p-ISSN: 0973-3558, e-ISSN: 0973-3566

Original Research Article

Development and Validation of a Presumptive Colour Test for Diagnosing Hair Dye Poisoning

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Article Info

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How to cite this article : Vaishnavi Vinu, Arathy SL, Anagha Chandran, Devika Rajeev, Development and Validation of a Presumptive Colour Test for Diagnosing Hair Dye Poisoning
J Ind. Soc. Toxicolo 2025;21(1):10-15

Abstract :

Aim: To develop and validate a modified Simon's test as a simple, rapid, and cost-effective method for detecting paraphenylenediamine (PPD) in hair dyes and biological samples.

Materials and Methods: Twelve commercial hair dye formulations (black and dark brown) and natural controls (henna, indigo) were obtained from the local market in Kochi, Kerala. Urine samples spiked with PPD (50 ppm) were also tested. The modified Simon's test involved adding sodium nitroprusside (10%) and sodium carbonate (2%) to prepared dye or urine solutions and observing color changes. The appearance of a deep blue color was interpreted as a positive result for PPD.

Results: All 12 commercial hair dye products tested positive for PPD at both 50 ppm and 100 ppm. Natural henna and indigo controls were consistently negative. Spiked urine samples also yielded positive results, confirming the test's sensitivity to detect PPD at 50 ppm.

Conclusion: The modified Simon's test is a rapid, inexpensive, and sensitive presumptive screening tool for PPD detection in cosmetic formulations and biological samples. Although qualitative in nature and limited by sample diversity, the method holds promise for application in clinical toxicology and forensic practice where advanced instrumentation is unavailable.

Keywords- Hair dye, Paraphenylenediamine, PPD, permanent hair dye, colour test, presumptive test

Introduction

Hair dye, a cosmetic product used for altering hair colour, has been popular since centuries for enhancing physical appearance.Commercial hair dyes contain pigments, developers, carriers and conditioners. Pigments add colour, while developers, like hydrogen peroxide oxidise the hair, allowing dye penetration. Common pigments in hair dyes include azo dyes, anthraquinone dyes, and nitro dyes. One widely used pigment is para-phenylenediamine (PPD), a coal-tar derivative known for producing natural-looking, long-lasting dark shades.

Paraphenylenediamine (PPD) containing hair dyes accounts for three-quarters of global use.[1] Temporary hair dyes, on the other hand, generally do not contain PPD. They rely on pigments that wash out over time, making them a safer option for those with sensitivities or allergies.In India, Paraphenylenediamine (PPD) based permanent hair dyes are common due toaffordabilityand accessibility.PPDis a known irritant and allergen.[1] Paraphenylenediamine consists of a benzene ring with two amino (-NH2) groups.PPD is metabolized by enzymes like cytochrome P450 and peroxidase, excreted mainly through renal clearance.

Acute exposure to high levels of PPD may cause severe dermatitis, eye irritation,

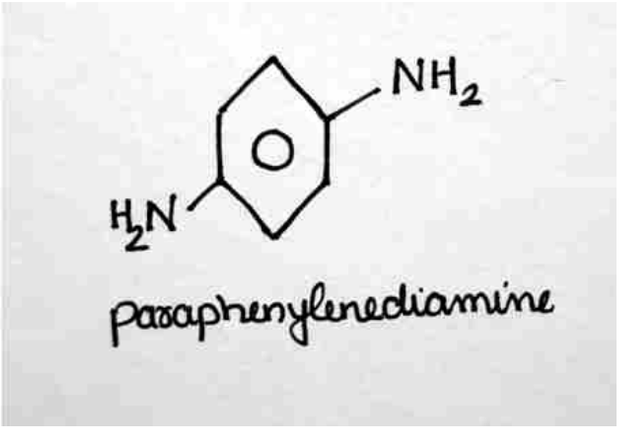

Fig 1 - Structure of Paraphenylenediamine

angioedema, asthma, renal failure etc.[2] Eczematoid contact dermatitis may result from chronic exposure.[2] The EU standards allows a maximum concentration of 6% PPD in hair dyes, while the Bureau of Indian Standards (BIS) allows a maximum of 4% and not less than 1.2% after dilution.[3] Lethal dose is estimated to be 7-10g in humans.[4]

In India, higher incidence of PPD poisoning is reported in states like Andhra Pradesh and globally, significant cases of PPD poisoning (linked to self-harm), occur in countries like Sudan and Pakistan.[5]Children, although less commonly affected than adults, account for a significant 12–18% of PPD poisoning cases, usually due to accidental ingestion.[6] Cases of deliberate self-ingestion are relatively less, but often have serious consequences. No specific antidote is available; treatment is mainly by gastric lavage with symptomatic and supportive measures.[7]

In the case of suspected hair dye poisoning, detection of PPD involves complex, sophisticated methods like High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS), requiring specialized equipment and expertise, making routine testing of biological samples (such as urine) challenging.[8]

This pilot study aims to develop and validate a simple color test modified Simon's test – a simple, rapid, cost-effective, colour test for PPD detection. This test was originally developed for detecting secondary amine compounds like methamphetamine and methylenedioxy-methamphetamine (MDMA).[9]

Paraphenylenediamine (PPD), widely used in hair dyes in India, poses significant toxic risks when ingested, with effects ranging from angioedema and respiratory distress to rhabdomyolysis, renal and hepatic failure, and cardiac complications.[10] Management is primarily supportive, as no antidote exists. Clinical features like angioedema and chocolate-colored urine can aid diagnosis, especially in resource-limited settings. A reported case of successful treatment in a young female adds to existing evidence on PPD toxicity and highlights the importance of early recognition and supportive care.[10]

"Super Vasmol", a widely used hair dye, has emerged as a significant contributor to cases of suicidal poisoning in India. The toxic components found in the dye include paraphenylenediamine, sodium ethylenediaminetetraacetic acid, resorcinol, and propylene glycol. Acute poisoning from ingesting the dye can lead to distinctive angioedema in the cervicofacial region, accompanied by multi-organ dysfunction.[11]. A case of systemic para-phenylenediamine (PPD) poisoning presented with angioneurotic edema, rhabdomyolysis, and intravascular hemolysis leading to acute renal failure. Two hours after ingesting ~20g of para-phenylenediamine (PPD), patient presented with recurrent vomiting, severe facial and neck swelling, tongue edema, and respiratory distress, necessitating an emergency tracheostomy. Within hours, he developed generalized muscle stiffness and tenderness, followed by oliguria and passage of dark brown urine. Laboratory findings showed elevated CPK (296,000 IU/L), hyperkalemia, metabolic acidosis, hemoglobinuria, and myoglobinuria, indicating rhabdomyolysis and intravascular hemolysis.[12]While rare in Western countries, such cases are increasingly reported in East Africa, the Indian subcontinent, and the Middle East.[12]In another reported case of a 14-year-old girl in Sudan who ingested para-phenylenediamine, the poisoning produced a typical triad of angioneurotic oedema, rhabdomyolysis, and acute tubular necrosis.[13] Similar case involved a 20-year-old male in New Delhi, India who ingested a large amount of Para-phenylenediamine (PPD) initially, experienced pain in the abdomen, vomiting, and giddiness.

Within hours, severe facial and periorbital puffiness (angioneurotic oedema) developed, and Later, about 14 hours post-admission, he developed pain and stiffness in his lower limbs, along with passing black coloured urine, indicating rhabdomyolysis and possibly intravascular haemolysis progressing to significantly decreased urine output , leading to oliguria and acute renal failure (acute tubular necrosis), accompanied by a rapid rise in blood urea and creatinine.[14]

A case study reports the death of a 24-year-old Indian housewife, from Puducherrywho ingested approximately 80 mL of hair dye emulsion in an impulsive act following a domestic dispute. She developed acute symptoms including angioedema, cervical swelling, rhabdomyolysis, and subsequently died of acute renal failure within 24 hours. Toxicological analysis confirmed the presence of paraphenylenediamine (PPD), and histopathological examination of the kidneys revealed acute tubular necrosis and myoglobin casts.[15]

A study conducted in United Arab Emiratesexamines the presence and concentration of para-phenylenediamine (PPD) in henna products from various salons across three cities. Using High Performance Liquid Chromatography (HPLC), they quantified the amount of PPD in each sample. The findings revealed significant levels of PPD, particularly in black henna, with many samples exceeding recommended safety limits for hair dyes. The study highlights the risk of adverse health effects, such as allergic contact dermatitis and potential complications for individuals with G6PD deficiency, linked to PPD exposure from henna use.[16]

A systematic review and meta-analysis of 32 studies, focusing on developing countries by Bhagavathula et al revealed that paraphenylenediamine (PPD)-containing hair dye poisoning is a significant public health problem, with a pooled prevalence proportion of 93.5% occurring with suicidal intentions. The analysis of over 5,500 subjects showed a strong female predominance among cases (73.2% female, sex ratio 2.7:1). Oral ingestion, facilitated by the product's low cost, wide availability, and ease of acquisition, leads to severe complications due to high PPD concentrations (ranging from 2% to 90% in some regions). Prominent early complications include severe angioneurotic edema (67.1% pooled occurrence) leading to respiratory distress often requiring emergency tracheostomy, while acute renal failure is a major late complication (54.7% pooled occurrence). With no known antidote, aggressive interventions are crucial, yet the pooled mortality rate remains high at 14.5%. These findings underscore the urgent need for awareness campaigns and stricter regulations on PPD concentration in hair dyes in developing countries.[5]

Materials and Methods

The study utilized a range of commercially available hair dye samples in black and dark brown shades, sourced from the local market in Kochi, Kerala. A total of 13 different dye products were selected, with prices ranging from Rs. 12 to Rs. 450. In addition to these commercial dyes, natural hair dye samples were also used as controls, including powdered henna leaves and indigo powder. The chemical reagents employed for analytical procedures included 10% sodium nitroprusside solution and 2% sodium carbonate solution. All chemicals used are of Extra Pure grade and Millipore water was used throughout the experimental process for dilution and preparation of reagents.

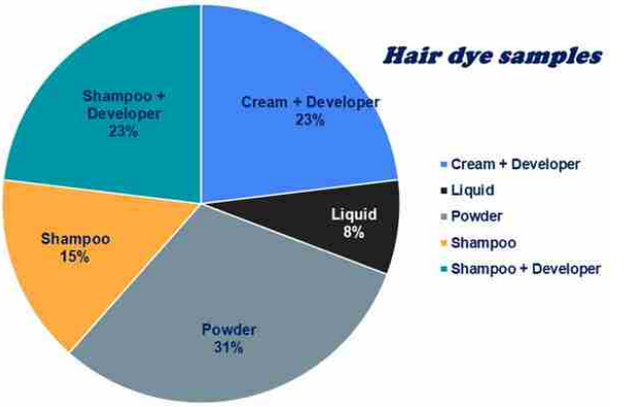

Fig 2 - Pie chart showing various formulations of hair dye

For the analysis, dye solutions and spiked urine samples were prepared at various concentrations ranging from 50 ppm and 100 ppm, with 3 mL of each solution used for testing. To these, 0.5 mL of 10% sodium nitroprusside

solution and 1 mL of 2% sodium carbonate solution were added sequentially. The reaction mixtures were then observed for colour development within 10-15 minutes. The appearance of a deep blue colour was interpreted as a positive indication for the presence of para-phenylenediamine (PPD).The modified Simon's test is based on the chemical reaction between para-phenylenediamine (PPD) and sodium nitroprusside in the presence of sodium carbonate. This reaction leads to the formation of a deep blue-coloured complex, which serves as a qualitative indicator for the presence of PPD in the sample.

Results

Al hair dye samples tested positive for PPD (presence of deep blue colour) at both 50ppm and 100ppm concentration levels. Absence of deep blue colour in homemade henna and indigo samples (control), demonstrating the validity of this test. The test also yielded positive results when dye samples were spiked into urine samples. These findings suggest the potential utility of the modified Simons' test in forensic investigations involving paraphenylenediamine exposure.

Table 1: Para-phenylenediamine details in different hair dye products

S N.	Name	Shade	Brand	Formulation	PPD	Price (Rs.)	Net content
1	Kali mehandi powder hair dye	Black	Black Rose	Powder	Not more than 3% after dilution, not less than 3% as powder	12.00	10 g
2	Expert original	Natural black	Godrej	Powder	Not more than 10%, not more than 3% after dilution	21.00	3 g
3	Black henna	Brazilian black	Banjaras	Powder	Not more than 3% after dilution, not less than 10% as powder	20.00	9 g
4	Henna for men	3 black	Veola	Powder	Not more than 1.5% after dilution, not less than 2% in powder form	120.00	60 g
5	Kesh Kala	Type-1 black	Super Vasmol 33	Liquid	Not more than 4%	35.00	50 ml
6	Excellence creme triple care color	3 Natural darkest brown	L'Oréal Paris	Crème and developer	PPD listed as ingredients. Concentration not mentioned.	289.00	25g+25ml
7	Men permanent hair color cream	1 Natural black	Garnier	Crème with developer	PPD listed as ingredients. Concentration not mentioned.	115	30g+30ml
8	Men's beard color B102	Brown black	Bigen	Crème and developer	PPD listed as ingredients. Concentration not mentioned.	450	40g
9	Hair color shampoo based	Natural black	Siso	Shampoo	Not more than 3.8% after dilution	30	20 g
10	Easy shampoo hair color	Natural black-1	Indica	Shampoo	Not more than 2%	30	18 g
11	Black hair magic	Type-1	BSY	Shampoo and developer	Not more than 4%	50	12 ml
12	Hair color shampoo	Black	VIP	Shampoo and developer	PPD listed as ingredients. Concentration not mentioned.	96	40 ml
13	Henna hair dye shampoo	Type-1	Melano	Shampoo and developer	Not more than 4%	100	30 ml

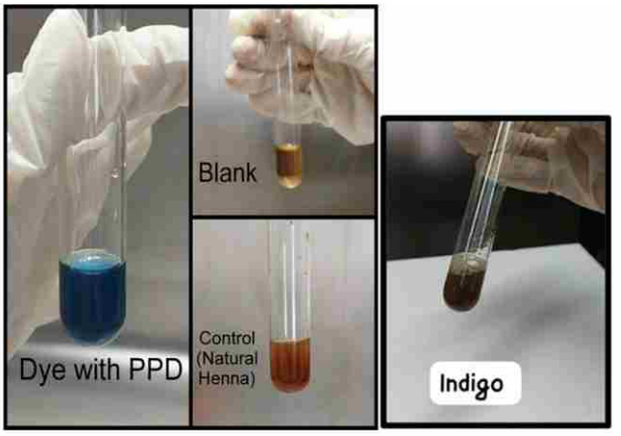

Fig 3 - Test tube showing positive result (dye with PPD), control samples (indigo and natural henna) and blank.

Discussion

In this pilot study, we found that the Simon's test acts as a valid and versatile colour test for detecting PPD in hair dye samples in powder, liquid, and cream formulations as well as in biological samples. It is a highly sensitive test that can detect PPD even at a concentration of 50ppm.

With regard to our samples, we found that hair dyes market themselves as henna, though they still contain PPD, falsely advertising themselves as all natural and giving themselves the appearance that they are a safer option to other permanent dyes in the market.While PPD has its adverse effects, outright banning it in cosmetic products is not necessary - as long as the levels stay below standard limits, and the product properly establishes that they contain PPD, its concentration and adverse effects.

Conclusion

The modified Simon's test presents distinct advantages as a screening method for the detection of para-phenylenediamine (PPD) in hair dye formulations. It is a rapid, cost-effective technique that requires minimal technical expertise, making it accessible for routine use. Furthermore, the test demonstrates high sensitivity, capable of detecting PPD at concentrations as low as parts per million (ppm), which enhances its practical utility in both clinical and forensic settings. However, the test is inherently presumptive qualitative test and does not provide quantitative measurements. Additionally, the presence of complex or compounded ingredients in certain formulations may interfere with test accuracy, leading to potential false positives or ambiguous results. A further limitation lies in the scope of our study, as the sample size does not encompass the full spectrum of hair dye products currently available in the market. Despite these constraints, our findings support the modified Simon's test as a reliable preliminary screening tool for PPD detection, with potential applicability in diverse analytical and diagnostic contexts.

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