The quantitative analysis of para phenylene diamine (PPD) compound in henna (mehndi) by using high performance liquidchromatography (HPLC)

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ABSTRACT

Objective: To determine the concentration of paraphenylene diamine in Henna (Mehndi) by using High Performance Liquid Chromatography in cases of contact dermatitis.

Methodology: A retrospective study was conducted to determine the presence and concentration of paraphenylene diamine (PPD) in emergency cone mehndi products commercially available in the city of Dera Ghazi Khan, Pakistan. In this study, 61 cases of contact dermatitis were examined with a history of excessive use of Henna (Mehndi) for making tattoo designs on arms and hands. Three (4.9%) children developed hemolytic anemia after five days. Suspected samples of henna were analyzed by using High Performance Liquid Chromatography (HPLC) to estimate the concentration of PPD in popular commercial brands of Henna (Mehndi). Data was analyzed by using MS Excel 16 and HPLC analyzer for graphical presentation of the results.

Results: It was found that all of the samples of Black Henna contain high concentration of paraphenylene diamine (11.2-26.9%). The color of pure Henna changes to maroon whereas black henna color changes to black due to the presence of PPD, more the amount darker will be the color. Most victims were young females of age 11 to 16 years, and children of age 03 months to 03 years with Glucose-6-phosphatase dehydrogenase (G6PD), who initially presented with contact dermatitis but later, after 5-7 days, developed fatigue, muscular rigidity, fever and angioedema. Tracheotomy was performed to clear airways.

Conclusion: It was concluded that the use of Black Henna is hazardous for human health and may cause localized and systemic effects depending upon the amount of Henna applied. It is recommended that addition of paraphenylene diamine to natural Henna should be prohibited in Pakistan. In addition, a public awareness program should be initiated especially in school and colleges about the risk of using Black Henna.

Keywords: Henna, paraphenylene diamine, contact dermatitis, renal failure, tattoo, Mehndi, acute renal failure.

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INTRODUCTION

Henna (Lawsonia Inermis) belongs to a family Lythraceaeis. It contains a lawsone (2-hydroxy-1,4-naphthoquinone), a burgundy dye molecule. It binds with proteins and gives maroon color to the skin. It is widely used to dye hair, and for body painting.[1] Paraphenylene diamine, when added, gives darker maroon color.[2] It has stringent tea like aroma. In ancient times Henna was used to cure dysentery due to its anti-bacterial action, and help to prevent excessive menstrual bleeding.[3] Natural Henna is not allergic to skin but when mixed with paraphenylene diamine (emergency cone mehndi), causes contact dermatitis.[4] Henna (Mehndi) is very popular in South Asian countries such as India, Pakistan and Srilanka. It has become an indigenous part of their culture and tradition. Henna gets absorbed in the skin when remains in contact for 4-6 hours.[5]

Paraphenylene diamine (PPD) is an amine; a chemical compound with a formula $C_6H_4(NH_2)_2$. It is a white colored compound that upon exposure to air oxidizes and turns reddish brown and finally black in color.[6] It is primarily used

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to dye hair when mixed with hydrogen peroxide. It may cause dermatitis, eye irritation, asthma, gastritis, renal failure, vertigo, tremors, convulsions and coma in humans.[7] When it comes in contact with skin for a longer period (8-12 hours) it causes type-4 hypersensitivity reaction. Cell-mediated hypersensitivity is initiated by T-lymphocytes by activating CD8+ cells causing allergic dermatitis, and CD4+ cells causing extensive tissue damage and renal tubular necrosis, especially in children due to genetic deficiency of Glucose-6-phosphatase enzyme.[8] Glucose-6-phosphatase is an enzyme that is mainly present in liver and kidney. Its deficiency may lead to acute renal failure and rhabdomyolysis.[9] PPD is used in tattoo industry, fabrics, dark makeup and printing inks.[10] Paraphenylene diamine is a very toxic compound. It is dose dependent with estimated lethal dose of 0.5mg - 0.8mg/kg or 17-21gm orally, or if remains in contact with the skin for at least 08 - 12 hours.[11]

METHODOLOGY

It was a retrospective study, the outcomes (complication) of excessive use of Henna had already occurred (i.e contact dermatitis) at the time the study was initiated. 61 known cases of contact dermatitis were identified with a history of excessive and repeated use of Henna. This study design was chosen because it allowed us to investigate and formulate an idea about possible associations of paraphenylene diamine compound in henna with contact dermatitis. The study was conducted at District Headquarter Hospital (DHQH) Dera Ghazi Khan over a period of one year from January to December, 2018. 61 cases of allergic dermatitis due to excessive use of Henna were included in the study. Ethical approval and permission to conduct study was obtained from Medical Superintendent, DHQ Hospital, Dera Ghazi Khan. The demographic statistics included gender, age, socioeconomic status, and information about hospital stay, treatment, discharge and mortality within first 48 hours of admission was recorded. The clinical laboratory findings were correlated with clinical manifestations. Ten suspected Henna samples were analyzed by using HPLC in analytical section of Forensic Toxicology laboratory Northwest School of Medicine (NWSM), Peshawar. The reason of using high performance liquid chromatography technique for determination of concentration of paraphenylene diamine (PPD) in emergency Cone Mehndi was that HPLC permits PPD characterization by its spectrum and retention time, avoiding any risk of false identification of the substance. To confirm the identity of PPD in our samples, 1ml of the standard was diluted to 5ml with 50% aqueous methanol solution before analysing any sample to determine its spectrum and its retention time.

Apparatus:

A buffer solution (Borate buffer) was used to maintain the pH at 8.0 for mobile phase. 10mg of each sample of Henna was dissolved in 10ml of 50% aqueous methanol solution. Samples were run twice to minimize the error. Control standard for PPD was prepared by dissolving 10mg PPD in menthol solution. Wavelength of HPLC was adjusted to 290 nm. Before running the test 0.51mg of pure PPD diluted with 5ml menthol was analyzed.

RESULTS

61 cases of contact dermatitis were reported due to repeated or excessive use of Henna (Mehndi). Out of these cases 16 (26.2%) patients were hospitalized due to angioedema, shortness of breath and fatigue, while 03 (4.9%) cases developed hemolytic anemia. 51 (91.8%) patients were females. The median age of the victims was 21 ± 4 years. Only 05(10.4%) were boys between age of 01 - 07 years. Initially they reported for contact dermatitis but later, after 5-7 days, readmitted due to shortness of breath, and developed fatigue, muscle weakness, confusion and changed urine color to dark brown.

On HPLC analysis the retention time (RT) for the PPD standard was found to be 4.49 minutes and range for the samples (TS1 - TS10) were found to be 4.41 and 4.68 minutes (Figure -1).

On spectrophotometric analysis, the absorption spectrum of each of our samples (the peak and the retention time) was the same as that of the PPD standard. The concentration of PPD in each sample was analyzed by determining the transmittance and absorption directly from the HPLC analyser.

A very high concentration of PPD was found in all the Black Henna samples (S1 - S10) that were commercially available in the market (Table -1). The concentration of PPD in commercial preparation was found to be 11.3-31.6mg in 100 grams of emergency Cone Mehndi (Black Henna). The onset of symptoms depends upon contact time (Tc) and concentration of PPD (CS) in the Henna. Initially, the patients presented within 24 - 48 hours with itching, redness and localized swelling, but later developed infection (08 cases, 50%), fatigue (12 cases, 75%), vertigo and angioedema (16 cases, 100%), dysphagia (10 cases, 62%) and dyspnea (8

cases, 50%). These patients were treated with antibiotics, anti histamines and supportive oxygen therapy. Tracheotomy was performed in 6 (37%) cases (Table-2).

Figure 1 - Absorption Spectrum of p-phenylenediamine in methanol

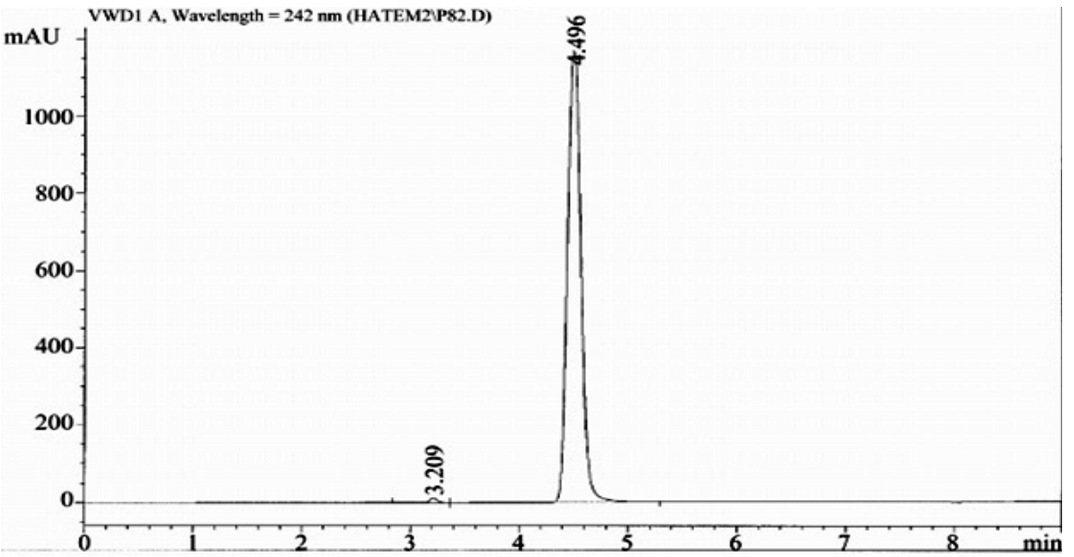

Table 1 - Quantitative analysis of Concentration (%) of paraphenylene diamine (PPD) in Henna samples

Sample of Henna	PPD	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Conc in % of PPD	97.6	21.6	25.9	12.1	17.6	11.3	23.6	30.9	19.8	23.2	26.1
Conc in 5mg/ml	0.92	0.21	0.17	0.9	0.13	0.8	0.21	0.16	0.19	0.23	0.23
Time interval	4.49	4.51	4.53	4.42	4.44	4.41	4.68	4.66	4.47	4.52	4.59
(TS1-10) in minutes	T_R	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}

Table 2 - Clinical manifestations in admitted patients of contact dermatitis (n=16)

Signs and Symptoms	N=16	Occurrence
Infection	8(50%)	4th day
Fatigue	12(75%)	4th day
Angioedema	16(100%)	4th day
Vertigo	16(100%)	3rd day
Dysphonic	10(62%)	4th day
Dysphasia	8(50%)	5th day
Tracheotomy (Outcome)	6(37%)	

DISCUSSION

Paraphenylene diamine is a highly toxic substance which can cause contact dermatitis, hemolytic anemia, and hepatic and renal failure. It gives permanent black color when applied to hair, skin, fabrics and leather. It is also used as printing ink on newspaper. The use of paraphenylene diamine is illegal in henna due to its adverse effects.

The concentration of PPD in our study was (11.3 - 26.1 %) which is significantly similar to as reported in New York University, New York, NY, USA (15.1%), and 29.2% as reported in Kyunghee University, Seoul, Korea.[12-13] Many cases were reported worldwide that Henna containing PPD causes life threatening hemolysis in glucose-6-phosphate dehydrogenase deficiency. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that is mainly present in liver and kidneys.[14] It provides glucose to the body to function normally during starvation. Its deficiency leads to hemolytic anemia. There is evidence that PPD and anti-malarial drugs cause acute hemolysis in people with G6PD deficiency.[15-16]

In all cases of suspected death, it is a duty of the state to identify the case, manner and mode of death. In the field of forensic analytical toxicology, it is necessary to determine

the nature and intention of poisoning.[17-18] A study conducted in Dubai had shown similar findings.[19] The commercial preparations of Cone Mehndi contain a very high amount of PPD that is not safe for human health. The European Union has prohibited the commercial sale of cosmetics containing PPD compound for skin, eyelashes or eyebrows.[20] Food and Drug Administration (FDA) has prohibited the use of PPD directly on the skin.[21] The FDA and other regulatory authorities mentioned that only legal use for paraphenylene diamine is in cosmetics as a hair dye only.[20,21] Drug Regulatory Authority of Pakistan (DRAP) has prohibited the use of paraphenylene diamine in Mehndi.[22]

CONCLUSION

The study indicated that Black Henna (Emergency Cone Mehndi) contains a very high amount of paraphenylene diamine. Most of the victims were young females who have developed contact dermatitis due to excessive use of Black Henna. It is recommended that addition of PPD to natural Henna must be prohibited in Pakistan. In addition, a public awareness program should be initiated, especially in school and colleges, about the risk of using Henna mixed with PPD (Black Henna).

Abbreviations:

PPD: Paraphenylene diamine, HPLC: High Performance Liquid Chromatography, G6PD: Glucose-6-Phosphate Dehydrogenase, TS1 –TS10: Retention Time for Each Sample (1-10), Tc = Contact Time, RT = Retention Time for PPD standard, Cs = Concentration of PPD in Henna.

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