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Determination of Heavy Metals and Other Toxic Ingredients in Henna (*Lawsonia inermis*)

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Abstract

Background: The plant Henna (*Lawsonia inermis*, family *Lythraceae*) is a naturally grown or cultivated plant allover Africa and Asia. Marketed Henna is a natural powdered material derived from dried and crushed leaves of the plant. Henna is very popular in many parts of Egypt as it is part of the culture and traditions, and it recently became very popular in touristic areas as Sharm El Sheikh-Egypt being used as a tattooing agent. This makes detection of heavy metal content and, other toxic ingredients in Henna marketed in Egypt of crucial importance.

Objective: To investigate heavy metal content and, other toxic ingredients as para-phenylenediamine (PPD) in Black Henna marketed in Egypt.

Methods: Fifteen Black Henna samples were collected randomly from Sharm El Sheikh-Egypt market and, analyzed for metal content by atomic absorption spectrophotometry (ICP-MS) after microwave acid digestion, also the presence of PPD in henna samples was determined qualitatively and quantitatively using High Performance Liquid Chromatography (HPLC).

Results: PPD was detected in all the black henna samples at concentrations ranging between 1.75% and 32.1%, which is higher than that recommended for hair dyes. The mean concentration of some studied metals as Aluminium, Lead, copper, nickel, and zinc were higher than permissible levels for cosmetics in some of the studied samples.

Conclusion: In conclusion, PPD is a common ingredient in black henna dye in the developing world. Physicians must be aware of the potential toxicity of this chemical and of the clinical signs of systemic poisoning. High concentration of some metals as Aluminium, lead, copper, nickel, and zinc might be encountered in some Henna products. Considering popular use of henna, the hazardous cumulative effects of prolonged exposure to low concentrations of such metals, especially in children, cannot be ruled out. Further studies are recommended.

Keywords: Henna; *Lawsonia inermis*; Heavy metals; Aluminum; Lead; Para-phenylenediamine; PPD; HPLC; ICP-MS; Cosmetics; Hair coloring; Traditional herbs; Allergic dermatitis; Poisoning; Toxicity; Cosmetics

Introduction

Henna (*Lawsonia inermis*) had been used as a hair colorant and decorative medium for long time [1,2]. Hair is a filamentous outgrowth of protein, whose main component is keratin [3]. Hair color is the result of pigmentation due to the existence of the chemicals eumelanin and pheomelanin. The hair coloring agents market is dominated by cosmetic companies mostly marketing chemical based colors [4]. It is an accepted fact that all chemicals have some side effects, convincing more discerning customers to use herbal based substitutes; that's why it was accepted that scientists and cosmetologist made use of henna as a chemical substitute for making hair colors [5].

Henna contains a wine-red dye molecule, lawsone (2-hydroxy-1,4-naphthoquinone) [6,7], which makes it useful for dying of hair, as well as colouring of palms, fingers, and soles by binding with proteins [8,9].

Henna paste is prepared by drying the henna leaves and grinding them to powder, and then mixing it with oil or water to form a paste. When this henna paste is applied to the skin the dye (lawsone) migrates from the paste to the outermost layer of the skin; more lawsone will migrate more if the paste is left on the skin for a longer time, thus creating a red-brown stain. Additionally, henna plant extract and essential oils can be used either in its concentrated or diluted forms and mixed with another materials in industrial products [2].

Besides its use in cosmetics, the henna leaves are used as a prophylaxis against skin diseases [10], and the stem is reported to be useful for jaundice, enlargement of the spleen [11], and skin diseases [12]. It is used also in therapy with oral administration, where the infusion of leaves is used in treating diarrhoea, renal lithiasis [13] and, abdominal pain [14].

Seeds have been reported to have deodorant action and are used in cases of menorrhagia, vaginal discharge and leucorrhoea [15]. Analysis of the chemical composition of Henna leaves indicates the presence of the active dye lawsone (2-hyroxyl,4-naphthaquinone), 1,2 dihydroxy coumarine, xanthones, flavones, flavanoids, gallic acid, steroids, tannic acid and light essential oils rich in phenols [16,17].

Para- Phenylenediamine (PPD), a derivative of Para-nitro aniline is widely used in hair dye formulations, in dyeing furs and in some industries [18]. It has also been used to deepen the color of henna and to accelerate the coloring process. Local application of PPD in vulnerable individuals may result in dermatitis, asthma, arthritis, lacrimation, exophthalmos or even permanent blindness when applied to the eyes. Oral ingestion of PPD might be fatal and results in renal failure, severe edema of face, tongue, neck and laryngeal edema with respiratory distress often requiring emergency tracheostomy [19].

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Some heavy metals have been used as cosmetics ingredients previously. Examples include the preservative thimerosal (mercury), the hair dye lead acetate and a number of tattoo pigments such as red cinnabar (mercuric sulphide). As the issue of heavy metals as intentional cosmetics ingredients has been thoroughly studied, awareness turns nowadays to the presence of these substances as impurities [20].

Methods

Heavy metals

The analyses for Heavy metal detection were performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave induced preparation of the 500 mg sample of henna with nitric acid in autoclave. The resulting solution was filtered and then diluted to 50 ml using demineralised water. Blanks were prepared in the same way.

Fourteen elements (Pb, Ba, Hg, Bi, Al, Cr, Mn, Co, Ni, Cu, Zn, Mo, Ag and Cd) in all prepared solutions were quantified subsequently by ICP-MS.

Organic substances

Selected henna products were analysed quantitatively for the content of PPD. The analysis for PPD was performed by HPLC.

Chemicals

All solvents were purchased from Sigma. PPD internal standard (IS) was prepared by weighing pure PPD substance (Sigma Life Science, 0.011 gram) and dissolving it in 50% aqueous methanol solution (100 mL). The HPLC instrument (Agilent; USA) comprised of a quaternary pump combined with photo diode array detector, vacuum degasser and an auto sampler injector. Chromatographic separation was performed on a Zorbaxeclips-C18 (150 mm × 4.6 mm, 5 µm) column (USA) maintained at a temperature of 25°C. The mobile phase consisted of acetonitrile: buffer (0.01M) potassium di-hydrogen phosphate (20:80, v/v), adjusted to pH 5.0 with 0.1M sodium hydroxide and a flow rate of 1.0 mL/min.

One gram of each of the collected samples was weighed into a 50 mL volumetric flask and diluted with 50% aqueous methanol solution (50 mL). This solution was then filtered after 15 minutes. Finally one mL of this solution was diluted to 5 mL with 50% aqueous methanol solution and analysed for PPD.

Statistical analysis

Data for metal estimation have been tabulated and the results were analyzed statistically and expressed as mean ± SD. The percentage concentration of PPD in all studied Henna Samples was tabulated.

Results

Case study (1)

An 18-years-old male sought medical consultation because of Erythema and vesicles on his back 2 days after the tattoo was applied with intense Erythema, vesicular formation and exudation. He did not undergo patch testing. The symptoms resolved with topical and systemic corticosteroids for two weeks (Figure 1).

Case study (2)

A 23-years-old female sought medical consultation because of Erythema, vesicle formation and pain on her left forearm one day after the tattoo was applied. She did not undergo patch testing. The symptoms resolved with topical corticosteroids and systemic antihistamines for 5 days (Figure 2).

Case study (3)

A 31-years-old female sought medical consultation because of Erythema, vesicle formation and intense pain on her upper right side of the back on the same day of the tattoo application, without previous patch testing. The symptoms resolved with topical and systemic corticosteroids for 10 days (Figure 3).

Laboratory studies

In this study, we have tested fifteen henna products for the presence of fourteen heavy metals using ICP-MS. The distribution of heavy metals in samples studied is shown in Table 1. Metals were detected in most of the samples in varying concentrations. However, we did not observe clear patterns indicating that metal concentrations were related to specific brands, colour, or cost. The distribution frequency of metals among the studied henna samples were; Al>Cr>Cu and Zn>Pb>Ni, Ba, and Mn>Mo, and Bi>Hg>Co, and Ag>Cd. Aluminium was detected in all the studied samples.

As shown in Table 2 and Figure 4, The PPD was found in all the studied black henna samples with concentration ranging between 1.75% and 32.1%. The concentration of PPD was more than 10% in ten

Figure 1: Erythema, vesicles, and inflammation on the back of one patient that started 2 days henna post application.

Figure 2: Erythema and inflammation on the left forearm of a patient that started 1 day post henna application.

Figure 3: Erythema, and inflammation on the upper right back of a patient that started the same day of henna application.

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Sample Number	Concentrations of Metals (ppm)													
	Pb	Ba	Hg	Bi	Al	Cr	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd
1	16.42 ± 1.62	10.37 ± 4.42	0.1 ± 0.14	21.30 ± 4.02	85.5 ± 3.99	8.05 ± 4.97	9.89 ± 3.39	ND	ND	118.9 ± 2.85	345.8 ± 3.27	ND	ND	ND
2	1.12 ± 0.78	18.9 ± 0.12	ND	ND	10.63 ± 1.52	7.3 ± 4.97	0.89 ± 0.13	ND	4.94 ± 0.18	118.6 ± 2.25	ND	ND	ND	0.14 ± 0.07
3	5.37 ± 1.72	36.04 ± 4.05	ND	ND	137.1 ± 1.24	7.20 ± 2.87	ND	ND	223.11 ± 0.25	95.92 ± 4.65	54.91 ± 14.16	ND	ND	ND
4	ND	ND	ND	66.15 ± 3.06	1017.3 ± 0.52	1.30 ± 1.11	ND	ND	29.65 ± 3.53	ND	270.122 ± 0.126	ND	ND	ND
5	11.51 ± 0.06	17.46 ± 2.21	ND	ND	56.75 ± 0.03	15.75 ± 0.28	3.02 ± 5.95	ND	ND	2.34 ± 0.25	5.39 ± 3.15	ND	ND	ND
6	15.34 ± 0.73	ND	2.4 ± 1.04	54.15 ± 3.1	19681.5 ± 0.17	9.51 ± 0.11	ND	ND	3.41 ± 0.14	ND	284.63 ± 0.304	ND	ND	ND
7	9.38 ± 0.10	10.20 ± 0.21	ND	ND	8803.25 ± 0.18	4.45 ± 0.13	0.70 ± 0.01	ND	18.45 ± 12.3	4.51 ± 0.19	1.676 ± 0.22	ND	ND	ND
8	5.18 ± 3.63	2.50 ± 0.151	ND	ND	69.85 ± 0.05	2.16 ± 0.32	ND	ND	ND	ND	ND	1.040 ± 0.026	0.82 ± 0.79	0.958 ± 0.24
9	5.78 ± 0.83	103.68 ± 0.6	ND	ND	33.26 ± 0.92	1.32 ± 1.02	11.04 ± 0.1	1.06 ± 0.09	ND	18.95 ± 14.29	36.99 ± 0.19	ND	ND	ND
10	1.85 ± 0.62	93.75 ± 1.01	1.95 ± 0.71	38.58 ± 3.26	478.9 ± 4.39	3.15 ± 0.12	4.01 ± 3.65	ND	ND	2.464 ± 0.484	89.93 ± 3.43	1.02 ± 0.36	ND	ND
11	5.71 ± 0.81	ND	ND	ND	10.98 ± 1.3	6.22 ± 0.84	13.54 ± 0.19	0.47 ± 0.21	3.22 ± 0.34	4.84 ± 0.16	16.69 ± 0.34	0.227 ± 0.108	1.780 ± 0.448	ND
12	ND	ND	ND	32.07 ± 3.51	142.1 ± 1.52	8.72 ± 0.04	ND	ND	7.12 ± 0.3	1.65 ± 0.23	ND	2.18 ± 0.26	ND	ND
13	2.12 ± 0.3	79.57 ± 0.66	ND	698.1 ± 4.05	10.98 ± 1.3	4.30 ± 0.12	5.76 ± 0.07	1.3 ± 0.3	ND	2.95 ± 0.11	10.62 ± 0.28	0.71 ± 0.28	ND	ND
14	ND	ND	ND	ND	694.5 ± 0.71	ND	ND	ND	18.45 ± 2.3	0.84 ± 0.02	996.3 ± 0.1	ND	ND	ND
15	ND	ND	1.43 ± 0.54	ND	62.17 ± 4.18	2.16 ± 0.32	1.19 ± 0.49	ND	10.53 ± 0.15	18.95 ± 4.29	54.91 ± 4.16	0.51 ± 0.06	0.07 ± 0.05	ND

Results are expressed as average ± SD for three samples (SD: standard deviation); All results are in ppm: Part per million; SD: Standard deviation; ND: not detected (<DL)

Table 1: Concentrations of metals in Black Henna samples.

Sample Number	PPD Concentration
1	32.104
2	18.214
3	11.251
4	7.457
5	11.124
6	15.016
7	9.784
8	1.753
9	25.682
10	16.739
11	2.187
12	29.518
13	26.511
14	20.503
15	4.249

Table 2: The Concentration of Para-Phenylenediamine in Henna Samples.

black henna samples, more than 6% in twelve samples, more than 2% in fourteen samples, and less than 2% in only one black henna sample. The differences in concentration of the PPD between the samples may be attributed to the difference in the origin of the Henna samples. HPLC chromatograms of a blank and one of the Henna samples are shown in Figures 5 and 6.

Discussion

Cosmetics have often been considered by many dermatologists, more dangerous than good [21]. They may contain more than 10,000 ingredients which are related to many diseases like cancer, birth defects, developmental and reproductive harms. Knowing such poisonous effects the (US FDA) Food and Drug Administration in 2011 [22] completely forbidden the presence of nine ingredients including coal tar colors, formaldehyde, glycol ethers, lead, mercury, Phenylenediamine, phthalates in cosmetic products.

Recently, awareness has been drawn to PPD use in many brands of black henna dyes and the possibility to cause hyper sensitivity reactions in many individuals [23]. Recently PPD has been mixed with natural henna to give an ebony dark black colour instead of the orange/reddish colour given by natural henna, and to hasten fixation time as well [24]. Natural henna staining takes an average of 4 to 12 hours but, addition of PPD can reduce this time to an hour or two, and also long-term effect will be seen as well. Thus, a new pattern of exposure to PPD has been noted through henna art which increases the risk of developing adverse health effects related to PPD [25].

Acute short-term exposure to high levels of PPD may cause severe dermatitis, pruritic, oedematous, erythematous scaly patches and vesicular dermal lesions, in addition to eye irritation and tearing [26]. Severe allergic reactions to PPD can result in asthma, gastritis, renal failure, vertigo, tremors, anaphylaxis, convulsions, and coma. PPD induced dermatitis on the hands is commonly seen in hair dressers. Other skin reactions, such as post inflammatory hyper or hypo pigmentation, can also ensue [27,28].

An increasing number of reports of contact allergic dermatitis from temporary black henna tattoos have appeared in many recent dermatologic literature, and most of these have implicated PPD as the

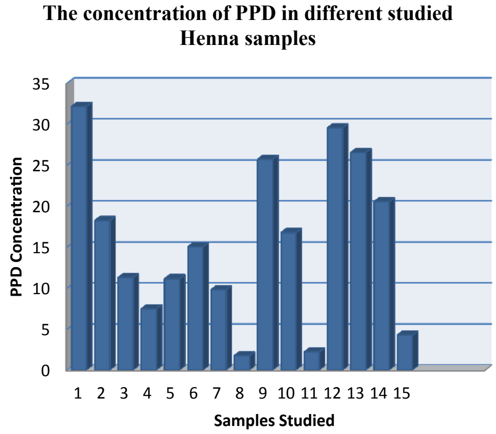

Figure 4: The Concentration of Para-Phenylenediamine in Henna Samples.

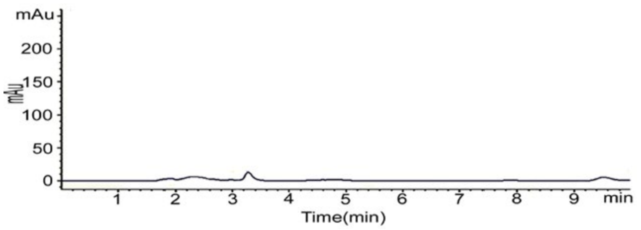

Figure 5: HPLC chromatogram for a blank sample.

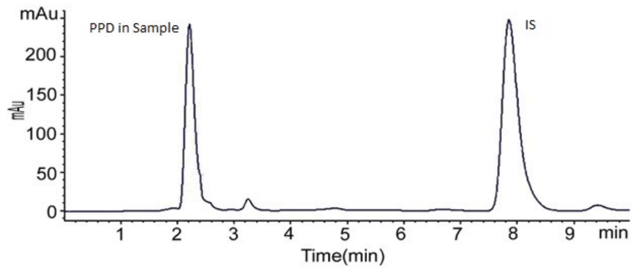

Figure 6: HPLC chromatogram for PPD in one of the samples and Internal Standard.

causative allergen, as the patients reacted to PPD on patch testing [29]. The highest concentration of PPD reported by this study (32.10%) was significantly higher than that reported by Ayesha Al-Suwaidi et al. [30] (29.5%), Brancaccio et al. [31] in their studies (15.7%), and also higher than that reported by Kang and Lee et al. (2.35%) [32].

The concentration of PPD in fourteen black henna samples was higher than the maximum concentration of PPD found in hair dyes when applied to hair as specified by Scientific Committee on Consumer Products (SCCP) [33] which is 2.0%. The concentration of PPD reported by this study for twelve black henna samples was higher than the permitted concentration of PPD in hair dye products established by the European Union, which is 6.0% [31], and this finding is consistent with other researchers who found the level of PPD in black henna used for tattooing was much higher than that found in hair color [23,31,34,35]. The European Union has banned the use of PPD directly on the skin, eyelashes or eyebrows, and FDA as well has prohibited the use of PPD directly on the skin [31].

The density of heavy metals is five times greater than that of water [36,37]. These includes: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc. Even the essential metals when present in higher concentration show toxic effects [37].

Exposure to metals may occur through the diet, medications, environmental exposure and cosmetics use [38]. The use of underarm anti-perspirant has been investigated as a possible cause of breast cancer. Basis for breast carcinogenesis may be due to the binding of various chemical constituents including metals to DNA and promotion of damaged cells growth [39], that's why some Directives banned the use of heavy metals, Cd, Co, Cr, Ni, and Pb as impurities in the preparation of cosmetics [40]. Information about dermal exposure to metal toxins is very scanty, and few data exist on the personal care products and their role in inducing toxicities [41].

The study of metal content in some black Henna products raise the concern about potential public health hazards; though, metals in cosmetic products are not currently regulated by the FDA. Although metal concentrations in cosmetic products have been reported by studies performed in many countries [42-49], interpreting how these reported concentrations may be related to potential health risk is challenging.

Apart from the demand of availability of cosmetic products in different markets, the increasing health awareness draws the attention of researcher and clinician [50] to find adverse effects related to heavy metal contamination [51,52]. Although some cosmetics are benign; others can cause or are supposed to cause harmful effects such as cancer, allergic reaction, mutations, as well as respiratory, developmental and reproductive problems. An increased level of cadmium has been reported to cause inhibition of DNA mismatches, Zinc as well has been reported to cause the same signs of illness as does lead, and can easily be mistakenly diagnosed as lead poisoning [53,54].

Keeping in mind their toxicity, the estimation of toxic metals in Henna products has encouraged us to carry out this study. Unfortunately there are no existing international standards for impurities like heavy metals in cosmetics except 20 ppm for lead and 5 ppm for cadmium [55]. The Canadian regularity limits for certain metals in cosmetics are 10 ppm for Pb, 3 ppm for As, Cd, Hg and 5 ppm for Sb [56]. The result of this experiment revealed that the distribution frequency of metals among the studied henna samples were; Al>Cr>Cu and Zn>Pb>Ni, Ba, and Mn>Mo, and Bi>Hg>Co, and Ag>Cd.

Most of the black Henna samples in this study were found to contain high concentration of metals, particularly, Al, Pb, Cu, Ni, and Zn which showed a wide variation among the samples. The differences in concentration of the studied metals between the samples may be attributed to the difference in the origin of the Henna samples. Aluminium was detected in all the studied samples. Significant concentrations of aluminium (19681.5 ppm) have been found in one henna product (sample 6) and 8803.25 ppm in sample number (7). Worth mentioning, Al-Saleh et al. [43] had highlighted the adverse developmental effects of aluminium toxic exposure on children and infants.

Very high lead concentration was measured also in four samples which reached 16.42, 15.34, 11.51 and 9.38 ppm in samples number (1, 6, 5, and 7) respectively. This high lead value poses an alert for all users of such products. It is likely that an exposure to more than 0.005 ppm of cadmium can be hazardous to human health. However, level of exposure that is well thought-out as a high limit is diverse because the cadmium effect may increase or decrease depending on other factors as the form, type and duration of exposure (ATSDR 2012) [57,58].

Sixty (60) percent of the tested samples contained nickel, with levels ranging from 3.22 ppm in sample (11) to 223.11 ppm in sample number (3). The presence of high concentrations of nickel (more than 5 ppm) was detected in six samples of studied henna products. Usually, nickel salts are used as dye mordant and pigments. Nickel allergy caused by eye shadow has been reported by Sainio et al. [58], who reported that even 1 ppm may set off a pre-existing allergy.

Maximum concentration of Copper was 118.9 ppm detected in sample (1) which is higher than many of the studies conducted for cosmetic products. The maximum value of cobalt (1.3 ppm obtained in this study in sample number (13) was found to be lower than those reported elsewhere [59].

Barium, Manganese, and Nickel were detected in nine samples. Cadmium was detected only in two samples (2 and 8), followed by Silver and Cobalt that were detected in only three samples. Fourteen of the tested samples contained chromium (ranging from 1.30 to 15.75 ppm) where 70% of the samples contained chromium with a concentration more than 5 ppm. Some directives banned the use of heavy metals, Cd, Co, Cr, Ni, and Pb as impurities in the preparation of cosmetics [40,60,61].

The metals analyzed in this study were not listed as ingredients on any of the tested products. Due to absence of manufacturer testing and regulatory monitoring of such products, it is possible that the producing companies are not even attentive that their products are contaminated. These metal contaminants likely get into the products when below standard ingredients are used. Manufacturers should test the used raw ingredients before using them in making the final products to be able to track the origin of such contaminants.

Conclusion

This study has revealed that continuous use of such contaminated cosmetics could result in an increase in some metal levels in human body beyond acceptable limits, as well as induction of severe allergic dermatitis in susceptible personnel due to unsafe constituents that were not mentioned on the constituent list of the black henna package.

Our findings call for an instant mandatory regular testing program to check heavy metals in cosmetic products that are imported to Egypt in order to limit their overabundance and protect consumer health. Only by studying the clinical types of adverse reactions to black Henna, it will be possible to gather a complete epidemiological data about the nature and extent of the problems related to such cosmetics' ingredients. Also further efforts should be made at enlightening the users and the general public on the dangers involved especially for unknown misbranded products that are pumped in large quantities to many markets in the Arab world, especially in touristic areas like Sharm El Sheikh, Egypt.

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