

Original Research Article

Determination of Mercury Contents in Some Commercially Available Skin-Lightening Creams in Benghazi-Libya Markets

Nagwa H. S. Ahmida^{a*}, Aziza Ahmida^b, Sami. H. Almabrok^b, N. H. Towier^c, Randa. S. El-zwaeya^a, Salah A Gadalla^a

^a Department of Environmental Health, Faculty of Public Health, University of Benghazi, Benghazi, Libya

^b Department of Chemistry, Faculty of Science, University of Benghazi, Benghazi, Libya

^c Chemistry Department, Art& Science Faculty-Tokra, University of Benghazi, Benghazi, Libya

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ABSTRACT

Mercury and its compounds are well known to have toxic effects on human body. Many research studies have reported in this field. These studies found that the prolong using of the skin whitening and lightening creams lead to accumulation of mercury in human body. As a result of that, the damage to the brain, nervous system and kidneys, can occur. In this study, the concentrations of total mercury metal in six top selling skin lightening creams sold in local markets in Benghazi, Libya were analyzed. The Cold Vapor Atomic Absorption Spectrophotometric (CV-AAS) method was used for mercury determination after suitable wet digestion process. In all analyzed samples, mercury was detected, with concentrations ranged between 0.2500 to 2.0950 ppm. The concentration of mercury in four samples exceeded the maximum permissible value for mercury in cosmetic products set by World Health Organization (WHO) and Food and Drugs Administration (FDA). The results of the present work revealed that the analyzed creams were contaminated with mercury; making risk of toxicity for consumers.

* Corresponding author: Ahmida, Nagwa H. S.

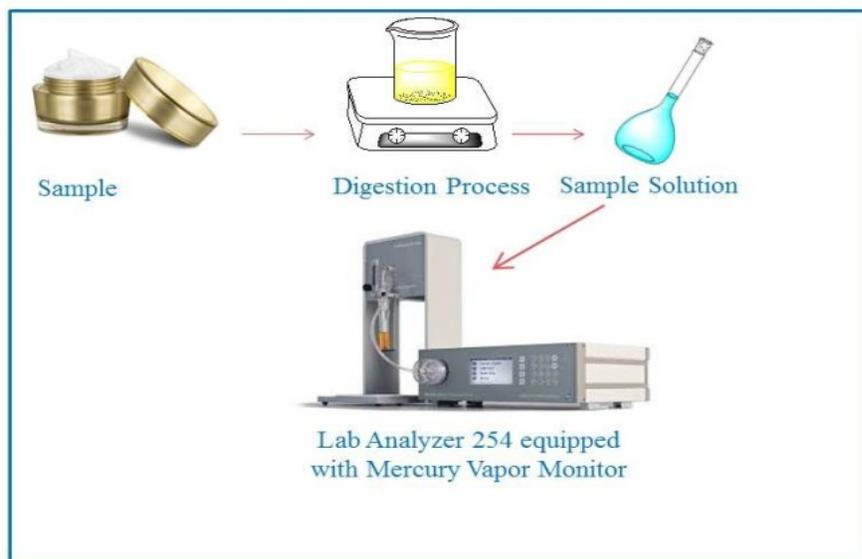
✉ E-mail: najwa.ahmida@uob.edu.ly

☎ Tel number: 00218-61-22195

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GRAPHICAL ABSTRACT



Introduction

Definitely, the skin is a critical and extensive organ of the human body. It is the barrier that establishes the body's first line of defence against outer environmental risks [1]. As a result, the body maintains a safe internal environment conducive to normal physiological function [2]. In fact, any harm done to the skin, such as skin bleaching, can harm the body [3]. The skin bleaching process is also known as skin whitening or skin lighting [4]. Historically, Skin bleaching was known since ancient time in the Mediterranean, Asia and parts of Europe where women have used it to lighten their skin. The skin bleaching process is now considered as a widespread global phenomenon [5]. The main reason for ladies to use skin whitening creams is to treat problems of the skin damage, such as; post inflammatory, hyper-pigmentation, melasma and acne. Another reason is to make their skin color more light and bright, as many ladies around the world believed that the lighter skin tone is more attractive. Recently, several studies have revealed that many ladies in African,

Asian and Caribbean countries, apply the lighting skin agents regularly. Besides, dark skinned ladies in Europe and North America also use lightening skin product [6].

The skin bleaching products use different items, such as; chemicals, soaps, herbs, fade creams and other substances that are strong enough to cause a quick change of the skin color by inhibition of melanin biosynthesis via different mechanisms [3,7]. Most of these bleaching products composed of chemical substances, such as mercury, hydroquinone, kojic acid dipalmitate, azleic acid, arbutin, bearberry, vitamin C, magnesium ascorbyl phosphate, calcium ascorbate, and ascorbic acid. However, the most common cosmetic products contain mercury and its compounds, as bleaching agent [6].

Actually, the consumers in the world prefer to use the bleaching creams that commonly used to lighten the complexion of skin. Those creams have been marketing with different brands, such as Skin-evening creams, Skin lighteners, Skin brighteners, Skin whiteners, Skin toners, Fading

creams, or Fairness creams [3, 8, 9]. In general, ammoniated mercury compounds are used in skin lightening creams and soaps [3]. Mercury can lighten skin by the displacement of copper ions from the active site of the melanin formation enzyme, tyrosinase, rendering it inactive. Therefore, mercury containing skin-lightening creams could be potentially dangerous since stripping melanin, leaves the skin more susceptible to sun damage and increase the risk of developing malignant melanoma

[7, 10, 11,12]. In addition, mercury and its compounds are considered as high risk ingredients used in cosmetics. The presence of mercury and its compounds in cosmetics can lead to serious problems to consumers including renal, fatigue, nervousness/ irritability, severe headaches, insomnia, memory loss, loss of leg strength, tingling or burning sensation, tremors or shaking of the hands, depression [13, 14]. Moreover effects such as; burning of face, contact dermatitis, grey-blue-black facial discoloration, flushing, erythrodermas, purpura, and gingivostomatitis, nephritis or neurological disorder have been reported [15]. Mercury also has side effects on the development of the fetus if the pregnant women were using the skin lightening products that contain mercury. This metal can accumulate in the placental membrane and cause decrease the fluidity of this membrane, which then affects membrane function [1]. Mercury in soaps, creams and other cosmetic products is eventually discharged into waste water. Consequently, the mercury enters the environment, where it becomes methylated and can enter the food chain as highly toxic methyl mercury in fish [16]. Owing to harmfulness of mercury and its compounds to human health, their level in cosmetic products are prohibited or strictly regulated by different global regulations in the world. According to World Health regulation (WHO), the levels of mercury in cosmetic products must be less than 1ppm [17,18]. The Food and Drug Administration

(FDA) set the highest acceptable level of mercury (as unavoidable contaminant) at 1mg mercury/kg, but when mercury compounds are used on purpose as preservatives in cosmetics applied to the periocular area the acceptable limit is 65mg mercury/kg [19]. Japan Standard Regulation and European Union Directive prohibited mercury and its compounds in cosmetic products (with exception of 0.007% of thimerosal and phenyl mercuric salts that used in eye products as preservatives) [10, 20]. Health Canada has established the maximum acceptable limits of mercury metal concentration in cosmetics at the 3mg mercury/kg [10]. Many other countries have regulations banning mercury-containing cosmetics. These countries include Ghana, Nigeria, Uganda and Philippines. On the other hand , many countries, including the two biggest markets, India and China, have no regulations on ingredients contained within the cosmetic products [18]. Because of the toxic effects of mercury, it is necessary to monitor commercially available cosmetics regarding the concentrations of mercury. The available literature data show that mercury is present in many types of skin lightening products produced and used in various parts of the world. There are plenty of scientific articles about the detection of mercury in different body care and cosmetic products. The most recent and important articles emphasized that the problem of presence the mercury metal in cosmetics, is still current. Based on the available literature, some reference values for mercury metal in skin lightening products, in other parts of the world, were demonstrated in Table 1 [2, 21-27]. In these articles, different methods of analysis have been used, included; Atomic emission spectroscopy [21], partial-inductively X-ray Spectroscopy [22], X-ray fluorescence [23], Cold-Vapor Atomic-Absorption Spectrometry (CV-AAS) [2,24,26,27,28,29] and electrochemical methods [25,30], to determine mercury metal in different skin lightening samples.

Table 1. Mercury Contents in Some Skin Lightening Creams In Different Countries around the World

Country, year	Concentration of Total Mercury (ppm)	Reference
Indonesia, 2021	0.42261-44.96079	[21]
Indonesia, 2020	0.12-7834.4	[22]
Jamaica, 2020	0.05-17,547	[23]
Pakistan, 2018	0.74-44,292	[24]
Malaysia, 2017	2.7-2.3	[25]
Trinidad, 2017	0.437-.766	[26]
Iraq, 2016	0.482-29.54	[27]
Ghana, 2014	0.001-0.327	[2]

In all these research works, prior to mercury quantification, the analyzed sample should undergo to digestion processes, to convert the components of sample matrix into simple chemical forms [31].

The literature review indicates the lack of information concerning mercury level in skin lightening creams sold by the Libyan markets. On the other hand, there is a wide popularity and availability of skin whitening and lightening creams in body care and local markets in Benghazi, Libya. The aim of this study is to determine levels of mercury in the top selling skin lightening creams available in Benghazi-Libya markets using CV-AAS. The results of mercury levels in the collected samples will compare with WHO Standard Limits [18], then determine if Libyan women are at risk of mercury toxicity.

Experimental

Materials and Methods

Sampling

In this study, six best selling lightening creams were purchased from randomly selected pharmacies and local markets in city of Benghazi.

The information on the product container labels were carefully examined visually. The products codes, date of manufacture (Mfd.) and of expiry (Exp.), preservative used, if any, as well as ingredients listed on the labels of these products were noted and recorded in Table 2.

Chemicals and Reagents

All chemicals and reagents that used in the experimental work, were of analytical grade and were obtained from well qualified chemical companies. These chemicals included; nitric acid (65%) was obtained from CODEX (Milan, Italy), perchloric acid 70%, was obtained from MERCK (Germany), and Mercury standard solution of concentration 1000ppm for AAS, were purchased from SCHARLAU (Spain). This standard was used to prepare the calibration solutions. De-ionized water was used for the preparation of all solutions.

All glassware and plastic containers were washed with liquid soap, rinsed with water, soaked in 10% nitric acid for 24hours. The glassware then cleaned thoroughly with distilled water and dried, to ensure that any contamination does not occur. Blanks and samples were processed and analyzed simultaneously [32,33]. Vapor Monitor model VA-3000 (Mercury Instruments GmbH, Analytical Technologies, Germany) was used to measure the absorbance.

Digestion Process

The skin lightening creams were digested for total mercury determination by an open flask procedure [29, 31]. Two grams of lightening cream sample were weighed out in a test tube. Ten mL of nitric acid and 10mL of perchloric acid were added. The digestion tube was heated to at least 100°C for 30 minutes until clear solution was obtained. On completion of digestion process, the digested sample was allowed to cool to room temperature.

Table 2: List of Selected Skin Lightening Creams

Sample Code	Weight& Uses	Country of Manufacture	Manufacture& Expiry Dates	Ingredients
S1 (Topicalcream)	15g, topical cream for skin hyper pigmentation disorders	Egypt	Mfd. Date: January 2020 Exp. Date: -	Each 100g cream contains Hydroquinone (3g), Trelenolin (0.02g), Hydrocortisone acetate (1g), Eusolex(4g).
S2 (Face Cream with Milk acid & Lemon Juice)	18 g, cream for spots and teenage skin Problem	Egypt	Mfd. Date: - Exp. Date: Best before 3 years from Mfd.	
S3 (Advanced multivitamin TH)	25g, high definition Glow-Career Foundation create your own identity	India	Mfd. Date: May 2019 Exp. Date: April 2021	Water, palmitic acid, Stearic acid, Glycerin, Titanium, Aluminium hydroxide,dioxane,butylmethoxydibenzoyl methane, Sodium ascorbyl phosphate.
S4 (Saffron& Pure milk-natural Fairness for all skin types)	50g, Natural fairness	Thailand	Mfd. Date: May-2017 Exp. Date: May-2022	Saffron, Pure milk, Wheat-gem oil which is rich in vitamin E.
S5 (Beauty Cream)	12g, Shine effect	Taiwan	-	Celanol, Zinc oxide, Glycerine, Mineral oil, Petroleum jelly, Lecitin, Lanotin, Vitamin A, Aloe Vera, Pearl powder, perfume.
S6 (Pearl Beauty Cream)	12g-Extra Special	Taiwan	-	Glycerin, Glycerlymonolearate, Lanolin, Arbutin, Vitamin E, Aloe Vera, Pearl Powder, Perfume.

Then the solution was transferred to 100mL volumetric flask, and the volume made up to the mark with deionized water. The solution then filtered using Whatman No. 41 filter paper. Then, the digested solutions were subsequently analyzed to detect mercury by using Lab Analyzer 254 equipped with Mercury Vapor Monitor model. In CV-AAS method the mercury metal in the sample was chemically reduced with stannous chloride, to convert the mercury to its elemental form, which carried into an optical cell made of fused silica, using a stream of air. Then, the liberated mercury vapor is determined by measuring the absorption at 254 nm. The

working solutions were freshly prepared by diluting an appropriate aliquot of the commercial stock standard solution through intermediate solutions, using blank solution [34].

Data Analysis

All the experiments of mercury metal analysis were performed in duplicate. The results were expressed as the mean values (mg/kg) with standard deviation (mean \pm SD), which calculated using Microsoft Office Excel 2007. Statistical analysis of data was carried out using statistical Package for Social Analysis (SPSS) (SPSS version 19.0, IBM, Chicago, III., USA) Program, adopting the significance level of 5% ($P < 0.05$). The mean

values of samples analysis were compared using dispersion analysis (ANOVA). For the multiple comparisons, a Least Significant Difference (LSD) test was applied. Also, the Quality Control Test was performed.

Results and Discussion

In this study, six best selling skin lightening creams were purchased from randomly selected local pharmacies and cosmetic products shops in Benghazi City. After the sample had been wet digested by strong acids, the levels of total mercury were determined in these products using CV-AAS method, [29, 31, 34]. Many chemical analysts have used this method to determine mercury in skin lightening creams [26-29]. This method provides high sensitivity and selectivity for mercury metal in different samples [28].

The results in Table 3 showed that, all the skin lightening cream samples were contained detectable amounts of mercury. The total mercury contents of the analysed skin lightening creams ranged between 0.2500-2.0950ppm. Although mercury was detected in all the skin lightening cream, it was not listed as an active ingredients on the product packaging. Instead, another active ingredients were listed on packaging included; the natural vitamin E, zinc oxide, hydrocortisone, hydroxyquinoline derivatives, titanium oxide, aluminum hydroxide and pearl powder, as shown in Table 3. There is only brand (sample S2) has no active ingredients on the printed packaging label. On the other hand, S1 and S2 samples are pharmaceutically product, that medically prescribed for hyper-pigmentation treatment [35]. In the printed packaging labels of these samples, there are no precautions of topical application and no warning information of medicine external uses [36]. Besides, advices were not given to apply the product without medical prescription or with a sunscreen, as it is stipulated by law on the sale of cosmetics and toiletries in South Africa [37].

Table 3: Mean Levels of Mercury Contents In Skin Lightening Cream Samples

Sample Code	Total Mercury (ppm) ^a
S1	1.0450 ±0.035
S2	0.2500 ±0.042
S3	1.0200 ±0.071
S4	0.7400 ±0.47
S5	1.4950 ±0.25
S6	2.0950 ±0.035
P value	0.002

^aEach value is the average of duplicate separated measurements.

The highest level of total mercury was detected in S6, as presented in Table 3. Statistically, the analysis of variance (ANOVA) between the lightening cream samples showed a significant difference in the total mercury contents ($P=0.002$). The intergroup comparison for mercury contents among skin lightening cream samples showed that a significant difference was found between the total mercury content of sample S6 with all other analyzed samples.

In this study, the concentration of mercury at 1.02 ± 0.07 ppm was detected in S3. Despite, the manufacture company of this sample claims that its special patented formulation safely and gently controls the dispersion of melanin in the skin without the use of harmful chemicals frequently found in other skin-lightening products [37]. On the other hand, the concentration of total mercury in S3 was higher than the mercury concentration detected in same products that reported by other authors in different countries [11, 12, 38].

Al-Saleh *et al.* have detected mercury at 0.304ppm in same S3 products that widely available in Saudi markets [11, 38]. However, our results indicated that some of these products that are purchased in the Benghazi markets were not original products.

The concentration of Total mercury in our selected skin lightening samples from Benghazi markets were compared with other recent

research articles on skin lightening creams conducted in different countries and presented in Table 1. The total mercury concentration range in the analyzed samples was much lower than the concentration range of total mercury in skin lightening cream samples collected from Indonesia [21, 22], Jamaica [23], Pakistan [24], and Iraq [27]. While, the concentration range of total mercury in studied samples was higher than the range of total mercury in skin lighting samples collected from Trinidad (Caribbean Country) [26] and Ghana [2] markets.

Figure 1 presented the comparison of the levels of mercury metal in the investigated samples with the maximum allowed limit of this metal recommended by WHO in lightening creams. As shown in Figure 1, Both S2 and S4 have concentrations of total mercury fall below the maximum permission limit (less than 1 ppm) set by WHO and U.S. FDA [17- 19] for mercury in skin lightening creams.

Although the total mercury concentrations of S2 and S4 had acceptable concentrations of mercury within the permission limits set by global regulations, excessive utilizing of skin lightening creams becomes a threat to both human health and environment due to the accumulation of mercury compounds [24].

Conclusions

The skin lightening creams containing mercury and its compounds still have high selling power in local markets. In the present study, this metal was detected in all the six selected skin lightening cream samples which were purchased from pharmacies and cosmetic shops in Benghazi, Libya.

Four of the analyzed products contained mercury exceeded the permissible limits that set by different global organizations.

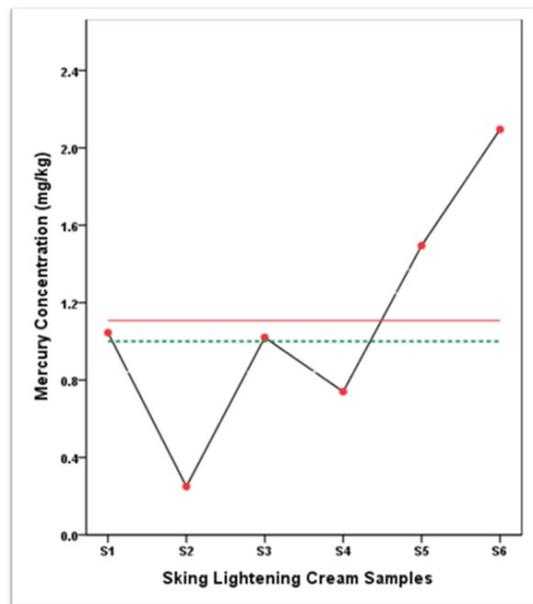


Fig. 1: Comparative total mercury concentration (ppm) in skin lighting cream samples (●, each value is a mean of two values) with maximum allowed limit by WHO, represented by broken green line (---). The average of mercury concentration in six lightening samples is represented by solid redline (—).

Although two analyzed samples have mercury concentrations fall within the permissible limit set by WHO, the frequent application of these creams for a long period of time might lead to toxic effects to consumers.

Despite of the control procedures that imposed by different countries around the world, to protect the consumers, the skin lightening products containing mercury are still available for sale over the internet. For this reason, it is important to adopt continuous education and raising awareness among the consumers about the dangerous effects of using uncontrolled skin lightening creams, via Television, magazine and the social media.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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