Black Henna Dyes Containing paraphenylenediamine: Assessing the Risks of Exposure on Pruritus, Hematological and Biochemical Parameters

A. A. S. Salah [1, *]
S. M. Q. Mofleh [2]

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[1]Department of Biology &Chemistry, Faculty of Toor Al-Baha, Lahej University, Lahej, Yemen.
[2]Department of Chemistry, Faculty of Education, Aden University, Aden, Yemen.
*Corresponding Author's Email: aasalehsalah.74@gmail.com

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A. A. S. Salah
Department of Biology & Chemistry, Faculty of Toor Al-Baha, Lahej University
Lahej, Yemen
aasalehsalah.74@gmail.com

S. M. Qasem Mofleh
Department of Chemistry, Faculty of Education, Aden University
Aden, Yemen

Abstract— This study investigates the health risks associated with black henna dyes, specifically focusing on the presence and concentration of Paraphenylenediamine (PPD) in commercial products. Henna, historically used as a dye, has evolved to include chemically mixed variants that can cause allergic reactions skin. The research involved analyzing samples from 60 regular users of black henna and 60 control participants who had never used it. In our study most symptoms itching and the dark color urine, The study found hematological indices and changes in kidney function, liver function than group control. The hematological and biomarker may be indicated to toxicity among users, that is probably the health hazards linked to exposure to black henna dyes. The findings underscore the need for awareness regarding the risks associated with these products.

Keywords— Black Henna dyes; Paraphenylenediamine (PPD); Liver Enzymes; Kidney toxicity; Allergic reactions.

I. INTRODUCTION

In a culture that is fixated on beauty, people are drawn to improving their looks in order to boost their confidence and develop wonderful personalities. However, a lot of these cosmetics, which are supposed to help us feel healthier and more attractive, have a dark side because they include dangerous chemicals and harmful ingredients in excess [1]. Hair care products are a major source of lifestyle related chemical exposure in the general population, especially since hair dyes use is becoming an integral part of modern culture [2], these products serve various cosmetic purposes, such as covering gray hair, altering hair color, and enhancing color retention, which has contributed to the growth of the hair coloring market, particularly with an aging population [3]. Henna, scientifically known as Lawsonia inermis L., belongs to the Lythraceae family, (Fig.1) flowering plant it's also known Lawsone is commercially cultivated in several countries such as Morocco, Sudan, India, Pakistan, and Yemen. In these countries the henna leaves are ground into a paste with water or oil and using for body art and to color skin and hair during social events, particularly weddings. The compound 2-hydroxy-1,4-naphthoquinone is active dye responsible for henna's red color (Fig.1) [4,5].

Fig.1. Leaves of Lawsonia inermis

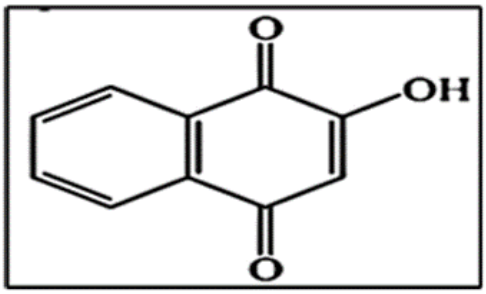


Fig. 2. Structure of Lawsone

Para-phenylenediamine (PPD), an aromatic amine compound and derivative of aniline (1,4-diaminobenzene, C6H8N2), has a molecular weight of 108.15 g/mol (Fig. 3). PPD typically appears as white crystals that oxidize in air, changing color from red to brown and eventually to black (Fig. 4). It is commonly used in various industries for dyeing fabrics and furs, as well as in the production of photographic developers, appliances, wheels, caoutchouc, cosmetics, and plastics [6-8].

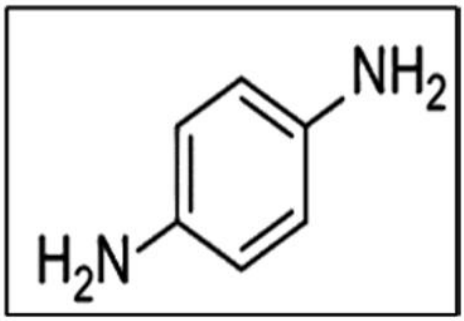


Fig 3: Structure of paraphenylenediamine

Fig. 4: Crystal form of PPD.

PPD is widely utilized as a chemical an ingredient of oxidative hair coloring products and black henna dyes. Currently, PPD is present in more than 1000 hair dye formulations marketed all over the world [9]. Moreover, the natural red henna is often combined with PPD to create black henna, commonly used for temporary black henna tattoos, which acts as: a chemical dye, enhancing the staining effect when mixed with henna paste [10], accelerates the dyeing and drying processes, intensifies the color, improves the design, and extends the tattoo's longevity [11]. PPD is prevalent in various hair dyes, with inexpensive formulations such as tancho and branded products like L'Oreal and Garnier typically contain lower percentages (2-6%) and from 70-90 % in Black Stone Hair Dye (BSHD) that is containing a high concentration of PPD, which are used for giving black color to hair [12].

In contrast, BSHD is available in North Africa and the Middle East, the traditional name of a commercial black hair dye and widely used because it is freely available and less expensive than pharmaceutical hair dye preparation [13]. It is also usually added in henna Lawsonia after crushing, which applied as popular custom to make black hair dye also to lessens the amount of henna needed, intensifies its color, and hastens the staining process [14]. In contra, the chemical reaction between a coupler agent (e.g., resorcinol) and the dye precursor (PPD) under oxidative conditions forms a colorful compound but may also lead to the formation of Bandrowski's base, that is consider a carcinogenic substance [15]. Recent studies have linked chemicals commonly found in permanent hair dyes of loss, contact dermatitis and toxicity from PPD-containing hair dyes both red and black henna products has been reported in Asia, Africa, and the Middle East, highlighting its potential as a means of suicide due to its lethality [12, 16, 17]. The studies have been dealt with several types of PPD poisoning, which can the local application cause skin allergies, lacrimation, cardiotoxicity, urinary toxicity, both allergic contact dermatitis and irritant contact [18]. Moreover, exposure to PPD foam, during its production or application in manufacturing of various products, may result in health problems affecting airway and skin, in addition, the direct toxic effects occurs following ingestion of PPD after some hours that has a more systemic damage has taken place (renal, lover), due to the absorption and distribution of the toxic metabolites throughout the body and death [19]. The first defined a strong allergen causing PPD in 1939 by the standard antigen group of the North American and European epidermal patch tests, as a result of PPD allergy symptoms, many strict legislation were put in in its use. In 1976 according the European Cosmetic Directive regulation the maximum permissible concentration of PPD in hair coloring was 6% [20] then in 2009 modified to 2% for direct application with the oxidizing agent on the hair dyes [21] and due to its health risks has been banned in countries like France, Germany, and Sweden [7]. On the other hand, less toxic of PPD poisoning in developed countries the maximum percentage does not exceed 2% per 100 ml of color solution but PPD concentrations in thiscountries may be reach to 90% due to a lack of stringent regulations. On the contrary, in the USA, there is no regulation of the limits for concentrations of PPD in hair dyes [21] as a result may lead to the addition of high PPD concentrations due to the lack of sufficient legal restriction on the use of PPD concentrations [22]. The authors studied four Black henna samples that, which considered a more available, very cheap price and commonly used, from shops selling in Aden Governorate. The result confirmed the presence of PPD in the four samples and according to the following concentrations of PPD in Black Henna samples: 1.9, 6.98, 14.33, and 70.35% for Brand-2, Brand-1, Brand-3 and Black Stone respectively. The PPD levels in the last three samples higher than permissible levels in black henna dyes and proven that the black stone dye contains large amount of PPD [23]. After proven the presence of PPD in the four samples, this search aims to study evaluate the toxic effects of these dyes on human hematological and biomarkers on blood cells and renal and liver functions, and contributing to improved consumer safety and environmental protection with facilitate better monitoring of Black Henna samples.

II. 2. Materials and Methods

A. Materials

The Chemicals, Equipment and Instruments: All chemicals used in the study were of analytical grade and highly pure, and it is also use micropipit, test tubes anticoagulant samples, syringes, gloves for collected blood samples. The Instruments used to Blood samples analysis: Siemens Healthineers, high-volume hematology analyzer, the ADVIA 2120i Hematology System and Biochemical Roche Hittachi Diagnostics CobasC 311 analyzer.

B. Samples

Four samples Black Henna, more available and commonly used were collected from shops selling different areas randomly in Aden Governorate, and these samples were

labeled as Brand-1, Brand-2, Brand-3, and Black Stone Dye instead of their commercial names.

C. Study Period

This study was done in the period September 2023 to January 2024 in Aden Governorate.

D. Study Population.

The study population was comprised adult Yemeni women that use Black henna dyes in Aden Governorate, Republic of Yemen. This prospective cross sectional analytical study involved 120 adult women who were randomly selected and used black henna dyes in beauty salons. 120 participants were selected after application of exclusion criteria of participation. All participants were confirmed to have no medical history of kidney, liver, diabetes, or hypertension conditions, and not taking any vitamin supplements.

A day before sample collection, volunteers were stop taking any food after 9 pm, where the blood samples collected after 24 hours from applying the dyes to the scalp, then placing into tubes and marked by code numbers identify the volunteer's personal identity, then the samples were transferred to the National Center for Central Public Health laboratories

The participants were divided into three groups:

a. Sixty subjects as the control group (without any history of black henna use).
b. Thirty subjects who used various dye brands (Brand 1, Brand 2, Brand 3).
c. Thirty subjects who used black stone dye.

E. Biochemical Measurements

Two parameters analysis used to measure effects hair dyes on volunteers:

a. The hematological analysis:

the blood samples were performed to assess the following parameters: Hemoglobin (HGB), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC), Blood Platelet (PLT), Lymphocyte, Neutrophil were conducted using the using ADVIA 2120i (Siemens Healthineers Germany).

b. The Biochemical analysis: the blood samples were collected to assess the renal function tests, measuring blood urea and creatinine levels and to liver function tests performed to assess total bilirubin, total protein, albumin, Alanine Aminotransferase (ALT/GPT), Aspartate Aminotransferase (AST/GOT), and Alkaline Phosphatase (ALP) using by the Roche/Hitachi Cobas 311 analyzer.

F. Ethical Consideration

Ethical approval for the study was obtained from the Ethical Scientific Research Committee of the University of Aden (Rec-57-2024).

G. Statistical Processing of Results

Statistical analysis was performed using Origin 8, Excel, and the Statistical Package for Social Science (SPSS) version 20 for Windows. Continuous and categorical variables were tested for significance using T-tests and One-way ANOVA, with a p-value of < 0.05 considered statistically significant.

III. Results

The study effects of hair dyes on volunteers used by biochemical and hematological indices. It was analyzed statistically and the following statistical analysis results were obtained:

A. Hematological assays:

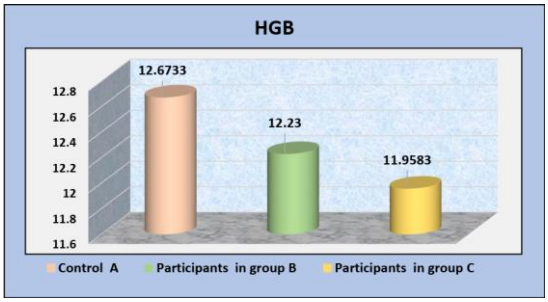
The results of the statistical analysis revealed are a decrease in mean values of Hemoglobin (Hb), and Mean Corpuscular Hemoglobin (MCH) in the blood with a statistical of (P=0.013), (P=0.016) in groups B and C compared with the control respectively, but these result of the Hb (12.23 and 11.96) and MCH (28.52 and 28.48), in the B and C groups respectively, were slightly lower than the control group (12.67 and 28.53) respectively. In Red blood cell counts (RBCs) decreased in the value of (P=0.011) in group B and C compared to the control group and the average RBCs was low in the sera of participants in the B and C groups by in mean (4.30 and 4.23, respectively) compared to control group (5.31). While decreased in the value of mean corpuscular volume (MCV) in the blood with a statistical of (P=0.019) in group B and C compared to the control group, the MCV was low in the sera of participants in the B and C groups by in mean (85.80 and 85.63, respectively) compared to the average rate in the sera of participants control group (86.17). The result in Mean Corpuscular Hemoglobin Counts (MCHC) was different in the statistical analysis revealed no statistically significant was (P=0.239) of MCHC in groups B and C compared to the control group where the MCHC was equal in the B and C groups (33.29 and 33.27 respectively) but lower than the average control group (42.84). The results in the value of White blood cell counts (WBCs) revealed in a decrease in a statistical of (P=0.014) in group B and C compared to the control group, but differences the results WBCs between B and C groups (6.12 and 5.92, respectively) while lower in compared to the control group (6.84). In the Blood Platelets (PLT) the results that was no statistically significant (P=0.046) in groups B and C compared to the control group but the result of PLT in groups B (297.37) was slightly higher than control group (291.63) and significantly lower in C group (257.60) than control group. The results of the statistical analysis revealed was no statistically significant was (P=0.256) in the value of Lymphocytes (LYMP) in the blood in group B and C compared to the control group, the results related of LYMP was equal in the B and C groups (43.65 and 43.15 respectively) but lower than the control group (40.47). In the last results of Neutrophil (NEUT) in the blood the statistical analysis revealed was decreased in the value statistical of (P=0.012)) in group B and C compared to the control group, which differences the value NEUT low in C groups from B groups (41.79 and 43.23, respectively) and the two group lower than control group (46.19).

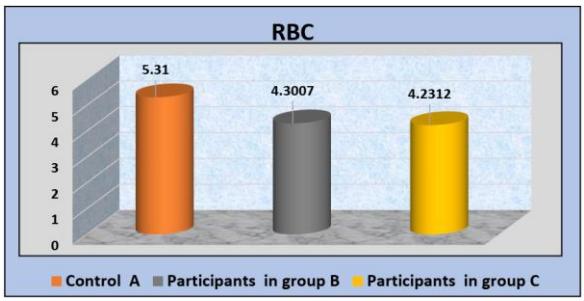
All results of hematological analysis are summarized in Table 1 and illustrated in Fig.5.

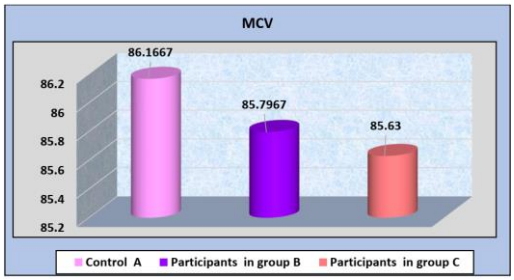
Table (1): Hematological parameters among study groups.

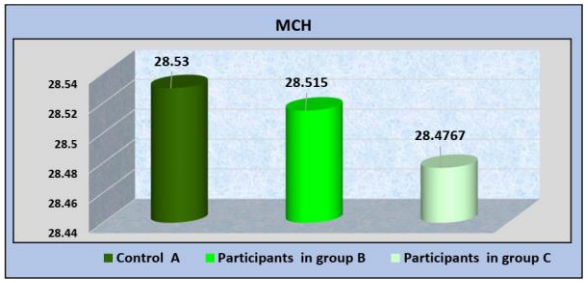
Hematological parameters	Group A Control (mean± SD)	Group B (mean± SD)	Group C (mean± SD)	p-value
Hb	12.67 ± 1.75	12.23 ± 1.75	11.95 ± 1.23	0.013*
RBC	5.31 ± 4.69	4.30 ± 0.52	4.23 ± 0.43	0.011*
MCV	86.17 ± 9.00	85.80 ± 10.73	85.63 ± 9.68	0.019*
MCH	28.53 ± 3.37	28.52 ± 3.84	28.48 ± 2.90	0.016*
MCHC	42.84 ± 5.35	33.29 ± 0.60	33.27 ± 2.13	0.239*
WBCs	6.84 ± 5.30	6.12 ± 1.84	5.92 ± 1.67	0.014*
PLT	291.63 ± 61.19	297.37 ± 91.433	257.60 ± 54.92	0.046*
Lymphocyte	40.47 ± 8.42	43.65 ± 11.83	43.15 ± 9.88	0.052*
Neutrophil	46.19 ± 11.29	43.23 ± 13.46	41.79 ±11.75	0.012*

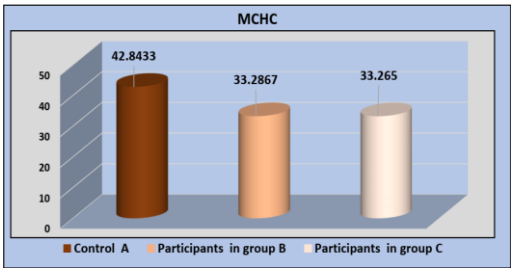
*P≤0.05

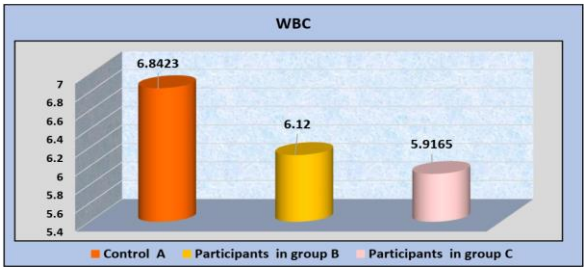


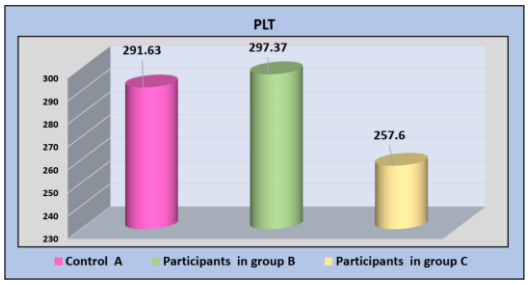


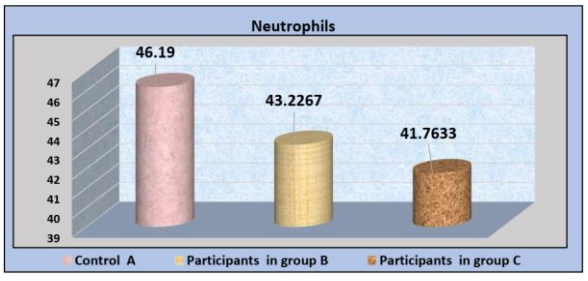












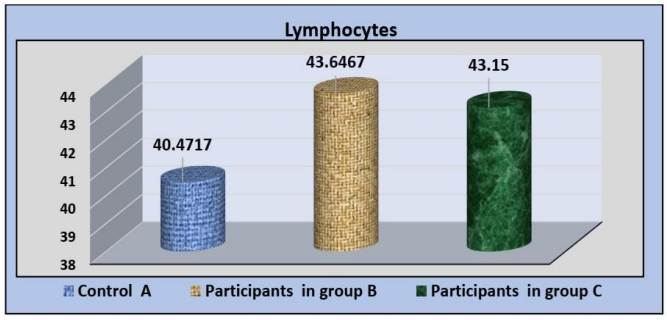


Fig .5: The Hematological parameters tests for groups B and C compared with control A,

B. The Biochemical analysis

a. Liver Function Tests

The liver function tests assessed included total protein and albumin levels, which exhibited a significant decrease ($P \leq 0.05$) in the tested groups B and C. Conversely, plasma enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels showed a significant increase ($P \leq 0.05$) in groups B and C compared to the control group. These results are summarized in Table 2 and illustrated in Fig. 6.

Table 3: The Liver function tests among study groups.

Biochemical parameters	Group Acontrol (mean± SD)	Group B (mean± SD)	Group C (mean± SD)	p-value
T.Protein	7.33 ± 0.69	6.83 ± 0.69	6.26 ± 0.89	0.015*
Albumin	4.61 ± 0.31	4.04± 0.55	3.36 ± 0.87	0.028*
T.Bilirubin	0.29 ± 0.15	0.740 ± 1.62	1.26 ± 1.68	0.413
AST	17.62 ± 4.66	28.56 ± 16.48	35.57 ± 31.44	0.049*
ALT	11.73 ± 6.48	14.67 ± 10.17	32.70 ± 38.20	0.035*
ALP	78.07 ± 23.51	129.07 ± 90.91	155.23 ± 114.98	0.020*

*P≤0.05

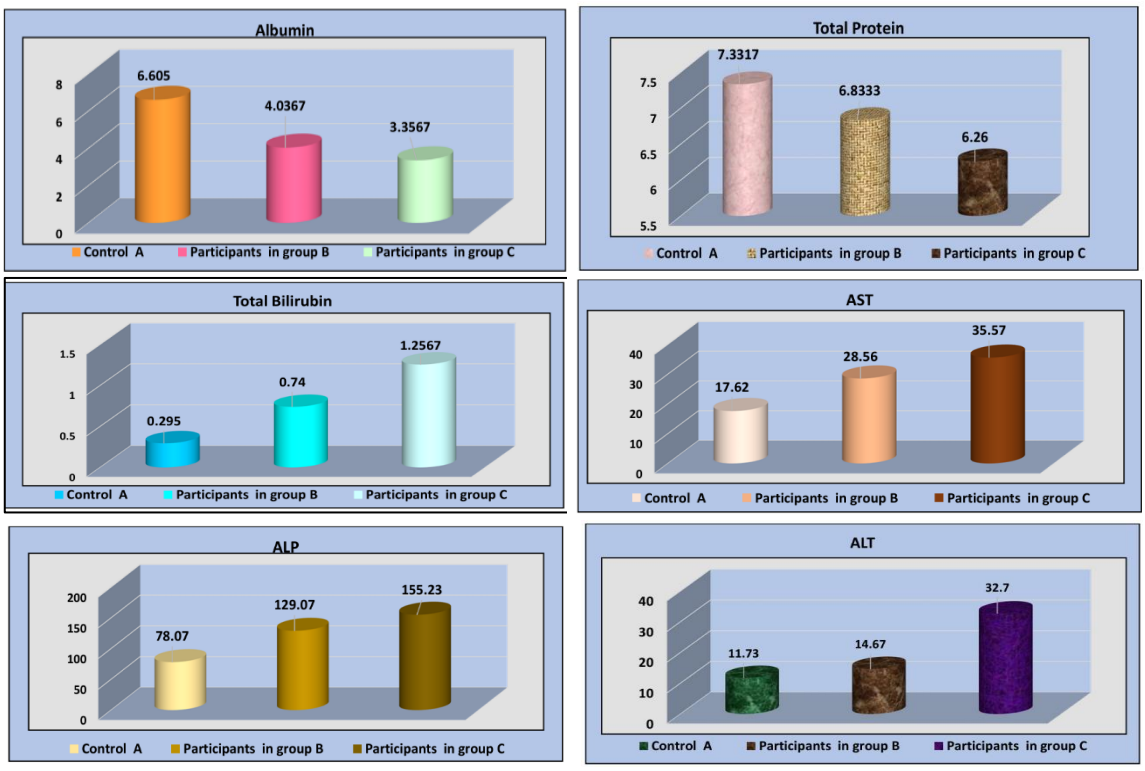


Fig .6: illustrated the liver function tests.

b. Kidney Function Tests (KFT)

The key indices for assessing kidney function include serum urea and serum creatinine levels. The results presented in Table 3 and Fig.7 indicate that women exposed to para-phenylenediamine (PPD) over an extended period are at significant risk for kidney-related issues. Specifically, the measurements for blood urea and creatinine showed a significant increase ($P \leq 0.05$) in the tested groups compared to the control group, highlighting potential kidney dysfunction in these individuals.

Table 3: Kidney function tests among study groups.

Biochemical parameters	Group A (mean± SD)	Group B (mean± SD)	Group C (mean± SD)	p-value (mean± SD)
Urea	19.567 ± 16.34	31.30 ± 21.16	103.60 ± 57.43	0.012*
Creatinine	0.57 ± 0.09	0.85 ± 0.34	2.24 ± 1.18	0.014*

*P≤0.05

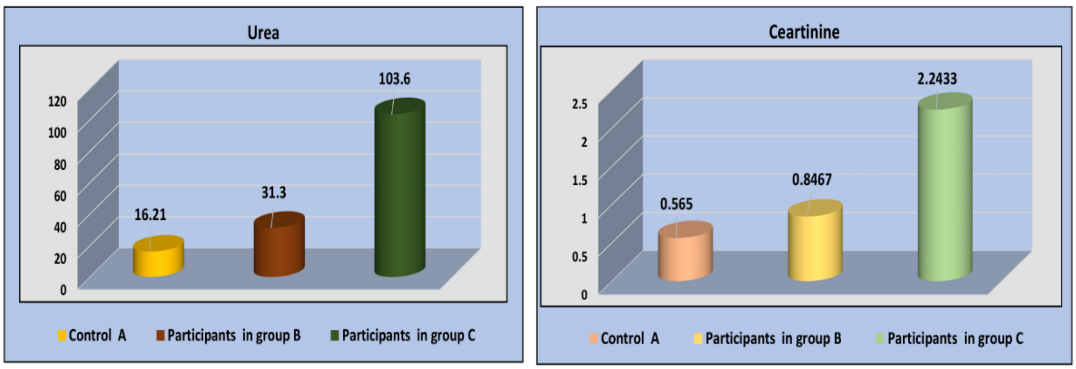


Fig .7: Kidney function tests

C. Symptoms appeared after application black henna:

In Table.6 and fig.8 most symptoms itching 13.3% in group B and 40 % in of group C, and the skin darkening was complained by 6.7 % for group B and 10 % in group C, while the darkening of urine no appeared in group B and 13.3 % in of group C. in contrast, 86.7% from group B and 60 % group C did not have any reaction on skin after application of hair dye, but 13.3 % group B and 40 % group C they had itch on the skin immediately after application of hair dye complained by 23.3 % of group B and 60 % of group C.

Table 4: Distribution of the study subjects according to previous history of reaction after dye application (n=60).

Reaction after dye use	Group B %	Group C
Darkening of urine	**0.0%**	**13.3%**
Itching	**13.3%**	**40.0%**
No effect	**76.7%**	**40.0%**
Pain/darkening of skin	**6.7%**	**10.0%**

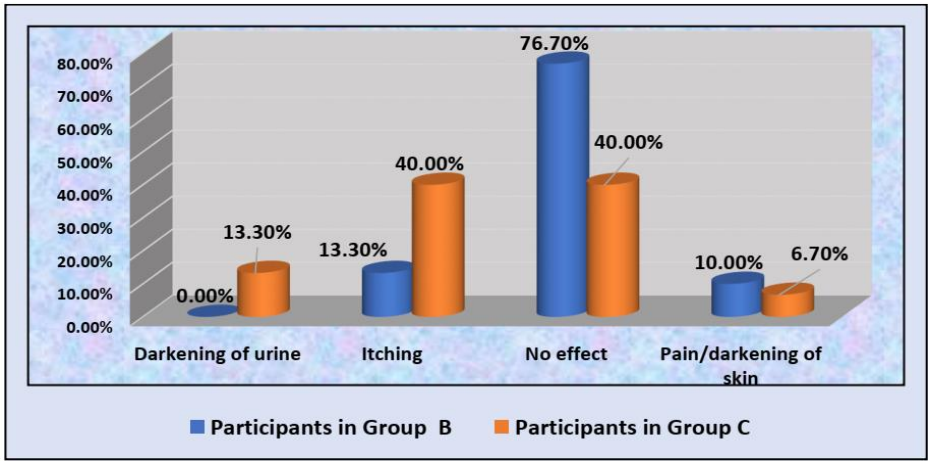


Fig.8: Distribution of the study subjects according to previous history of reaction after dye application (n=60).

IV. Discussion

A. The Hematological Assays

The skin acts as a protective barrier, preventing external substances from disrupting the body's intricate internal processes but applied some compounds such hair dye can penetrate the skin layer without resistance. therefore, the human exposure to PPD during hair dyeing process, regular hairdressers, handling hair dye, industrial workers etc. after absorption during layer skin PPD can reach systemic circulation, where some studies reported the causal association of hair dye usage may be cases: leukaemia, bladder cancer risk and a strong allergen [24].

In this study the effect of the exposures to PPD contented in black henna dye was significantly correlated with blood parameters, the tested groups B, and C showed significant decrease ($P \leq 0.05$), mainly Hb, RBCs compared to control group. The MCV was found to be elevated in the Group B and C, but no statistical significance was observed. While that of MCH, and MCHC measurements of tested group B did not show significant difference ($P \geq 0$ .05), whereas the tested C showed significant decrease ($P \leq 0.05$), while that of WBCs in the blood of tested C showed significant decrease ($P \leq 0.05$) compared to control group, with little decrease in group B. On the other hand, PLT in the blood of tested group B but no statistical significance was observed, whereas the tested C showed significant decrease ($P \leq 0.05$). while that of mean Neutrophil percentages in the blood of tested B, and C little decrease ($P \leq 0.05$) compared to control group. On the other hand, the lymphocytes percentages in the blood of tested C showed significant increase ($P \geq 0.05$) compared to control group, with little decrease in group B. Despite the significant increases ($P \leq 0.05$) in the percentage of neutrophils count, the lymphocytes showed significant ($P \leq 0.05$). We observed from all results the group C more consider effect of the dye from group B counts and compared to the control group.

One of the results obtained in this study is increased in lymphocytes percentages, this result identical with Sieben et al., (2002) where illustrated that is both PPD and its auto-oxidation lead to motivate lymphocyte diffusion which may cause of inflammation or allergies reactions [25]. Our result showed decreasing in WBC this corroborated with Zhang et al., (2023) that is decease WBC probably for exposed to higher PPD concentration [20]. The decreasing in in RBC count, Hb and WBC that reported by [24,26]. The effect of hair dye containing PPD on decrease Hb, RBCs, MCH, MCV and PLT that is possible to associated with macrocytic anemia and iron deficiency, while the significant increase in lymphocyte and decreased in neutrophil and in WBCs indicates to the effect of PPD lead to may injurious the immune system and reduces its efficiency [27]. From hematological assays for all groups group B and C with compare control group shown that the more effect is group C from B which applied black stone dye that the high concentration of PPD and this result compatible with information above. Therefore, the continue application of henna mixed with PPD may be led to severe hemolytic anemia and posing a risk of systemic toxicity [26].

B. The Biochemical assay

The traditional henna used as form a paste henna leaf for cosmetic purposes like: colouring skin, hair, and hand or body, this natural henna appears to be non-toxic effects and biosafe. But before being used, the dye is frequently combined with PPD. While natural henna reactions are rare, allergic contact dermatitis is more likely to occur when PPD is added to natural henna. The idea that drugs applied to the skin can be readily absorbed through the dermal blood vessels and may have an impact on the tissue of the liver and kidneys is supported by several research [28]. Therefore, biomarkers are important tools for measurement, knowing, evaluating the toxicity and adverse health effects that reflect of the health disease as a result of exposure to hair dyes. When exposed to hair dye, chemicals or their biological metabolisms are absorbed and distributed throughout the organism. The toxicity of these substances is assessed by urine, saliva, blood, serum, etc., which reflect the severity of exposure, risks and adverse effects on the skin, kidney, bladder, liver and other organs along with the immune system [29].

a. Liver Function Test:

Our study observed elevated liver enzymes for participants (AST, ALT, ALP) and the increased in Bilirubin with decreases in total protein and albumin, after applied black henna hair that containing of PPD, this increases in liver enzymes and albumin and deceased in total protein and albumin indicating potential poisoning liver or liver dysfunction due to exposure for black henna hair. This study indicated that PPD significantly increased liver enzymes while decreasing total protein and albumin levels, consistent with previous research [30]. it is supported this finding, noting similar higher enzyme behavior in cases of PPD exposure to the cause's hepatotoxicity due to muscle damage associated with PPD toxicity, this results consistent with [31]. The decreased in total protein synthesis by hepatic cells that is reflects the synthetic function of the liver, elevated liver enzymes and in increased total bilirubin due to a breakdown of red blood cells indicate sensitivity and toxicity to liver tissue, even following brief exposure of PPD may lead to hepatotoxicity [32]. El-Amin et al., (2014) when their administration of PPD to rats revealed a significant increase in liver enzymes GOT, GPT, and ALP, and a significant decrease in the total protein, albumin associated with the increase of the commercial hair dye dosage [27]. Workers exposed to high PPD concentrations exhibited increased AST, glutamyl transpeptidase, and total bilirubin levels, while albumin and total protein levels decreased, indicating potential liver dysfunction [33].

b. Kidney function tests

Our result indicate that use of black henna dyes adversely affects urea and creatinine levels, with increases observed in two groups compared to the control group. furthermore, the group C higher levels in urea and creatinine then group B these influences may be due to correlating the concentration of PPD in hair dyes on urea and creatinine, therefore the PPD's toxicity on kidney tissues, leading to reduced renal function and potential renal failure [33,34]. Our result consistent with Amiri (2023), was study effect of mixed henna with PPD users from the relationship between serum creatinine measurement and kidney function, where the study was revealed a high creatinine in blood with significant effect on the of kidney function which indicated possibility to be caused the developing disease Henna-induced pigment nephropathy by consuming hair dye [35]. Moreover, very high serum levels of creatinine and urea making PPD the only cause of nephrotoxicity, this occurs due to the aromatic

structure of PPD which makes it readily absorbable and concentrated in the tubules which cause of acute tubular necrosis in PPD poisoning is due to concentration of PPD in renal tubules [36]. Therefore, the applied of black henna hair dye containing on PPD for long times probably to related with acute or chronic renal disease, which results to cause arrhythmia, myocarditis, and ventricular tachycardia that may causes death [19].

C. Symptoms appeared after application black henna

Patients who are allergic to PPD are usually for the people who have no experience using hair dyes at home, and usually take longer to apply the dye to the scalp, and the risk of allergic contact dermatitis may appear within for as 5-30 minutes, the symptoms are usually very dramatic, and they take the form of acute eczema on the scalp, face, neck and sometime cases of hair loss [21]. In our study In Table.6 and fig.8 most symptoms itching 13.3% in group B and 40 % in of group C, and the skin darkening was complained by 6.7 % for group B and 10 % in group C, while the darkening of urine no appeared in group B and 13.3 % in of group C. in contrast, 86.7% from group B and 60 % group C did not have any reaction on skin after application of hair dye, but 13.3 % group B and 40 % group C they had itch on the skin immediately after application of hair dye that is effect may be due to allergic contact dermatitis, this result same results reported by Han et al., (2018), where study to identify the clinical characteristics of hair dye contact allergy and to assess the relationships between hair dye contact allergy, exposure time to PPD and PPD positivity. Hair dye allergy was appeared on most patients 80% where the diagnosis associated skin diseases, involved Allergic contact dermatitis area and pruritus was the most common symptom; erythematous macules and patches and with the face and non-specific eczema and urticaria [2]. Similar result to irritant reaction and change morphology that looks on skin with Young, et al., (2019), that was found 88% subjects study reacted to PPD at 1.0%, while 69% reacted to 0.32% PPD. Therefore, they recommended to reduce the risk of active sensitization and elicitation or decreased effect of contact allergy may be lowering the patch test concentration of PPD from 1.0% to 0.32%, it also found the elicitation and sensitization dependent on the concentration on the dose per unit area of allergen delivered to the skin and exposure time [37]. This was consistent with the studies of Asghar et al,. (2022) The dark color urine (82.5%) with declining urine output and inclining serum creatinine, it was suggestive of acute kidney injury which was 22.3% in this study. Renal replacement therapy in the form of hemodialysis was done in 4.8% of our patients. where rhabdomyolysis was evident in (74.5%) patients, acute kidney injury in 30% out of which 16 patients required renal replacement therapy [18].

V. Conclusions

- Our results the changes in the biological parameters indicate possible hepatic toxicity, pointed out by significant a decrease in the protein and albumin and increases in liver enzymes (GOT, GPT, ALP) and, in groups C more than groups B compared with the control groups associated with the increase of PPD the hair dye.
- Our result underscores the use of black henna dyes adversely affects in urea and creatinine levels, as the increased urea and creatinine are observed in groups B and C compared to the control group, which may be due to the of PPD concentration in hair dyes therefore the PPD's toxicity on kidney tissues, leading to reduced renal function and potential renal failure
- The exposure to black hair dye effect of PPD on the changes in hematological where the result appearance significant decrease in Hb, RBCs, MCH, MCV, RBCs, MCHC and PLT that is possible cause the hemolytic extend to bone marrow leading to iron deficiency and insufficient produced of red blood cells lead to anemia. Furthermore, the increase in lymphocyte cells and decrease in neutrophil cells associated with significant decrease WBCs count, where observed in group C more group B compared with the control groups that is due to effect of PPD concentration on the immune system.
- The contact allergy related to exposure hair dye with PPD observed from the symptom of itching and dark spots.
- Our study experimental exhibit the correlation between concentration of PPD in black hair dye with in biochemical and hematological parameters and the resulting indication to an incidence of liver and kidney functions which can cause serious health problems.

VI. Recommendations

- Increase public awareness regarding the toxicity of black hair dyes containing PPD.
- The hairdressers should use protective plastic gloves to prevent direct skin contact and the user black henna dyes for the use long time and conduct routine health tests for individuals to reduce the medical effects of PPD on their bodies.
- Encourage further investigations and research to explore the health impacts of PPD and other ingredients in black henna and hair dyes.
- This study recommended to minimize of PPD to the premesibale levels in black henna dyes, and the decreased usage of hair dyes containing PPD, to safe to use and potentially reduced the hazardous.
- The PPD poisoning due to easy access and low priced, especially in poor and popular and rural areas living in rural areas, so the study reflects the importance of public awareness regarding dyeing, and government legislation must restrict the sale of commercial hair dyes and control the use of PPD in hair dye products.

VII. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that have influenced, or could be perceived to influence, the work reported in this article.

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