

ASSESSING PARA-PHENYLENEDIAMINE (PPD) ALLERGENICITY IN HENNA: A SURVEY STUDY

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ABSTRACT

The potential allergenicity of henna products containing Para-Phenylenediamine (PPD) is the primary objective of this survey. Henna-based dyes and temporary tattoos frequently employ PPD to create darker colors with less application time. However, for some people, PPD might trigger allergic reactions and skin problems. This survey aims to collect data on people's experiences and opinions of henna products that include PPD. It will ask about any negative reactions that people have had, how bad they were, and if they sought medical help. The poll also asks how well people understand the possible dangers of PPD in henna products to human health and how they feel about the accessibility of information about these dangers to the general public. This survey's results will help researchers better understand the frequency and severity of PPD-induced allergenicity in henna users, which in turn could guide efforts to raise awareness about the dangers of henna and improve consumer safety.

Keywords: Henna, Patch test, High-performance liquid chromatography, Spectrophotometric technique, Dermatitis, Allergies, Paraphenylenediamine

INTRODUCTION

The henna plant, whose scientific name is *Lawsonia inermis* and which belongs to the Lythraceae family, is a shrub or flowering plant native to the tropics and subtropics of Asia and Africa. Grown in a number of nations, including Yemen, India, Pakistan, Sudan, and Morocco. Lawsonone (2-hydroxy-1, 4-naphthoquinone), a burgundy color molecule found in henna, has the ability to form protein bonds. So, henna has been used for a long time to color hair, skin, and fingernails, and it has also been used to dye wool, silk, and leather. The process of creating henna body art is decorating one's skin with henna paste. Henna is commercially sold as a green powder, derived from crushing dried leaves and combining them with water and oil to form a paste. When applied to the skin, natural henna produces a brownish-orange hue initially (known as red henna), which darkens over time to a brown shade. Different coloring effects and a thicker consistency can be achieved by incorporating additives like lemon oil, vinegar, eucalyptus oil, and coffee into the paste. The lawsone dye in henna paste moves to the skin's outermost layer when applied to the skin. Lawsonite will migrate and leave a reddish-brown stain on

the skin if kept on for an extended period of time. As an alternative to the more common orange or red shades, some have begun combining natural henna with para-phenylenediamine (PPD) to create a black shade. Adding PPD to natural henna can reduce the usual staining time from four to twelve hours down to only one or two hours. On the other hand, this innovative technique exposes users to PPD through henna, which can lead to health complications. Injuries such as burns, skin irritation, kidney problems, and gastrointestinal disorders are among these. An analysis of the para-phenylenediamine (PPD) allergenicity risk in henna products, focusing on real-life customer reports. To learn more about the frequency and kind of allergic responses linked to henna that had PPD infused into it, the research used a structured questionnaire that was sent out to a wide range of participants. The poll sheds light on the variables impacting allergic reactions—such as length, frequency of application, and individual skin type variations—through careful data analysis. The results investigate the link between consumer knowledge and the frequency of allergic reactions, in addition to illuminating the spectrum and

intensity of adverse reactions. Furthermore, the study evaluates the effectiveness of existing warning labels and preventative measures, providing actionable suggestions for better consumer safety and well-informed decision-making. Giving a Structural activity relationship (SAR) of the compound, this study provides a comprehensive overview of the structural activity relationship (SAR) of Paraphenylenediamine (PPD) in henna, focusing on allergenicity from the consumer standpoint. This information is valuable for informing regulatory initiatives, enhancing product labeling, and raising public awareness about health risks.

Relationship between paraphenylenediamine's structure and activity (SAR):

In order to minimize the toxicity and allergic properties associated with paraphenylenediamine (PPD), various alterations to its chemical composition may be suggested:

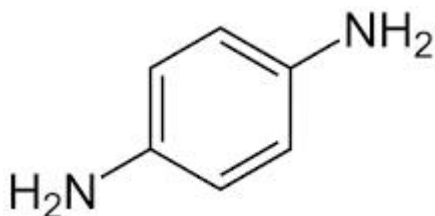


Figure 1: paraphenylenediamine (PPD)

1. Substitution on Aromatic Rings: By substituting certain functional groups like alkyl or hydroxyl on the benzene rings, one might change the electrical characteristics and maybe reduce the allergenicity of the molecule by making it less reactive with skin proteins.

2. Modification of Amino Groups: Reducing the molecule's allergenic potential involves changing or blocking the amino groups linked to the benzene rings, which reduces its capacity to react with skin proteins.

3. Conjugation Breaking: Reduce the molecule's reactivity and toxicity by changing its structure to reduce the degree of conjugation.

4. Steric Hindrance: The goal is to decrease toxicity by introducing large substituents that prevent the molecule from interacting with biological targets.

LITERATURE REVIEW:

Lawsonia inermis (Lythraceae family) included a wide variety of bioactive compounds, including carbohydrates, flavonoids, alkaloids, terpenoids, quinones, coumarins, xanthenes, tannins, resin, and lipids. Lawsonone, a 2-hydroxy-1, 4-naphthoquinone, was also present. Many alkaloids, naphthoquinone derivatives, phenolics, and flavonoids were extracted from various parts of Lawsonia inermis. Scientific investigations on Lawsonia inermis have revealed a wide array of pharmacological effects, such as those against microbes, fungi, parasites, mollusks, antioxidants, liver damage, central nervous system, pain, inflammation, fever, wound healing, immunomodulatory, urolithiatic, diabetes, hypolipidemia, ulcers, diarrhea, diuretics, cancer, and many more. Black henna is synthetically made from the original henna and a number of other chemicals; it doesn't exist in nature. Paraphenylenediamine (PPD) is one of these chemicals; it both deepens the tattoo's color and makes the design more detailed. It is well-known that PPD is a potent sensitizer, and its exposure increases the likelihood of allergic contact dermatitis [1]. The chemical formula for the aromatic amine molecule para-phenylenediamine (PPD) is $\text{C}_6\text{H}_8\text{N}_2$, and its molecular weight is 108.15 g/mol. As the powder undergoes oxidation, its initial white hue gradually shifts to a subtle purple tone, eventually darkening to black upon exposure to air [2, 3]. It exhibits only marginal solubility in water. The concentration of paraphenylenediamine (PPD) in various types of black henna spans a wide range, from as low as 0.38% to as high as 29.5% [4]. PPD and similar compounds play a role in accelerating drying, enhancing definition, and intensifying the red coloration of henna. Within the literature, there is a notable trend indicating a rise in reported instances of sensitivity to PPD present in henna. This increase corresponds with a growing prevalence of allergic contact dermatitis attributed to paraphenylenediamine exposure [5]. In recent years, temporary henna tattoos have become increasingly popular in Western countries, especially among teenagers and young people. Natural henna is entirely safe to use, creates a brownish-orange color, and takes many hours to penetrate the skin. It also has very few adverse reactions. The powerful allergen para-phenylenediamine

(PPD) is added to henna mixes (black henna tattoo) to make the application time shorter and the color intensity greater. The use of henna therapy is frequently associated with various dermatological concerns, including localized and generalized contact dermatitis, hypertrophic or keloid scarring, and temporary or permanent hyper- or hypopigmentation. Type I hypersensitivity incidents, such as urticarial, angioedema, and anaphylaxis, are extremely rare but potentially deadly. Those who suffer from PPD allergies are particularly vulnerable to cross-reactivity caused by azo dyes in textiles and hair colorants, among many other substances. Perform PPD patch testing with a diluted solution (0.01% in Vaseline) to avoid sensitization and extremely severe reactions [6]. Historically, natural henna has imparted a reddish or orange hue; however, para-phenylenediamine has been included to the mixture in recent times, resulting in ebony or "black henna" colors. If you want your tattoo to be done faster, mix PPD with natural henna. Consequently, the use of henna in art projects is an innovative way to expose people to PPD, which increases the likelihood that they may have negative health effects [7]. Research has shown that there are serious health risks associated with using henna that contains PPD, sometimes known as black henna. These risks include chemical burns, dermatitis, acute renal failure, severe angioneurosis edema, abdominal discomfort, vomiting, and a host of other symptoms [8-14]. Furthermore, certain individuals have documented instances of allergic responses to black henna, a substance that contains polyphenolic dibenzoate (PPD), as well as subsequent cross-reactivity with oxidized hair dyes and clothing pigments [15, 16]. The use of henna is well ingrained in Pakistani tradition. People of various ages use henna to paint their skin, and it's an essential component of weddings and other social gatherings [4]. So, the purpose of the study was to find out if black and red henna that can be bought at the store contained PPD and, if so, at what amounts. On the ninte the literature has analytical methods that can be used to determine PPD. Voltametric analysis [17], emission spectroscopy [18], gas chromatography/mass spectrometry [19], and high-performance liquid chromatography [4] are all examples of such algorithms. Removes the guesswork from drug identification by allowing spectra and retention time to

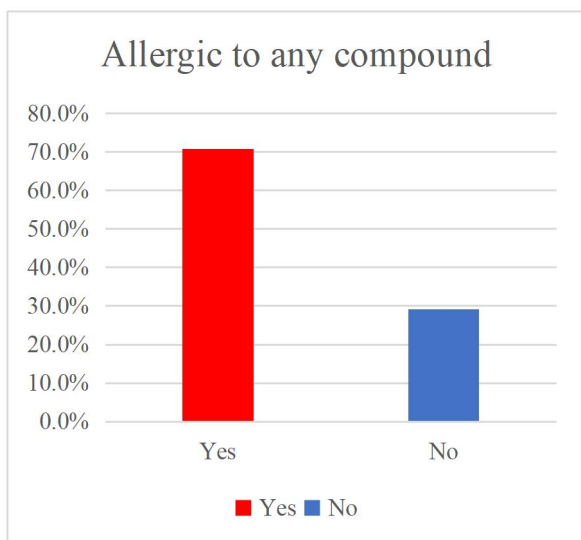
define PPD. The use of HPLC allowed for the quantitative and qualitative analysis of PPD in the henna sample.

METHODOLOGY:

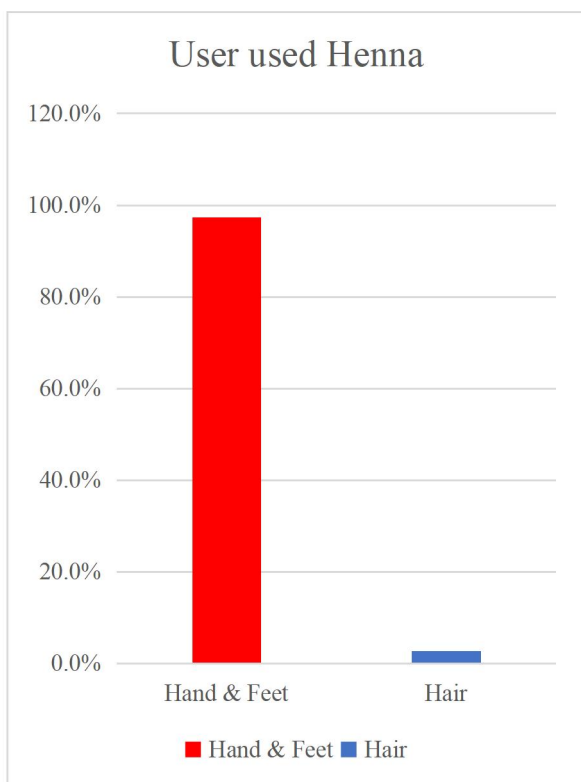
The development of a survey questionnaire constitutes the methodology for the present inquiry. We aim to measure the frequency and intensity of allergic reactions to PPD in henna products, as well as the level of knowledge and awareness among its consumers. Pick a representative cross-section of henna artists and their demographics at random. Get people of different ages, genders, and places represented. Create a questionnaire with multiple-choice questions covering topics such as demographics, henna use (how often and for how long), history of allergic reactions (if any), henna knowledge of PPD, and safety precautions taken. Ensure that all participants understand the survey's aim and give their informed consent. We used internet platforms and social media to distribute this survey form and collect responses from customers. We also gathered information from classmates, friends, and family to make it widely accessible. We collected information from more than a hundred people. Use both closed- and open-ended questions to glean qualitative and quantitative information. We employ statistical methods to examine numerical data and theme analysis to examine qualitative replies in order to provide a more accurate picture of our work. When conducting surveys, it is important to do so in an ethical manner. Verify it by administering a pilot survey to a select set of people to see if there are any questions or problems. In order to get sufficient information, choose a certain survey duration. From the consumer's point of view, we may learn a lot about the allergenicity of PPD in henna using the aforementioned methods to gather and analyze data.

RESULTS

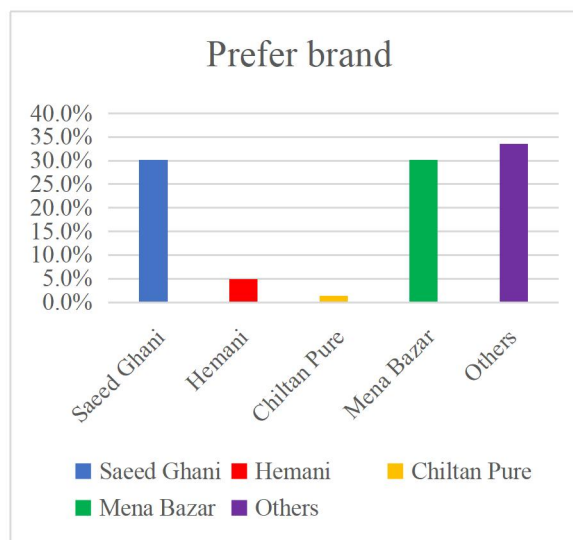
Among the 113 consumers that registered, 99.99% were female. 14.2% of the ladies were under the age of 18, 72.6% were over the age of 18, 8% belonged to the age group over 30, and 5.3% of the females were over the age of 40. All individuals has a background in literacy. A total of 70.8% of individuals do not exhibit any allergies towards any compound, whereas the remaining 29.2% of individuals do possess allergies to any compound. Based on the findings of this survey

**Figure 2:** Allergic to any compound

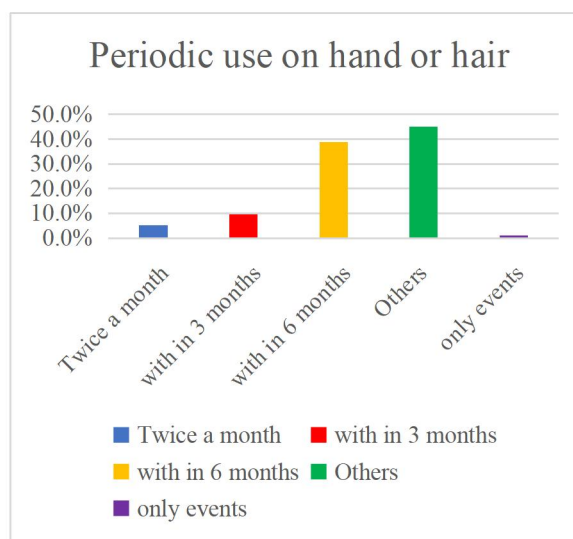
The prevalence of henna application on hands and feet is 97.3%, whereas 2.7% of individuals apply it on their hair.

**Figure 3:** User used Henna

The majority of individuals, accounting for 30.1%, expressed a preference for Saeed Ghani and Mena Bazar. For the remaining 33.6%, Hemani received 5%, and Chiltan Pure received 1.2%.

**Figure 4:** Prefer brand

5.3% of participants utilize the product on a monthly basis, whereas 9.7% employ it within a span of 3 months. Furthermore, 38.9% of individuals employ it within a span of 6 months, while 45.1% are classified under the "Others" group. Just 1% of participants indicated that they solely utilized the products for occasions.

**Figure 5:** Periodic use on hand or hair

Out of the whole sample, 19.5% of participants reported instances of etching, while 9.7% indicated scorching, and an equivalent proportion reported the formation of scars on the skin. 55.8% of the respondents had no negative experiences. In addition, 5% of participants noticed skin redness, while 0.3% had swelling.

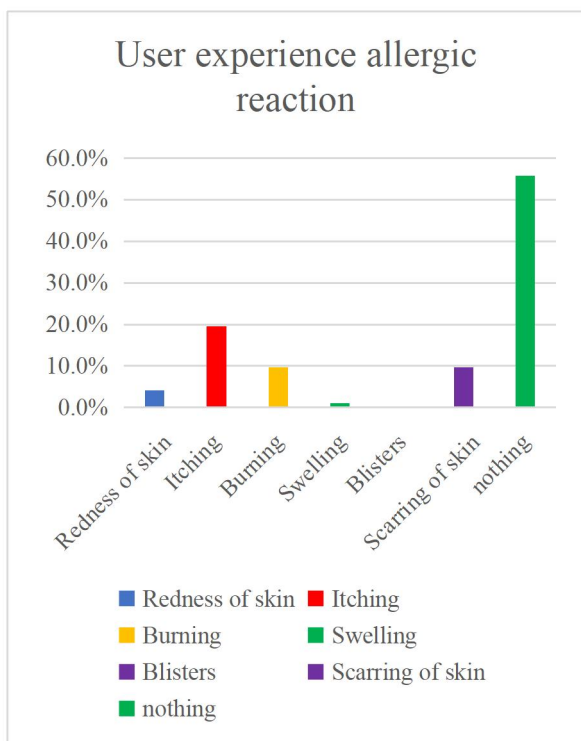


Figure 6: User experience allergic reaction

The product was utilized by 26.5% of the participants for a duration of 30 minutes, whereas 15.9% used it for a period of 1 hour. Furthermore, 22.1% of participants indicated a usage duration of 2 hours, while 28.3% reported a usage duration of 5 to 6 hours. Merely 5% belonged to the "Others" classification. In addition, 1.1% of respondents reported using it for a duration of 3 hours, while another 1.1% reported taking it for 1 hour.

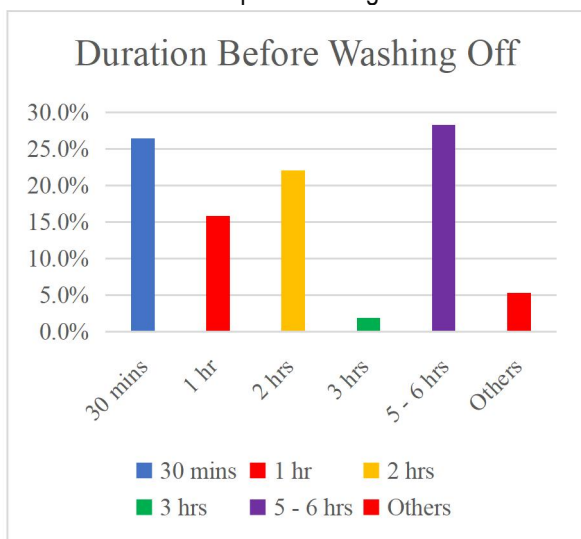


Figure 7: Duration Before Washing Off

A total of 83.2% of users weren't performing a patch test prior to usage, whereas 16.8% of users did. Out of the respondents, 71.7% are unaware of the components used in henna, whilst 28.3% reported being aware of them

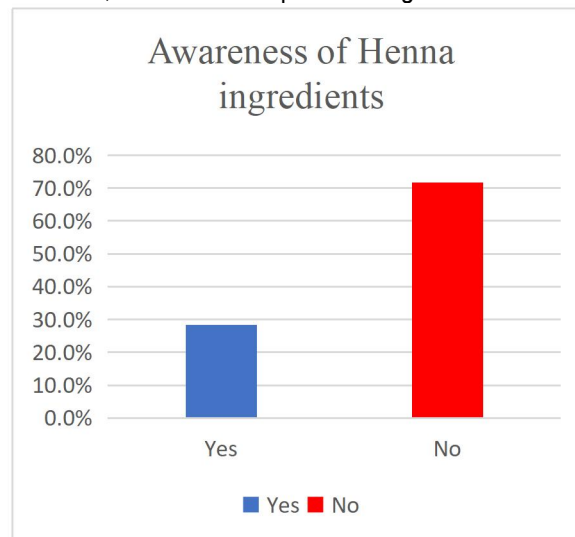


Figure 8: Awareness of Henna ingredients

75.7% of people Preferred natural products, while 16.2% for synthetic ones. Additionally, 8.1% chose any other type of product.

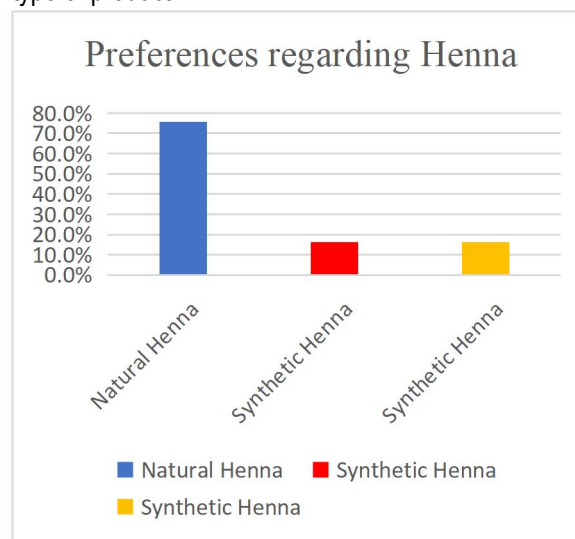


Figure 9: Preferences regarding Henna

17.1%. The buyer took into account the price, but in the case of 30.6, they prioritized the brand. In addition, 26.1% of the results concentrated around components, while another 26.1% were classified under the "Others" category.

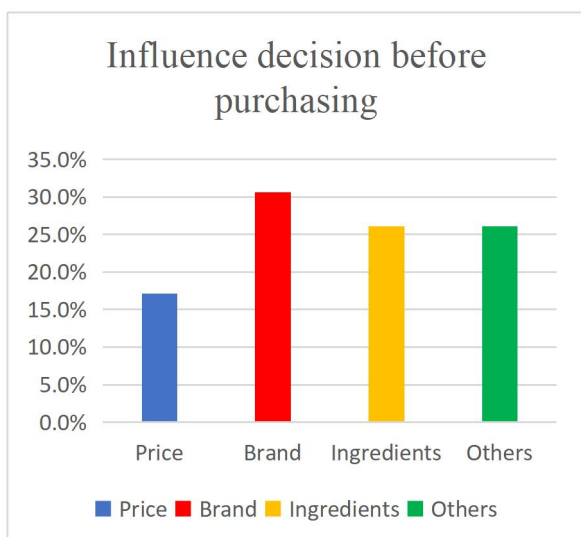


Figure 10: Influence decision before purchasing

A majority of the the participants, specifically 82%, reported a lack of knowledge of the PPD compound, whilst 18% indicated their awareness of the information related to PPD.

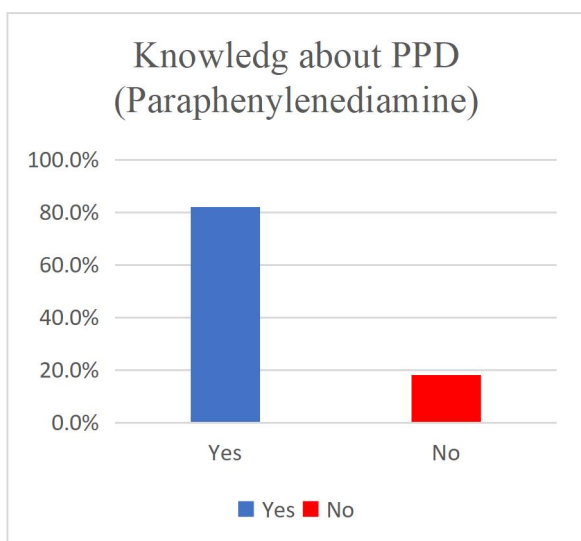


Figure 11: Knowledg about PPD

DISCUSSIONS:

Henna has a rich history of traditional use both for its medicinal properties and as a cosmetic. Medicinally, it has been consumed as a bitter tea to alleviate stomach or intestinal issues, treat fever and headaches, and applied as a paste to remedy conditions like ringworm or nail fungus. Additionally, it has been utilized to reduce chafing, prevent blisters, and soothe irritated or dry skin. On the

cosmetic front, henna has served as a hair thickener, a nail colorant and conditioner, and a decorative body stain. It has also been employed as a brown dye for various textiles such as wool, cotton, and silk. Paraphenylenediamine (PPD) also used in henna to intensify its effect due to this it cause some harm to skin barreier and produce allergenicity.

The outer layer of the skin, referred to as the epidermis, is penetrated by Para-Phenylenediamine (PPD) through diffusion or other methods. PPD forms a bond with proteins, specifically keratin proteins present in epidermal cells, within the skin. The oxidation of PPD occurs when it is exposed to air or oxidizing substances, resulting in the formation of reactive intermediates. These intermediate compounds have the ability to establish covalent bonds with amino acids present in proteins, leading to the creation of hapten-protein complexes. These complexes are recognized by the immune system as foreign substances, which then initiates an immunological response facilitated by dendritic cells, T lymphocytes, and inflammatory cytokines. Prolonged exposure to PPD results in tissue damage, inflammation, and a weakened skin barrier. In addition, PPD has the ability to cause oxidative stress in skin cells, which worsens the harm to lipids, proteins, and DNA.

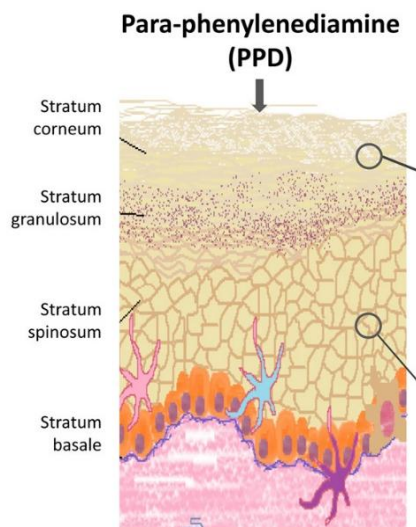


Figure 12: Structure of PPD

Several cases of contact allergy have been reported after using black henna. This Image shows a person who developed hand sensitization after using black henna.



Figure 13: Hand sensitization after using black henna); Dermatitis

The performance of a patch test prior to the application of henna is of the highest priority in order to detect possible allergic responses and verify that the natural dye does not induce skin irritation or other unfavorable consequences. Certain individuals may fail to consider this particular step, either as a result of misconceptions regarding the safety of natural goods or a limited understanding of potential allergic reactions. Raising awareness on the importance of patch tests is crucial in order to prevent unanticipated adverse effects associated with the use of chemicals such as henna.

CONCLUSION

In summary, the incorporation of henna is firmly embedded in the cultural fabric of the nation of Pakistan, playing a crucial role in numerous traditional practices. In order to evaluate the frequency of negative responses, specifically contact dermatitis, among those who use henna as a result of the presence of Paraphenylenediamine (PPD), an extensive survey was conducted. The findings of this survey indicate a significant proportion of patients experiencing allergic reactions associated with postpartum depression (PPD) in henna formulations. The range of reactions includes moderate symptoms like redness and itching, as well as more serious dermatological diseases marked by swelling, blistering, and the formation of skin lesions.

Based on these results, it is crucial to be careful, especially for individuals who have a past history of being sensitive to hair dyes or other chemical substances, while using henna products that contain PPD. To address this concern, it is vital to explore alternative formulations of

henna that are devoid of PPD, thus mitigating the associated risks. Moreover, further research endeavors and regulatory initiatives are warranted to gain a deeper understanding of the potential hazards linked to PPD in henna products. These efforts should also focus on implementing stringent labeling requirements and safety guidelines to safeguard consumers from the potential adverse effects of PPD-induced allergic reactions. By adopting these measures, we can promote safer usage of henna products while preserving the cultural significance and heritage associated with their traditional use in Pakistani ceremonies

Encouraging patch testing among henna users is vital for safe use. This involves organizing workshops and social media campaigns to raise awareness about patch tests before henna application. Dermatologists and cosmetologists can offer expert advice on conducting effective patch tests. Distributing informational materials in salons and community centers and adding warnings on henna product labels remind users of the importance of patch testing. Collaborating with beauty influencers can help spread the message widely, while offering free patch testing services at community events promotes safer henna practices. Lastly, promoting peer communication reinforces the significance of patch testing and adherence to safety measures, ensuring safer henna use overall.

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