

Quantification of para-phenylenediamine and heavy metals in henna dye

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Henna (*Lawsonia inermis*, family Lythraceae) is a shrub cultivated in India, Sri Lanka and North Africa and contains the active dye lawsone (2-hydroxy-1,4-naphthoquinone). Henna dye is obtained from the dried leaves, which are powdered and mixed with oil or water and are used to prepare hair and body dyes. Temporary henna tattoos are readily available worldwide, last on the skin for several weeks and offer a self-limited, convenient alternative to a permanent tattoo. The addition of para-phenylenediamine (PPD), which is widely recognised as a sensitizer, increases the risk of allergic contact dermatitis from henna tattoo mixtures, and a number of cases have been reported. We examined 15 henna samples available in Korea for the presence of PPD and heavy metals such as nickel, cobalt, chromium, lead and mercury using high-performance liquid chromatography (HPLC), atomic absorption spectroscopy (AAS), mercury analyser and inductively coupled plasma emission spectroscopy. PPD, nickel and cobalt were detected in 3, 11 and 4 samples, respectively.

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Henna dye is a dark green powder used in the Arab world and Indian subcontinent for skin decoration and hair dyeing and for social purposes (1, 2). It is obtained from the leaves of an indigenous shrub, *Lawsonia inermis*. The dry leaves are ground into a fine powder that is mixed with oil or water. It is applied on the skin using a soft, cone-shaped container and is left to dry for 2 h–6 h. The longer henna is left in contact with the skin, the darker the resulting colour. For this reason, darkness-enhancing substances are added to shorten the time of application. These substances include tannin concentrates obtained from tea leaves, instant coffee powder, charcoal powder, lampblack and 'secret' formulas that contain varying amounts of para-phenylenediamine (PPD), lemon oil, vinegar, eucalyptus oil and clove oil (2, 3).

Although there have been some reports of allergic contact dermatitis caused by PPD in henna tattoo mixtures, there have been no reports on the effects of heavy metals, such as nickel and cobalt, in henna. We observed positive reactions to nickel, cobalt and PPD in a patch test done on a patient with allergic contact dermatitis to henna (Figs 1 and 2). Therefore, we realised the importance of a further evaluation of the effect of PPD and heavy metals, such as

nickel, cobalt, chromium, lead and mercury, in the development of contact dermatitis resulting from henna tattoo mixtures.

Materials and Methods

Materials

We investigated samples of 15 henna dyes used for tattooing in Korea. The samples were bought from 15 different tattoo shops selling henna dyes for temporary tattoos.

Method

Analysis of PPD

Accurately weigh 0.5 g of sample into 50 ml volumetric flask and dilute with 50% aqueous methanol solution (the dilution ratio depended on the amount of material detected). This solution was sonicated for 15 min and passed through a membrane filter. A 100 ml solution was produced by mixing a stock solution (Rodol D, Lowenstein and Sons, Brooklyn, NY, USA) containing 10 mg of PPD and 50% methanol. It was then diluted to 50 µg/ml, 10 µg/ml and 1 µg/ml. High-performance liquid chromatography (HPLC) was performed using a Waters



Fig. 1. (a) Raised or swollen and geometric erythematous patches matching the designs on the left upper arm. (b) Focal dark-brownish coloration of a henna tattoo mixture.

Alliance 2695 HPLC system equipped with a photodiode array detector. The HPLC conditions were mobile phase, 0.05 M acetic acid (adjusted to pH 5.9 with ammonia)/methanol (95/5); flow rate, 1 ml/min and column, LiChrosphere RP Select B (250 × 4.0 mm, 5 µm). A standard curve was made using the 50 µg/ml, 10 µg/ml and 1 µg/ml. The raw integration values of the samples at 290 nm were converted to the percentage weight of PPD in the samples based on the standard curve.

Analysis of cobalt and nickel

A 0.4-g sample was added to a 15-ml Falcon tube with pure water to make 10 ml of solution. The solution was heated in an 80° C–90° C water bath for 30 min. After cooling, it was sonicated for 15 min and filtered to make the test solution. Stock solutions of cobalt and nickel were made by diluting standard solutions of 1000 mg/l from Fluka. Atomic absorption spectroscopy (AAS) (Varian spectra AA220FS) was performed at a wavelength of 232.0 nm and a lamp current of

4 mA for nickel and at a wavelength of 240.7 nm and a lamp current of 7 mA for cobalt.

Results

The HPLC and AAS results are summarised in Table 1. Standard calibration curves were constructed in 1 µg/ml–100 µg/ml for PPD, 0.1 µg/ml–1 µg/ml for cobalt and 0.2 µg/ml–2 µg/ml for nickel. The response was linear throughout the calibration range with the coefficient (r^2) greater than 0.99 for all cases. The limits of detection were estimated by successively diluting the concentration of the prepared standards. The absolute detection limits of PPD, cobalt and nickel were found as 0.2 µg/ml, 0.05 µg/ml and 0.1 µg/ml, respectively. When the dilution factor is considered, the detection limits of samples were 1.25 p.p.m. for cobalt, 2.5 p.p.m. for nickel and 20 p.p.m. for PPD. Of the 15 henna samples used in Korea, PPD, nickel and cobalt were detected in 3, 11 and 4 samples, respectively. PPD was detected at concentrations from 0.25% to 2.35%; nickel was detected from less than

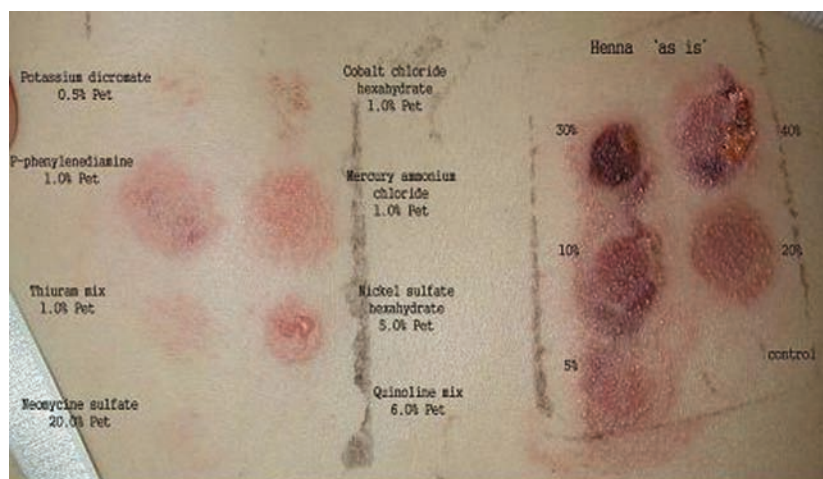


Fig. 2. Positive reactions to PPD, nickel, thiuram, mercury, chromium and cobalt and reactions resulting from using henna 'as is' at 96 h.

Table 1. Identification and quantification of para-phenylenediamine (PPD) and heavy metals in henna tattoo mixtures

Sample number	PPD (%)	Nickel (mg/kg)	Cobalt (mg/kg)
1	N.D.	3.90 ± 0.54	3.54 ± 0.32
2	N.D.	< 2.5	N.D.
3	N.D.	3.96 ± 0.32	N.D.
4	N.D.	N.D.	N.D.
5	N.D.	3.71 ± 0.19	N.D.
6	2.351 ± 0.020	<2.5	N.D.
7	N.D.	N.D.	N.D.
8	0.245 ± 0.009	N.D.	2.96 ± 0.24
9	1.760 ± 0.054	3.52 ± 0.71	3.36 ± 0.30
10	N.D.	3.83 ± 0.19	N.D.
11	N.D.	N.D.	3.25 ± 0.30
12	N.D.	2.94 ± 0.55	N.D.
13	N.D.	3.33 ± 0.57	N.D.
14	N.D.	<2.5	N.D.
15	N.D.	<2.5	N.D.

N.D., not detected.

HPLC was used to measure PPD, nickel, chromium and cobalt in henna tattoo mixtures. ICP-AES (Varian) and a mercury analyzer (M-6000A, Cetac) were used to detect lead and mercury, respectively. Data were described as the mean ± standard deviation of triplicate experiments.

2.5 p.p.m.–3.96 p.p.m. and cobalt was detected from 2.96 p.p.m. to 3.54 p.p.m.

Discussion

Henna has been used to make temporary tattoos in the Middle East and southern Asia since medieval times. Henna leaves contain the active substance naphthoquinone, which is relatively harmless (3). Nowadays, henna is used extensively for hair treatment and as a substitute for chemical hair dyes. Recently, temporary tattoos have become popular among young people. As the use of henna alone limits the extent of the colour and requires prolonged skin contact, various substances are added (2, 4, 5).

The addition of PPD, as widely recognised sensitizer, increases the risk of allergic contact dermatitis from henna tattoo mixtures, and a number of cases have been reported (2–7). According to the Food and Drug Administration, the only legal use of PPD in cosmetics is as a hair dye. It is not approved for direct application to the skin (5). Furthermore, the Scientific Committee for Cosmetic Products and Non-Food Products Intended for Consumer Use has also stated in their laws that lawsone is not suitable for use as a non-oxidising colouring agent for hair dyeing and, by extension, is not suitable for any other cosmetic use (8). Allergic contact dermatitis resulting from henna tattoo mixtures can occur in people who have been sensitised to PPD as well as in people who have not, and the

risk increases with the amount of PPD contained in the henna dye. Moreover, the lack of an oxidising agent like hydrogen peroxide in the mixture can lead to prolonged contact with PPD, which dramatically increases the risk of active skin sensitisation and subsequent allergic reactions (4, 5).

Out of the 4 patients that (9) experienced allergic contact dermatitis due to henna tattoos, 2 patients showed positive reactions to PPD after 10–14 days of contact, and they have reported in their literature that the dye substance sensitises the skin when in contact with it for a long period of time. The concentration of PPD detected in our experiment was 0.25%–2.35%, significantly lower than 15.7% that Brancaccio *et al.* (5) have reported, but even with low concentration, it is thought to be sufficient enough to induce sensitisation when in long-term contact with the skin.

We experienced a case of allergic contact dermatitis from a henna tattoo with positive patch test results in nickel, cobalt and PPD. Also, PPD and nickel were detected in the results when HPLC was employed to quantitatively analyse the henna dye the patient used (see sample number 6 in Table 1). This led us to postulate that heavy metals such as nickel or cobalt play a role in the occurrence of allergic contact dermatitis caused by henna tattoo mixtures. It is interesting to note that nickel and cobalt are present in most samples, but the role of metals in allergic contact dermatitis from henna tattoo mixtures is less probable. Allenby *et al.* reported that patients with normal skin previously sensitised to nickel can develop contact dermatitis at 1 p.p.m.–10 p.p.m. and at 0.5 p.p.m. if the skin has been irritated with sodium dodecyl sulphate (10). The concentrations of nickel and cobalt we found in the henna tattoo mixtures were relatively low, from less than 2.5 p.p.m.–3.96 p.p.m. and 2.96 p.p.m.–3.54 p.p.m., respectively. But as the substances in henna tattoos remain on the skin for a prolonged period of time, it is hard to completely rule out the possibility of heavy metals playing a role in inducing contact dermatitis. Also, even if these heavy metals are irrelevant to the development of contact dermatitis by henna tattoos, they are thought to provoke sensitisation, contributing to the occurrence. Therefore, we believe that additional research with clinical examinations should be done for further evaluation.

Also, we quantitatively analysed the existence of chromium, lead and mercury in addition to nickel and cobalt in henna tattoo mixtures. HPLC, ICP-AES (Varian, Palo Alto, USA) and a mercury analyser (M-6000 A, Cetac, CA, USA) were used to detect chromium, lead and

mercury, respectively. The detection limits of chromium (III), chromium (IV), lead and mercury were found as 0.2 µg/ml, 0.1 µg/ml, 0.1 µg/ml and 0.1 µg/ml, respectively. But, these metals were not detected in the samples.

Recently, henna dyes have been widely utilised in skin decorations; this replaces the more traditional use of chemical hair dye containing PPD. Therefore, patch tests for PPD and heavy metals should be conducted when henna-related allergic contact dermatitis occurs, along with additional tests as necessary.

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