 2 ppm mercury standards while 0, 2, 4, 6, and 8 ppm for titanium.

2.6 Statistical calculation

Standard regression curve analysis was performed by using of origin 7.5 and Microsoft Excel 2010 software without forcing through zero. Standard deviations and other statistical parameters were calculated by this software.

2.7 Analytical parameter

The samples were then analyzed for mercury and titanium by ICP/MS (7500cx, Agilent, Japan). Each sample was analyzed three times and the results are expressed as mean ± SD (SD: standard deviation). Least significant difference-LSD test was used to significant compare between means and found to be less than 5% ($P \leq 0.05$) for all samples in this study. Relative standard deviation (RSD) of the three results are calculated and found to be less than 5% for all samples for all metals analyzed in this study, reflecting the precision of the method for the analysis of these metals. Calibration curves for metals analyzed were constructed by plotting the ratio of the intensity of the analyte metal to that of the internal standard (In) vs. concentration of the metal (in ppm), and results showed that the calibration curves are linear with correlation coefficient (r2) greater than 0.99 for the Hg and Ti analyzed.

3. Results and Discussion

A total of 10 different samples of creams from different brands were studied for the presence of mercury and titanium. Levels of mercury and titanium in skin-whitening creams on the Yemeni market were determined using Inductive Coupled Plasma with Mass Spectrometry (ICP-MS) (7500cx, Agilent, Japan). The results of the analysis are shown in Table 2.

As can be seen, the mercury content of five analyzed creams was less than 0.0167 µg/g (4A = 10A = 3A = 8A = 7A). However, the mercury content of the five other whitening creams varied between 93 and 47151 µg/g. These values are extremely high and represent a serious health hazard [24]. It was observed that all the samples studied have reported levels of mercury more than Maximum Permissible Limits by specifications of the USFDA [32, 33], German [26, 27] and Canada [25] (maximum acceptable limit of 1µg/g) (Table 3). According to Saleh *et al.*, [30], it was found that 30% of analyzed cream samples contained hydroquinone levels higher than the international limits, as WHO, EU and USFDA. The use of such creams may lead to health hazards. In another study, it was found that 90% (of the same studied creams) contained nickel above the permissible limit [31].

Cumulative effect of prolonged low-level mercury exposure could lead to nephritic syndrome. Mercury can also be transferred from the mother to the fetus during pregnancy. Mercury from soap and cream has been reported to be readily absorbed through the skin and via inhalation [7].

The highest concentration of mercury has been found in "9A" cream, manufacturer from Egypt. The mercury

Table 2: Results of Hg and Ti analysis of some skin whitening creams in commonly used in Yemen

(Mean values ± standard deviations are significantly different (P < 0.05)

Sample Code	Source	Hg (ug/g)	RSD	Ti (ug/g)	RSD
1A	**Saudi Arabia**	**7385.03±0.002**	2.70818E-05	**59.442±0.000**	0
2A	**Saudi Arabia**	**2109.18± 0.001**	4.74118E-05	0.358±0.000	0
3A	U.S.A	<0.0167±0.000	0	0.408±0.0014	0.343137255
4A	India	<0.0167±0.001	5.988023952	39.092± 1.054	2.696203827
5A	**Yemen**	**92.98±0.000**	0	44.767±0.0067	0.014966381
6A	**-**	**714.19± 0.001**	0.000140019	46.767±0.0059	0.012615733
7A	Jordan	<0.0167±0.001	5.988023952	<0.0083± 0.000	0
8A	England	<0.0167±0.000	0	0.025±0.001	4
9A	**Egypt**	**47151.03± 0.004**	8.48338E-06	<0.0083±0.000	0
10A	Taiwan	<0.0167±0.000	0	43.142±0.000	0
Least significant difference (LSD)		**1.2978×10^{-4}**		0.0235	

concentration in various brands of creams in decreasing order is as following:

9A (Egypt) > 1A (Saudi) > 2A (Saudi) > 6A (Unknown) > 5A (Yemen) > 4A (India) = 10A (Taiwan) = 3A (USA) = 8A (England) = 7A (Jordan).

1A(Saudi)>6A(Unknown)>5A(Yemen)>10A(Taiwan)>4A(India)>3A(USA)>2A(Saudi)>8A(England)>9A (Egypt) = 7A(Jordan).

Table 3: Compare the levels of Hg and Ti of the study with Maximum Permissible Limits

Sample Code		Source	Hg (µg/g)	Ti (µg/g)
1A		**Saudi Arabia**	**7385.03±0.002**	**59.442±0.000**
2A		**Saudi Arabia**	**2109.18± 0.001**	0.358±0.000
3A		U.S.A	<0.0167±0.000	0.408±0.0014
4A		India	<0.0167±0.001	39.092± 1.054
5A		**Yemen**	**92.98±0.000**	44.767±0.0067
6A		-	**714.19± 0.001**	46.767±0.0059
7A		Jordan	<0.0167±0.001	<0.0083± 0.000
8A		England	<0.0167±0.000	0.025±0.001
9A		**Egypt**	**47151.03± 0.004**	<0.0083±0.000
10A		Taiwan	<0.0167±0.000	43.142±0.000
Maximum Permissible Limits	USFDA [33, 34] GERMANY [26. 27] CANADA [25]		**1 ppm (µg/g)**	**50 ppm**[*][35, 36]

*The Japanese Standards of Cosmetic Ingredients (JSCI) give the level Titanium Dioxides less than 50ppm

Table 4: Compare the levels of Hg and Ti of the study with previous studies.

Country	Hg	Ti	Reference
	Concentration rang (µg/g)		
Saudi Arabia	0.637 – 2745	2.52 - 2749	**[21]**
Saudi Arabia	2.46-23222		**[22]**
Saudi Arabia		16 - 187	**[12]**
Hong Kong	660-57000		**[28]**
Mexico	878-36000		**[24]**
Nigeria	0.09 – 0.207		**[23]**
Ghana	0.006 – 0.549		**[27]**
India	ND - 1.97		**[7]**
USA*****		4.64 - 1418	**[34]**
USA**	56000		**[29, 33]**
Yemen	**<0.0167 – 47151**	**<0.0083 – 59.44**	**Current study**

*****Titanium level in Lip Products**; **** The California Department of Public Health (CDPH) is investigating several cases of mercury poisoning due to an unlabeled face cream from Mexico used for lightening the skin, fading freckles and age spots, and treating acne. The cream contained very high levels of mercury: 56,000 parts per million (ppm) or 5.6%).

The concentration of titanium in the creams ranged between 0.0083 to 59.442 µg/g, with the highest value in 1A cream and the lowest in two creams (7A and 9A). The Japanese Standards of Cosmetic Ingredients (JSCI) give the level titanium dioxides less than 50ppm [35, 36].

The titanium concentration in various brands of creams in decreasing order is as following:

The comparison with literature of current study (Table 4) revealed that the concentrations of Hg and Ti in skin whitening creams are:

The highest concentration of Hg found in 9A cream (47151 µg/g) was found to be lower than maximum value (57000 µg/g) for California Department of Public Health [29,33], higher than 2 and 18 times that the maximum value (2745µg/g ; 23222 µg/g) for ALqadami *et al.*,[21] and I-Ashban *et al.*,[22] , respectively, and more than to be found in other reports described in the literature [7, 23, 27]. Were also compared to results obtained with the results of previous studies as shown in Table 4.

The highest concentration of titanium found in 1A cream (59.44 µg/g) was found to be lower than maximum value of skin whitening cosmetics (2749 µg/g, 187 µg/g, and 1418 µg/g) for ALqadami *et al.*, [21], Faten [12], and Sa Liu *et al.*, [34], respectively.

4. Conclusions

The results indicate that mercury content in skin-whitening creams was extremely high in five analyzed samples and represents a serious health risk. Skin creams containing mercury are obviously still available and commonly used in the local marketplace and its contents are poorly controlled. Therefore, it is recommended that all skin whitening creams should be checked for mercury levels and other toxic metals before marketing. Efforts should be made at enlightening the consumers about the hazards of mercury-containing skin lighteners.

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مقالة بحثية

تقدير محتويات الزئبق والتيتانيوم في كريمات تبييض البشرة باستخدام جهاز طيف الانبعاث الذري المرتبط بمطياف الكتلة (ICP-MS)

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الملخص

في هذه الدراسة، تم تحليل عشرة (10) عينات من كريمات تبييض البشرة لتحديد الزئبق والتيتانيوم. تم جمع العينات من العديد من الاسواق التجارية والصيدليات ومحلات التجميل في السوق المحلي اليمني. تم تحديد مستويات الزئبق والتيتانيوم في الكريمات باستخدام جهاز طيف الانبعاث الذري مع مطياف الكتلة (ICP-MS). تراوح تركيز الزئبق في الكريمات من 0.0167 إلى 47151 ميكروغرام/غرام، وتركيز التيتانيوم تراوح من 0.0083 إلى 59.442 ميكروغرام/غرام. بينت الدراسة بأن خمسون بالمائة (50٪) من عينات الكريمات الخاصة بالزئبق بها تركيزات تزيد عن الحدود القصوى المسموح بها حسب مواصفات إدارة الغذاء والدواء الأمريكية (USFDA)، الألمانية وكندا (الحد الأقصى المقبول هو 1 ميكروجرام/جم). قد يؤدي استخدام هذه الكريمات إلى مخاطر صحية. لذلك توصي الدراسة، بفحص جميع كريمات تبييض البشرة بحثًا عن مستويات الزئبق والمعادن السامة الأخرى قبل التسويق. وتوصى بمزيد من البحث لفهم مصادر الزئبق والمعادن السامة الأخرى بشكل أفضل في كريمات التبييض ومنتجات التجميل الأخرى.

الكلمات الرئيسية: الزئبق، التيتانيوم، كريمات تبييض البشرة، جهاز طيف الانبعاث الذري المرتبط بمطيافية الكتلة، اليمن.