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## Para-phenylenediamine toxicity in Henna product in Libya

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### Abstract

Henna is very famous inside the Libya; it's a part of the style of life and traditions. Allergy to natural henna isn't familiar; however the addition of para-phenylenediamine (PPD) to the natural henna increases the chance of allergic touch dermatitis. The objectives of the study were to identify the presence and determine the concentration of PPD in henna available in Libya and identity of heavy metals in these samples. To determine quantitatively and qualitatively presence of PPD in samples of henna we used High Performance Liquid Chromatography (HPLC) and nine elements (Pb, Hg, Al, Cr, Mn, Co, Ni, Mo and Ag) were quantified subsequently by Mass Spectrometry. Natural henna and jawee el henna and henna mix with unknown addition components were determined by a by GC/MS. The study showed that PPD was present in jawee el henna sample at concentration 100% and henna mix with unknown addition samples at concentrations ranging between11.01% and 24.66, At the same time as all of the black henna samples at concentrations ranging among 9.74% and 16.34% which is higher than that red henna. In some studied metal as Aluminum, Lead, and nickel mean concentration of red henna samples was lower than that black henna.

Key words: Henna; Heavy metals; Para-phenylenediamine; PPD; HPLC; GC/MS; Toxicity; G6PD deficiency

#### Introduction

Henna is a flowering plant belonging to the Lythraceae family. Henna is traditionally and extensively utilized in North Africa [1]. In Libya, these are freely bought, which increases their use henna were used as a hair colorant and ornamental medium for a long time. Henna is commercially cultivated in Morocco, Sudan, India, Pakistan, Yemen, and other international locations. Henna contains a burgundy dye molecule, lawsone (2-hydroxy-1,4-naphthoquinone).

This dye molecule has the potential to bind to proteins, and therefore has been extensively utilized in body art to dye pores and skin, hair and fingernails, and to dye silk, leather, and wool.

Henna frame artwork is made with the aim of making use of henna paste to the skin.

Henna paste is ready by means of drying the henna leaves and grinding them to powder, after which this powder is blended with oil or water to form the paste. When this henna paste is carried out to the skin the dye (lawsone) migrates from the paste to the outermost layer of the pores and skin; more lawsone will migrate if the paste is left on the skin for a longer time, hence creating a pink-brown stain [2]. Henna has been used to enhance ladies's our bodies in the course of marriage ceremonies and other social celebrations since the Bronze Age. In the Arab world, Egypt, Libya and Indian subcontinent henna is used for skin decoration and hair loss of life at some stage in social celebrations, and all through marriage ceremonies people have a good time by using decorating the bride, and sometimes the groom, with henna [3].

P-Phenylenediamine (PPD), Synonym: 1.4-Diaminobenzene, Chemical Formula: (C6H8N2) (fragrant amine compound), has many business programs. In cosmetology, it's far usually added to hair dyes (Henna, Lwasonia inermis) and is used as everlasting body dye (Black Henna or Harkous) to supply a darker colour [4]. Local utility of PPD in vulnerable individuals might also bring about dermatitis. asthma. arthritis. lacrimation. exophthalmos or even everlasting blindness while implemented to the eyes. Oral ingestion of PPD might be fatal and outcomes in renal failure, intense edema of face, tongue, neck and laryngeal edema with respiratory distress often requiring emergency tracheostomy [5].

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#### **Potential Acute Health Effects of PPD:**

Very risky in case of ingestion, of inhalation (lung irritant). Hazardous in case of pores and skin touch (irritant, permeator), of eye touch (irritant). Severe over-publicity can bring about death. Repeated exposure to an exceedingly poisonous cloth might also produce popular deterioration of health via an accumulation in a single or many human organs. [6] Short-term exposure to high ranges of PPD (acute outcomes) might also purpose extreme dermatitis, eve irritation and tearing, allergies, gastritis, renal failure, vertigo, tremors, convulsions, and coma in human beings. Eczematous touch dermatitis may also result from lengthy-term publicity (chronic impact) in people [7]. According to Scientific Committee on Consumer Products (SCCP), para-phenylenediamine is a completely sturdy potential pores and skin sensitizer and it's miles blanketed as such in the European Standard Series for diagnostic patch trying out for eczema sufferers [8]. Para-Phenylenediamine (PPD) is an allergen; although a person does no longer have a response to the primary time they are exposed to it, they could come to be "sensitized" to PPD over the years and might have destructive reaction upon re-exposure [9].

In UAE many cases of touch hypersensitive reaction had been reported after Black Henna usage. Picture 1 shows one of these cases where the patient has developed hand sensitization few days after the use of black henna.



# Picture 1. Hand dermatitis, reaction to black henna.[5]

Picture 2 shows a positive result for a patch test done on the patient with a PPD concentration of 0.1 [10]. In addition, hospitals in UAE have been reported that person suffered from vomiting and stomach ache after the laboratory takes a look as she was diagnosed

with poisoning because of henna. The affected person also had glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency, a genetic sickness which is greater commonplace inside the Gulf vicinity [10]. Usually, G6PD deficiency ends in an ordinary rupture (breakage) of crimson blood cells called haemolytic anemia. There is evidence that many materials are probably harmful to people with G6PD deficiency and they can motive acute haemolysis in human beings with G6PD deficiency. These materials encompass: anti-malarial medicines (together with chloroquine, primaquine), sulfonamides (consisting of sulfamethoxazole, sulfanilamide). PPD has a comparable structure to those substances and thus it'd cause the identical effect in people with G6PD deficiency

PPD has a similar shape to those substances and as a result it might purpose the identical effect in humans with G6PD deficiency



#### Picture 2. Positive reaction to patch test done using 0.1% PPD.[10]

In addition, it is said within the literature that henna has been unknown to cause haemolytic disaster in G6PD-poor babies and others [11-12]. In the UAE, Raupp and his colleagues have stated the occurrence of haemolytic crisis in 4 G6PD-deficient youngsters following topical utility of henna; of these a woman neonate recovered after exchange transfusion, and a male infant died regardless of receiving a transfusion [13].

Several heavy metals had been used as cosmetics substances previously. Examples consist of the preservative thimerosal (mercury), the hair dye lead acetate and some of tattoo pigments together with crimson cinnabar (mercuric sulphide). As the issue of heavy metals as intentional cosmetics components has been very well studied, focus turns in recent times in the presence of those substances as



impurities [14]The objectives of the study were to identify the presence and concentration of PPD in henna available in Libya and determination of Heavy metals in these samples.

#### Materials and Methods Samples

The Natural henna, jawee el henna, henna mix with unknown addition, black henna and red henna were purchased from super market in (Benghazi, almarge, Albayda and Ajdabiya) cities in the Libya.

#### Chemicals

p-Phenylenediamine (PPD) was supplied from Sigma company. All solvents were obtained from the Judicial experience and research center in Albayda.

#### Heavy metals determination :

Analyses for heavy steel detection have been carried out by using Inductively Coupled Plasma Mass Spectrometry after microwave precipitated preparation of the 500mg pattern of henna with nitric acid in an autoclave. The ensuing solution was filtered and then diluted to 50 ml using demineralized water. Blanks has been prepared in the equal manner. nine elements (Pb, Hg, Al, Cr, Mn, Co, Ni, Mo and Ag) in all organized solutions were quantified finally by Mass Spectrometry[15].

# Analysis of Samples for PPD by HPLC chromatography

PPD standard (0.11 mg per mL) was prepared by weighing pure PPD substance (Sigma Life Science, 0.011 gram) and dissolving it in 50 % aqueous methanol solution (100 mL) [ 2 ]. The HPLC conditions were: mobile phase, 0.05 M acetic acidmethanol (95/5) and adjusted to a pH of 5.9 with ammonia; the temperature was 30°C; flow rate was 1.5 mL/min; wave- length 290 nm; pressure 174 bar; and column, LiChrospher RP18 5 $\mu$ m, 250 mm × 4.6 mm. 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 analyzed for PPD. To confirm the identity of PPD in our samples, one mL of the standard was diluted to 5 mL with 50% aqueous methanol solution and analyzed before analyzing any sample to determine its spectrum and its retention time.

### Gas chromatography/ Mass spectra.

Thermo Scientific, Trace GC Ultra & ISQ Single Quadruple MS, DB-5 bonded-phase fused-silica capillary column was used in for GC/MS analysis of both henna and jawee el henna. **Statistical analysis**  Data for metal estimation have been tabulated and the results were analyzed statistically and expressed as mean  $\pm$  SD. The percentage concentration of PPD in all studied Henna Samples was tabulated.

#### **Results and Discussion**

The observe showed that PPD become found in jawee el henna pattern at concentration 100% and henna blend with unknown addition samples at concentrations ranging between11.01% and 24.66, while all of the black henna samples at concentrations ranging between nine.74% and sixteen.34% that is higher than that red henna Table 1.

In this take a look at, we've examined ten henna products for the presence of nine heavy metals the use of Mass Spectrometry. The distribution of heavy metals in samples studied is shown in Table 2.

They imply a concentration of some studied metals as Aluminum, Lead, and nickel in black henna samples had been better than that pink henna Table 2.

Due to the high polarity and low volatility of the hydrophilic materials in the henna, gas chromatography is restricted to use to identify these substances. On the running of GC/MS technique to determine the PPD obtained from commercially available henna, many peaks were obtained in the chromatograms. This could be due to the thermolabile nature of PPD. In the natural henna sample there was no detected peak for PPD as shown in Figure1, however, those peaks were obviously shown in all of the other henna samples such as Jawee, magona, and red henna (sample No . 3) as shown in Figures 2, 3 and 4 respectively. Those samples showed peaks with retention times between 15-19 min which indication of the present of PPD compound.



Figure 1. The GC spectra of the natural henna

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Figure 2. The GC spectra of the Jawee henna



Figure 3. The GC spectra of the Magona henna



# Figure 4. The GC spectra of the red henna (sample No . 3)

Moreover, the very best attention of PPD stated by this have a look at (16.34%) changed into appreciably higher than that suggested by means of Brancaccio and his colleagues of their look at (15.7%) [16], and additionally higher than that said by Kang and Lee (2.35%) [5].

When evaluating the attention of PPD in henna blend and black henna to that during red henna we discovered that the attention of PPD in Black Henna turned into as expected higher due to the fact the black coloration suggests a high attention of PPD [17].

The attention of PPD reported with the aid of this observe for all black henna samples become better than the authorized awareness of PPD in hair dye merchandise established through the European Union, that is 6.0% [16], and this locating is steady with other researchers who located the level of PPD in henna tattoos changed into plenty better than that determined in hair shade [16–17]. However, the European Union has banned using PPD directly at the skin pores, eyelashes or eyebrows, and Food and Drug Administration (FDA) has prohibited the use of PPD directly on the skin pores [16].

Cosmetics have frequently been taken into consideration by many dermatologists, greater risks than right [18]. They might also contain extra than 10,000 ingredients that are related to many sicknesses like most cancers, delivery defects, developmental and reproductive harm. Knowing such toxic results of the (US FDA) Food and Drug Administration in 2011 [19] completely forbidden the presence of 9 which include coal components tar hues. formaldehyde, glycol ether, lead. mercury, Phenylenediamine, phthalates in cosmetic merchandise.

Recently, awareness has been attracted to PPD use in many brands of black henna dyes and the opportunity to cause hypersensitivity reactions to lots of individuals [20]. Recently PPD has been mixed with herbal henna to provide an ebony dark black color in preference to the orange/reddish colour given by herbal henna, and to hasten fixation time as property [21].

Natural henna staining takes a mean of four to 12 hours but. The addition of PPD can reduce this time to an hour or two, and also long-term impact will be visible as nicely. Thus, a brand new pattern of publicity to PPD has been cited through henna art which increases the chance of developing damaging fitness consequences related to PPD [22].

Acute quick-term exposure to high levels of PPD may additionally motive extreme dermatitis, pruritic, edematous, erythematous scaly patches and vesicular dermal lesions, further to eye irritation and tearing [23]. Severe allergic reactions to PPD can result in bronchial asthma, gastritis, renal failure, vertigo, tremors, anaphylaxis, convulsions, and coma. PPD caused dermatitis at the palms is usually seen in hair





dressers. Other skin reactions, together with put up inflammatory hyper or hypopigmentation, can also happen [15].

An increasing number of stories of contact allergic dermatitis from temporary black henna tattoos have regarded in many current dermatologic literature, and most of those have implicated PPD because the causative allergen, because the sufferers reacted to PPD on patch testing [24].

The highest awareness of PPD is said by means of this take a look at (sixteen.34%) turned into extensively better than that mentioned by using Brancaccio et al. [25] of their research (15.7%), and also higher than that proposed by means of Kang and Lee et al. (2.35%) [5].

The density of water is five times lesser than that of heavy steel [26]. These include: 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 toxic effects appear when the essential metal present in higher concentration

Exposure to metal might also occur thru the weight loss program, medicinal drugs, environmental exposure and cosmetics use [27]. The use of underarm anti-in keeping with spirant has been investigated as a likely cause of breast most cancers. Basis for breast carcinogenesis can be due to the binding of diverse chemical components which include metals to DNA and promoting of broken cells boom [28], that's why some Directives banned the use of heavy metals, Cd, Co, Cr, Ni, and Pb as impurities inside the preparation of cosmetics [29]. Information about dermal exposure to steel pollutants may be very scanty, and few records exist on the nonpublic care merchandise and their function in inducing toxicity [30].

The take a look at of metal content material in a few black henna merchandise increase the concern about capability public fitness hazards; though. Metal in cosmetic products isn't presently regulated by means of the FDA. Although metallic concentrations in cosmetic products were suggested by means of studies performed in many nations [15], deciphering how these mentioned concentrations can be related to capacity health danger is difficult.

Apart from the call for of availability of cosmetic merchandise in distinctive markets, the increasing health awareness draws the attention of the researcher and clinician [50] to find detrimental results related to heavy metallic infection [15]. Although some cosmetics are benign; others can purpose or are imagined to cause harmful outcomes inclusive of cancer, hypersensitive reaction, mutations, in addition to respiratory, developmental and reproductive troubles. An improved degree of cadmium has been said to motive inhibition of DNA mismatches, zinc as properly has been pronounced to purpose the same signs and symptoms of contamination as does lead, and can effortlessly be mistakenly recognized as lead poisoning [15].

#### Conclusions

The observe shows that the samples of jawed el henna contained 100% concentration of paraphenylenediamine, henna blend with unknown addition and black henna sold from the first rate market protected in this observe contained high awareness of para-phenylenediamine. Therefore, recommended not to use these materials

City	Black Henna		Red Henna		Natural henna		jawee el henna		Henna mix with unknown addition	
	Sample PPD		Sample	PPD	Sample	PPD	Sample	PPD	Sample	<b>PPD</b> (%)
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	
Benghazi	1	12.07	1	0.15	1	ND	1	100%	1	13.75%
	2	9.74	2	ND					2	21.39%
	3	16.34	3	0.22					3	15.12%
Almarge	1	11.85	1	0.099	1	ND	1	100%	1	24.66.%
	2	10.12	2	ND					2	14.72%
	3	15.95	3	0.189					3	16.49%
Albayda	1	12.05	1	0.153	1	ND	1	100%	1	11.24%
	2	10.03	2	ND					2	14.16%
	3	15.90	3	0.20					3	11.57%

 Table 1. The Concentration of para-Phenylenediamine in black henna , red henna (the same but from different cities), natural henna, jawee el henna and henna mix with unknown addition(N = 3).

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Ajdabiya	1	12.06	1	0.12	1	ND	1	100%	1	12.51%
	2	10.0	2	ND					2	11.37%
	3	16.10	3	0.150					3	13.01%

#### \*ND :Not Detected

# Table 2. The Concentration of metals(ppm) in black henna , red henna ,natural henna and henna mix with unknown addition. Mean ± Standard Deviation (N = 3).

Sample		Pb	Hg	Al	Cr	Mn	Co	Ni	Mo	Ag
Natural henna		ND	ND	3.66±0.16	ND	<b>0.1</b> ±0.001	ND	ND	ND	ND
Black Henna	1	11.26±0.32	0.01±0.004	478.9±7.26	10.09±1.20	1.02±0.008	ND	177 ± 3.21	ND	0.16±0.001
	2	12.72±1.04	<b>0.041</b> ±0.02	1017.3±10.47	13.44±3.01	1.84±0.001	ND	203.1±5.99	ND	0.37±0.003
	3	11.44± <b>1.33</b>	0.01 ±0.007	821±5.26	10.91±1.70	1.75±0.001	ND	119± 2.97	ND	0.18±0.004
Red Henna	1	0.21±0.001	$0.05{\pm}0.001$	10.63±3.15	2.21±0.13	<b>0.3</b> ±0.001	ND	ND	ND	ND
	2	ND	ND	8.93±1.27	1.39±0.10	<b>0.75</b> ±0.004	ND	ND	ND	ND
	3	0.71±0.003	0.1±0.004	10.73±4.10	1.86±0.08	<b>0.52</b> ±0.001	ND	ND	ND	ND
Henna mix	1	<b>0.32</b> ±0.002	0.02±0.0002	10.44±2.53	2.11±0.19	<b>0.2</b> ±0.003	ND	ND	ND	ND
	2	1 <b>.42</b> ±0.73	0.08±0.001	9.30±2.97	2.01±0.36	<b>0.2</b> ±0.001	ND	ND	ND	ND
	3	0.05±0.001	0.001±0.0001	10.56±2.01	1.67±0.03	<b>0.2</b> ±0.001	ND	ND	ND	ND

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