

Original Article

Paraphenylenediamine Hepatotoxic Effects in Adult Female Albino Rats

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ABSTRACT

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Background: Paraphenylenediamine (PPD) is a chemical product used in almost hair dye preparations and in combination with henna in temporary tattoos to fasten the dyeing process and intensify its results. PPD can cause local and/or systemic toxic effects when applied topically, inhaled or ingested. **Aim of the work:** This study was implemented to evaluate the toxic effects of PPD on liver of adult female albino rats. **Methodology:** Thirty-two adult female albino rats were divided into 3 groups. Group I (control group) 16 rats: equally subdivided into 2 subgroups: Subgroup IA (negative control) 8 rats: received regular diet and water, Subgroup IB (vehicle control) 8 rats: received 1 ml distilled water (vehicle of PPD) once daily by oral gavage, 5 days/week for 8 weeks. Group II (PPD treated group) 8 rats: Each rat treated with 10 mg/kg B.W of PPD in 1 ml distilled water once daily by oral gavage, 5 days/week for 8 weeks.

Group III (Follow up group) 8 rats: Each rat treated with 10 mg/kg B.W of PPD in 1 ml distilled water once daily by oral gavage, 5 days/week for 8 weeks, followed by two weeks without treatment (wash out period). After 8 weeks in group I & group II and after 2 weeks of follow up in group III, rats were subjected to blood sample collection to estimate serum levels of aspartate transaminase (AST), serum alanine transaminase (ALT) & serum alkaline phosphatase (ALP). The livers were then dissected out and divided into three parts. The first part was used for estimating oxidative stress markers malondialdehyde (MDA) and reduced glutathione (GSH). Second part was subjected for histopathological examination under light microscope. The third part was examined by comet assay to assess the degree of DNA damage. **Results:** The results revealed that PPD induced a substantial increase in (AST, ALT & ALP), hepatic MDA and a significant decrease in hepatic GSH. It also induced several histopathological alterations and significant DNA damage in liver cells. As well, cessation of PPD treatment for 2 weeks ameliorates its damaging effects. **Conclusion:** PPD induced hepatotoxic, oxidative stress and genotoxic effects. Cessation of treatment for 2 weeks revealed partial improvement.

Key words: Paraphenylenediamine, Hepatotoxicity, Oxidative stress, Genotoxicity.

I. INTRODUCTION

Paraphenylenediamine (PPD) or “black dye” is an organic compound that is not existing in nature and commercially produced by various industrial companies (Hummdi, 2012). It is a byproduct of paranitroaniline

which is available as white crystals when pure and easily turns brown once exposed to air (Nohynek et al., 2015).

PPD has been the key coloring component in more than 1000 hair dye formulas marketed worldwide, regardless of brand, since the end of the

19th century and until now. Even the so-called “natural” and “herbal” hair colors, though free of ammonia, contain PPD (Stanley et al., 2005). It is also used in combination with henna (leaves of *Lawsonia inermis*) in Africa, Middle east, and India, the so-called temporary black henna tattoo (TBHT) to intensify the black color and to reduce the time required for dyeing and decorating hands and feet (Panfili et al., 2017). It is used as well in a wide range of industrial products such as fabric or fur dyes, dark colored cosmetics, photographic processing, lithographic plates, printing inks, black rubber, oils, greases and petrol (Abdelraheem et al., 2010).

Human exposure to PPD mainly occurs via the skin, but accidental ingestion or inhalation of powdered particles during the dyeing process may also occur (ElHelaly and Shaker, 2014).

In many studies PPD has been reported to elicit its toxic effect by causing cell death together with lipid peroxidation and increased free radical formation which is responsible for marked tissue damage in muscles, kidneys, liver and skin (Chen et al., 2010). Additionally, upon PPD oxidation by cytochrome P450-peroxidase, numerous metabolites and intermediate products are released in a complex reaction. The most recognizable allergic, mutagenic and extremely toxic metabolite is known as Bandrowski's base (Punjani, 2014).

The most common serious health consequence of black henna tattoos is sensitization to PPD dye, the allergic reaction can be life-threatening and necessitate hospitalization (Sosted et al., 2006).

In addition to local effects, there is a risk of systemic toxicity. Transcutaneous absorption of PPD is rapid and can produce systemic effects including angioedema, gastrointestinal upsets, tremors, drowsiness, seizures,

dyspnea, tender palpable liver with features of acute hepatitis, acute oliguric renal failure, anuria and dark urine, cardiac arrest or even death (Sudulagunta et al., 2015).

Reports on the chronic exposure to PPD are scanty. However, lethargy, myalgia, anorexia, gastro-intestinal disorders, chronic renal failure, hepatosplenomegaly, progressive neurological symptoms and coma have all been linked to repeated and prolonged PPD exposure (Şik and Çitak, 2016).

Some studies pointed towards the hepatotoxic nature of PPD. Yet, lower volume intake resulted in hepatitis (Kondle et al., 2012).

So, the present study was carried out to ascertain the role of PPD-mediated hepatotoxicity in adult female albino rats, through a biochemical, histopathological and genotoxic study.

II. MATERIALS AND METHODS

II.1. Materials:

II.1.1. Chemicals:

Paraphenylenediamine (PPD) was purchased from Sigma-Aldrich pharmaceutical company, USA as a gray to light brown powder, molecular weight is 108.14 Dalton, CAS No. is 106-50-3 and purity >98%. It was dissolved in distilled water provided by El-Nasr Company, Egypt.

II.1.2. Animals:

Thirty-two adult healthy female albino rats were used in this study, each weighing 150-200 gm, with an average age of 6-8 weeks, obtained from the Animal House of Faculty of Medicine, Zagazig University. All animals were acclimatized for 2 weeks prior to the beginning of the experiment with free access to solid food and water in their home cages and proper ventilation. The room was maintained with 12h-light/dark cycle. The Institutional Review Board (IRB) committee for scientific research of Faculty of Medicine, Zagazig University approved the design of the experiment according

to the established guidelines for the care and use of laboratory animals.

II.1.3. Experimental design:

The rats were allocated into 3 groups as following:

- Group I (control group): consisted of 16 rats subdivided into:
Subgroup IA (negative control group) 8 rats: Rats received only diet and water for 8 weeks to test the basic parameters.
Subgroup IB (vehicle control group) 8 rats: Each rat was treated with 1 ml distilled water (vehicle of PPD) once daily by oral gavage, 5 days/week for 8 weeks.
- Group II (PPD treated group) 8 rats: Each rat was treated with 10 mg/kg B.W of paraphenylenediamine (PPD) (1/10 LD₅₀) dissolved in 1 ml distilled water once daily by oral gavage, 5 days/week for 8 weeks. The LD₅₀ of PPD in rats is 80-100 mg/kg following oral administration (Waggas, 2011).
- Group III (follow up group) 8 rats: Each rat was treated with 10 mg/kg B.W of paraphenylenediamine (PPD) dissolved in 1 ml distilled water once daily by oral gavage, 5 days/week for 8 weeks, Then PPD treatment was stopped and follow up for two weeks for probable improvement (wash out period).

The whole duration of the study was 10 weeks (8 weeks of PPD administration and 2 weeks for follow up). At the end of the experiment, rats were sacrificed after 8 weeks in group I & group II and after 10 weeks in group III.

II.1.4. Sampling:

At the end of the trial, rats were anesthetized with ether and venous blood samples were taken from the retro-orbital plexuses, centrifuged and supernatant sera were stored at -20°C for estimation of liver function tests (AST, ALT & ALP).

Then rats were sacrificed, the livers were immediately dissected out

and washed with cold normal saline. Part of each liver (about 1 g) was enfolded with aluminum foil then kept frozen at -20°C until used for estimation of oxidative stress markers (MDA and GSH). The remaining part of liver was subdivided into two parts, the first part was fixed in 10% formalin for histopathological examination under light microscope. The second part was put in normal saline then kept at -20°C for comet assay to evaluate genotoxic effects of PPD.

II.2. Methods:

II.2.1. Biochemical studies:

II.2.1.a. Liver function tests:

Serum AST, ALT & ALP were assayed according to the spectrophotometric technique of Murray (1984a), Murray (1984b) & Rosalki et al. (1993) respectively.

II.2.1.b. Oxidative stress markers (MDA & GSH) in hepatic tissues:

Malondialdehyde (MDA) and reduced glutathione (GSH) were assayed according to the spectrophotometric technique of Ohkawa et al. (1979) and Moron et al. (1979) respectively.

II.2.2. Histopathological study:

Samples of liver were fixed in 10% formalin. Then, they were embedded in paraffin, sectioned, stained and examined under light microscope (Bancroft and Gamble, 2008).

II.2.3. Alkaline single cell gel electrophoresis (comet assay):

Comet assay was carried out using the technique specified by Singh et al. (1988).

II.2.4. Statistical analysis:

Data were analyzed by statistical package of social science (SPSS) for Windows, software version 25.0. (IBM, 2017). Differences between multiple means were compared by One Way Analysis of Variance (ANOVA) test, followed by Least Significance Difference (LSD) test for multiple

comparisons among different groups. Probability (P value) was set at >0.05 for non-significant results, <0.05 for significant results, <0.01 for highly significant results and <0.001 for very highly significant results. All results were expressed as Mean \pm Standard Deviation (SD).

III. RESULTS

III.1. Biochemical results:

There was no statistically significant difference between negative control group and vehicle control group regarding the findings of biochemical parameters (liver function tests and oxidative stress markers) all over the entire study duration ($p>0.05$). Consequently, negative control group used as a standard reference for comparison with other groups.

As regard biochemical parameters of treated groups:

III.1.1. Liver function tests:

III.1.1.a. Serum aspartate transaminase (AST):

There was a very highly significant increase ($p<0.001$) in the mean values of AST of PPD treated group when compared with negative control group. Also, there was a very highly significant decrease ($p<0.001$) in the mean values of AST of follow up

group when compared with PPD treated group, but there was a non-significant difference ($p>0.05$) when compared with negative control group (Table 1).

III.1.1.b. Serum alanine transaminase (ALT):

There was a very highly significant increase ($p<0.001$) in the mean values of ALT of PPD treated group when compared with negative control group. Moreover, there was a very highly significant increase ($p<0.001$) in the mean values of ALT of follow up group when compared with negative control group, but there was a non-significant difference ($p>0.05$) when compared to PPD treated group (Table 1).

III.1.1.c. Serum alkaline phosphatase (ALP):

There was a very highly significant increase ($p<0.001$) in the mean values of ALP of PPD treated group when compared with negative control group. Moreover, there was a significant increase ($p<0.05$) in the mean values of ALP of follow up group when compared with negative control and a very highly significant decrease ($p<0.001$) when compared with PPD treated group (Table 1).

Table (1): Statistical comparison of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) mean values in -ve control, PPD treated group and follow up group by using ANOVA test & LSD test.

Group (N=8) Parameter	Group I -ve control	Group II PPD treated	Group III Follow up	F	P	LSD
AST (U/L) Mean \pm SD	68.9 \pm 4.6	104.3 \pm 19.1	75.1 \pm 10.6	17.2	< 0.001***	< 0.001*** ¹ > 0.05 ² NS < 0.001*** ³
ALT (U/L) Mean \pm SD	32.0 \pm 2.9	106.3 \pm 24.5	85.9 \pm 26.5	26.9	< 0.001***	< 0.001*** ¹ < 0.001*** ² > 0.05 ³ NS
ALP (U/L) Mean \pm SD	93.9 \pm 19.3	219.6 \pm 72.9	104.0 \pm 43.7	15.4	< 0.001***	< 0.001*** ¹ < 0.05* ² < 0.001*** ³

N = Number of sacrificed rats in each group was 8 rats. SD = Standard Deviation.

U/L= Units per liter. LSD = least significance difference. NS = Non-Significant (P>0.05).

* = Significant (P<0.05) ***= Very Highly Significant (P<0.001).

1: -ve control group versus PPD treated group. 2: -ve control group versus follow up group.

3: PPD treated group versus follow up group.

III.1.2. Oxidative stress markers (MDA & GSH) in hepatic tissues:

III.1.2.a. Malondialdehyde (MDA):

There was a very highly significant increase (p<0.001) in the mean values of hepatic MDA of PPD treated group when compared with negative control group. Moreover, there was a very highly significant increase (p<0.001) in the mean values of hepatic MDA of follow up group when compared to negative control group, yet there was a very highly significant decrease when compared to PPD treated group (Table 2).

III.1.2.b. Reduced glutathione (GSH):

There was a very highly significant decrease (p<0.001) in the mean values of hepatic GSH of PPD treated group and follow up group when compared with that of negative control group. On the other hand, the latter revealed a very highly significant increase (p<0.001) when compared to PPD treated group (Table 2).

Table (2): Statistical comparison of hepatic malondialdehyde (MDA) & hepatic reduced glutathione (GSH) mean values in -ve control, PPD treated group and follow up group by using ANOVA test & LSD test.

Group (N=8) Parameter	Group I -ve control	Group II PPD treated	Group III Follow up	F	P	LSD
Hepatic MDA (nmol/mg) Mean ± SD	0.11 ± 0.01	0.31 ± 0.04	0.23 ± 0.03	93.5	< 0.001***	< 0.001*** 1 < 0.001*** 2 < 0.001*** 3
Hepatic GSH (ng/mg) Mean ± SD	0.27 ± 0.02	0.15 ± 0.04	0.20 ± 0.02	36.3	< 0.001***	< 0.001*** 1 < 0.001*** 2 < 0.001*** 3

N = Number of sacrificed rats in each group was 8 rats. SD = Standard Deviation.

nmol/mg = nanomole per milligram. ng/mg = nanogram per milligram.

***= Very Highly Significant (P<0.001). LSD = least significance difference.

1: -ve control group versus PPD treated group. 2: -ve control group versus follow up group.

3: PPD treated group versus follow up group.

III.2. Histopathological results:

• Control group (I):

Histopathological examination of the liver sections of both negative control and vehicle control groups exhibited normal hepatic architecture without any observable alterations all over the period of the study (Figures 1 & 2).

• PPD treated group (II):

After 8 weeks of PPD administration, several histopathological alterations were detected. Central vein was markedly dilated & congested with proliferation of its lining epithelium. Hepatic lobules were disorganized with loss of normal hepatic lobular architecture,

hepatocytes displayed marked vacuolation, dark pyknotic nuclei and severe congestion in the sinusoidal spaces. Complete necrosis of some hepatocytes was detected (Figures 3 & 4).

• Follow up group (III):

Cessation of PPD treatment for two weeks revealed some improvement in histopathological changes indicated by normal central vein, minimal disorganization of hepatocytes, dark pyknotic nuclei with vacuolation and minimal blood sinusoidal congestion. Slightly congested portal vein with minimal cellular infiltration was detected (Figures 5 & 6).

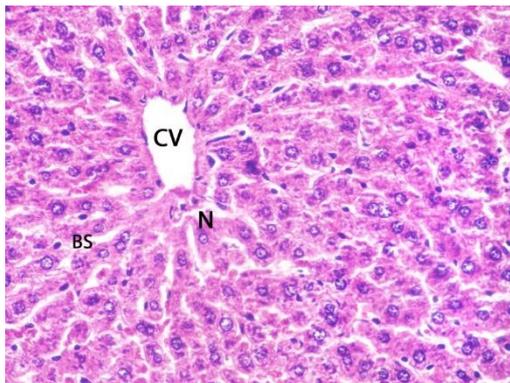


Figure (1): A photomicrograph of a section in liver obtained from an adult female albino rat of the negative control group showing normal hepatocytes containing pale nuclei (N) radiating from the central vein (CV), separated by blood sinusoids (BS) (H&E X400).

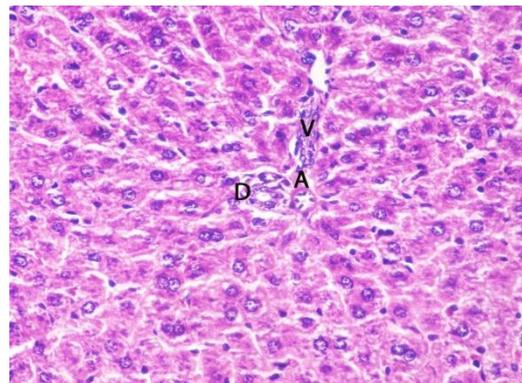


Figure (2): A photomicrograph of a section in liver obtained from an adult female albino rat of the negative control group showing normal portal area with hepatic artery (A), portal vein (V) and bile duct (D) (H&E X400).

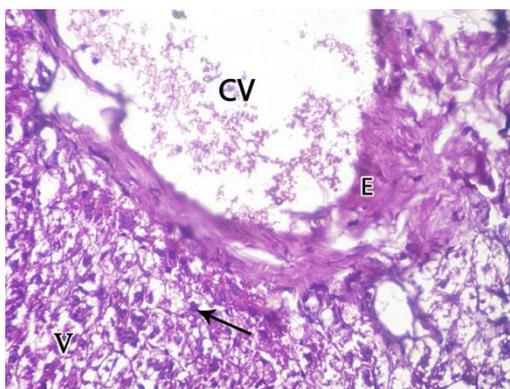


Figure (3): A photomicrograph of a section in liver obtained from an adult female albino rat of the PPD group (II) after 8 weeks of exposure showing dilated & congested central vein (CV) with proliferation of the lining epithelium (E), disorganization of hepatic lobules with loss of normal hepatic architecture, hepatocytes with dark pyknotic nuclei (black arrow) and marked vacuolation (V) (H&E X400).

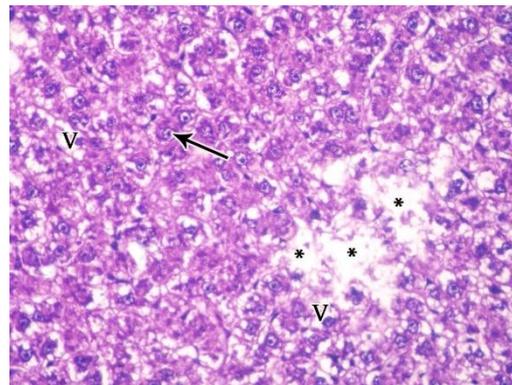


Figure (4): A photomicrograph of a section in liver obtained from an adult female albino rat of the PPD group (II) after 8 weeks of exposure showing marked disorganization of hepatic lobules, hepatocytes with dark pyknotic nuclei (black arrow) and marked vacuolation (V). Complete necrosis of some hepatocytes was demonstrated (star) (H&E X400).

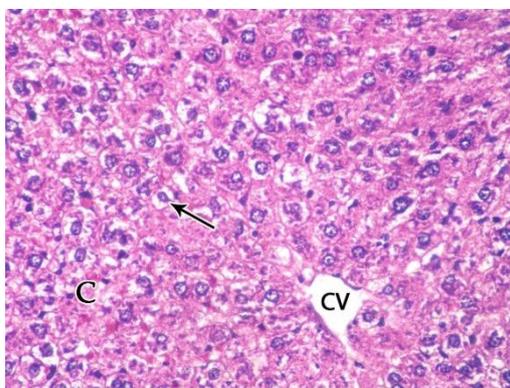


Figure (5): A photomicrograph of a section in liver obtained from an adult female albino rat of follow up group (III) showing normal central vein (CV), minimal disorganization of hepatocytes, dark pyknotic nuclei with vacuolation (black arrow) and minimal blood sinusoidal congestion (C) (H&E X400).

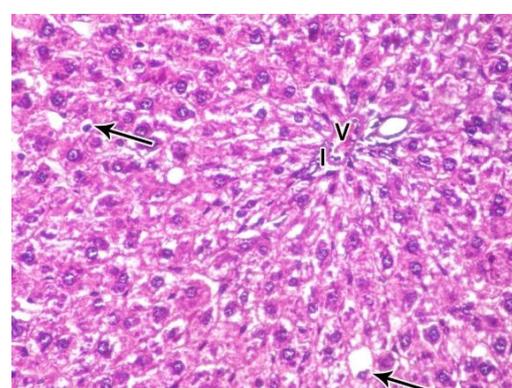


Figure (6): A photomicrograph of a section in liver obtained from an adult female albino rat of follow up group (III) showing slightly congested portal vein (V) with minimal cellular infiltration (I). Some hepatocytes showing dark pyknotic nuclei with intracytoplasmic vacuolation (black arrow) (H&E X400).

III.3. Comet assay results:

By comparing the comet results of liver specimens of negative control group and the vehicle control group, there was no statistically significant difference between them in all parameters ($p>0.05$) all over the period of the study. So, we use negative control group as a standard reference for comparison with other treated groups.

Liver specimens of PPD treated group revealed a very highly significant increase ($p<0.001$) in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a very highly significant decrease in % of untailed nuclei as compared with those of the negative control group. Hepatic nuclei of follow up group showed a very highly

significant increase ($p<0.001$) in % of tailed nuclei, tail length, tail DNA % and unit tail moment and a very highly significant decrease in % of untailed nuclei when compared with the nuclei of the negative control group (Table 3).

When comparing the mean values of comet assay measured parameters of hepatic nuclei of follow up group with the nuclei of PPD treated group it showed a non-significant difference ($p>0.05$) in % of tailed nuclei, % of untailed nuclei, tail length and a very highly significant decrease in tail DNA % & a highly significant decrease in unit tail moment (Table 3).

Results are illustrated in the form of fluorescent photomicrographs as shown in (Figure 7).

Table (3): Statistical comparison of comet assay results of liver in -ve control, PPD treated group and follow up group by using ANOVA test & LSD test.

Group (N=8) Parameter	Group I -ve control	Group II PPD treated	Group III Follow up	F	P	LSD
Tailed (%) Mean ± SD	4.5 ± 0.3	17.0 ± 1.9	15.6 ± 1.4	198.88	< 0.001***	< 0.001*** ¹ < 0.001*** ² > 0.05 ³ NS
Untailed (%) Mean ± SD	95.5 ± 0.6	83.0 ± 1.9	84.4 ± 1.4	189.82	< 0.001***	< 0.001*** ¹ < 0.001*** ² > 0.05 ³ NS
Tail Length (µm) Mean ± SD	1.1 ± 0.1	2.9 ± 0.2	2.9 ± 0.1	432	< 0.001***	< 0.001*** ¹ < 0.001*** ² > 0.05 ³ NS
Tail DNA (%) Mean ± SD	1.2 ± 0.03	3.0 ± 0.04	2.9 ± 0.1	1964.8	< 0.001***	< 0.001*** ¹ < 0.05* ² < 0.001*** ³
Unit tail Moment Mean ± SD	1.4 ± 0.05	9.0 ± 0.6	8.4 ± 0.5	699.55	< 0.001***	< 0.001*** ¹ < 0.001*** ² < 0.05* ³

N = Number of sacrificed rats in each group was 8 rats.

SD = Standard Deviation. % = percent - µm = micrometer.

LSD = least significance difference test. NS = Non-Significant (P>0.05).

* = Significant (P<0.05). ***= Very Highly Significant (P<0.001).

1: -ve control group versus PPD treated group. 2: -ve control group versus follow up group.

3: PPD treated group versus follow up group.

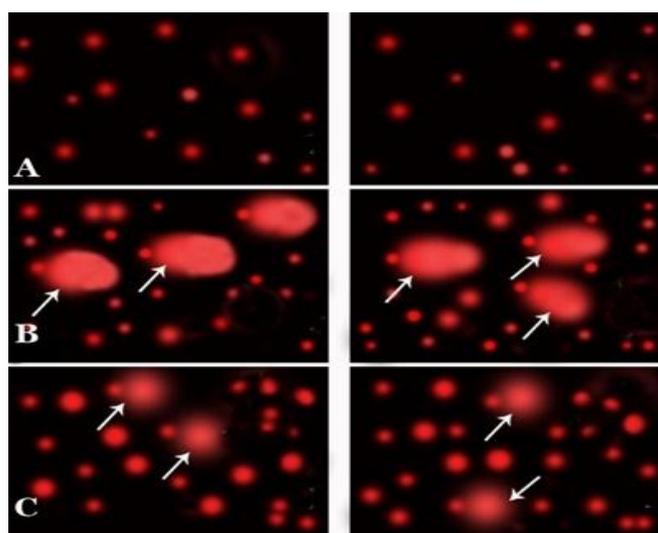


Figure (7): Photomicrographs by fluorescent microscope showing liver cells nuclei from adult female albino rats of: (A) control group, DNA in most cells is tightly compressed and maintains the circular disposition of normal nucleus. (B) PPD treated group (II), the comet has residual head and long dense tail pattern since most DNA migrated to tail (white arrow). (C) Follow up group (III), the comet with a residual head but shorter and less dense tail pattern as compared to PPD treated group (white arrow).

IV. DISCUSSION

In a society obsessed with beauty, people are attracted to enhance their appearance to create great on personality and build up confidence. But many of these cosmetic products, which are meant to make us feel healthy and beautiful have a deep dark side as toxic constituents and hazardous chemicals are being used beyond acceptable limits (Naveed, 2014).

Paraphenylenediamine (PPD) is an aromatic amine involved mainly in cosmetology. It is widely used in various types of commercially available hair dye formulations, dark colored cosmetics and temporary tattoos around the world. Also, it is found in dark blue and black clothing dyes, so virtually everyone can be exposed to contact with PPD (Mukkanna et al., 2017). Almost since its debut, it has been a problematic agent and is recently described as one of the dirty dozen among cosmetic products that can evoke toxic local and/or systemic effects when used topically, inhaled or ingested (DeLeo, 2006).

Findings of the current study disclosed that oral administration of PPD induced liver toxicity in the form of significant elevation of AST, ALT and ALP which go with those of Bharali and Dutta (2009) & Abd-ElZaher et al. (2012) who reported that topical application of PPD for 30 days caused significant dose dependent elevation of liver enzymes with chronic inflammation of the liver.

In other human studies, Tokumoto et al. (2003) reported that PPD based hair dyes induced hepatitis as an adverse effect. The patients were diagnosed with hepatitis and the damage was confirmed to be caused by hair dying after exclusion of all causes that typically induce liver damage. Episodes of hepatitis with impaired

liver function occurred shortly following recurrent use of hair dye. Also, Singh et al. (2008) observed hepatic affection in most of cases with PPD intoxication manifested by elevated plasma levels of hepatic enzymes.

In contrary, Alalwani (2013) reported that topical application of PPD caused a sharp rise in the serum ALP that followed a dose dependent pattern, but decrease in AST and ALT was recorded. This was clarified by Giannini et al. (2005) who declared that the ALP is an enzyme in the cell lining the bile duct of liver and it increases with bile duct damage, indicating that PPD contributed to bile ducts lesions. Giboney (2005) added that AST and ALT are associated with liver parenchyma cells so they are elevated in acute liver injury. While in chronic diseases or by the use of toxic substances, the level was decreased gradually as a result of cirrhosis of hepatic parenchyma.

The exact mechanism of PPD-induced hepatic affection is not entirely known. However, it can be due to the direct toxic effect of PPD or its byproducts (El-Amin et al., 2014). In accordance to Eyer (1994) PPD is an aromatic amine belonging to a group of compounds which cause toxicity after oxidative biotransformation in liver cells. Besides, PPD undergoes extra hepatic activation to free aminyl radical which rapidly become disproportionate and attach to thiol, resulting in further toxic items.

In agreement with El Shemy et al. (2017), results of follow up group in this study showed that the hepatic cells damage had almost stopped, but still the liver cell function was not fully restored.

Such findings can be approved by Tokumoto et al. (2003) who described a

case admitted to hospital due to three episodes of hepatitis following frequent use of hair dye and abnormal liver function started to improve after dye discontinuation.

In the present study, liver tissues of PPD treated rats showed evidence of oxidative damage indicated by elevated levels of hepatic malondialdehyde (MDA), and low levels of hepatic reduced glutathione (GSH).

These results coincide with Mathur et al. (1990) who stated that exposure to PPD caused a reduction in glutathione and an observed surge in malondialdehyde in serum, skin, liver and kidney of treated groups. Also, Sutrapu et al. (2010) observed a decline in glutathione levels and elevation of MDA levels indicating a decrease in antioxidant status and an overwhelming of oxidative stress respectively in PPD intoxicated patients at different time periods.

Additionally, Mathur et al. (2005) & Bharali and Dutta (2012b) confirmed that topical application of PPD caused oxidative stress. There was a rise in MDA, suggesting lipid peroxidation. Also, Elyoussoufi et al. (2013) noticed that PPD obviously increased lipid peroxidation of human cultured neutrophils due to higher level of MDA and alterations in neutrophils antioxidant defense system.

Furthermore, in a human study performed by Menicagli et al. (2018) to assess the level of oxidative stress in hairdressers and consumers exposed to hair dyes in Italy, the results showed a highly significant rise of salivary MDA (an indicator of oxidative stress) versus control group in hairdressers. A further analysis revealed that the level was higher for those working in a small workplace which was probably due to a more direct exposure to the gas produced by the dye, while consumers

who had used hair dyes for at least ten years also had an increased level of salivary MDA.

The oxidative stress caused by PPD could be explained by Chen et al. (2010) who proposed that PPD produced cell death at high concentrations and after a long period of exposure. This impact with lipid peroxidation can trigger the production of superoxide and hydrogen peroxide.

However, some studies by Corsini et al. (2013); Gibbs et al. (2013) and Zaroni et al. (2015) suggested that PPD used in hair dyes can cause oxidative stress and DNA damage through the formation of highly reactive hydroxyl radicals when exposed to the skin in human keratinocytes.

Results of follow up group in this study showed some improvement and this coincides with Sutrapu et al. (2010) who found that MDA and glutathione levels of PPD intoxicated patients started to improve following PPD elimination from their bodies and with symptomatic treatment, suggesting the recovery of the cellular antioxidant defense system.

The hepatic histopathological findings in the current work confirmed the results obtained for the effect of PDD on alteration of liver function tests (AST, ALT & ALP). As compared to control groups, PPD exposure induced several histopathological alterations in the liver. There was a markedly dilated & congested central vein with proliferation of the lining epithelium. Hepatic lobules were disorganized with loss of normal architecture, hepatocytes showed marked vacuolation and dark pyknotic nuclei with severe congestion in the sinusoidal spaces. Complete necrosis of some hepatocytes was also detected. However, cessation of PPD treatment for two weeks revealed some

improvement in these histopathological changes.

Such results were in line with the observations of Al-Shaikh et al. (2018) who documented that subchronic oral administration of PPD to rats was accompanied by deformed congested central veins with degenerated endothelial lining. Also, swollen hepatocytes with increase acidophilic staining of cytoplasm, small dark pyknotic nuclei, congested portal veins surrounded by inflammatory cells and marked degeneration of hepatocytes were detected.

One study conducted by Abd-ElZaher et al. (2012) recorded that liver sections of PPD treated rats revealed trivial chronic inflammation with a single necroinflammatory focus, which became multiple foci by increasing the dose.

In other human studies, Tokumoto et al. (2003) reported drug induced hepatitis as an adverse effect of PPD based hair dyes. Liver biopsy demonstrated centrilobular hepatocellular necrosis with minimal inflammatory reaction and inflammatory infiltrates of lymphocytes and eosinophils in portal tracts which were consistent with common histopathological presentations of drug induced liver injury.

Also, Ibrahim et al. (2006) confirmed PPD induced hepatic affection microscopically, observing mutable grades of focal and diffuse necrosis. Hepatocytes showed blow up with granular cytoplasm. Portal tract was expanded with large lymphatic spaces. Moreover, lining epithelium of bile duct demonstrated focal shedding.

Abdelraheem et al. (2010) observed characters of acute hepatitis on liver biopsies of some PPD intoxicated patients. In a study by Alugonda et al. (2013), the

histopathological examination of liver tissues of twenty-five hair dye poisoning deaths revealed scattered parts of fatty changes and central vein dilatation in 48% of cases.

Bharali and Dutta (2009) claimed that the mechanism of hepatocytes toxicity caused by PPD can result either directly from the interruption of intracellular function and membrane integrity, damage affecting endothelial and bile duct cells as seen in cholestasis or indirectly from immune-mediated membrane damage.

Alternatively, Copple et al. (2002) attributed the apparent hepatotoxicity to the fact that plasma half-life of PPD calculated to be 43.5 h. Since even a small fraction of PPD absorbed through the skin may be retained for a long time to cause toxicity to hepatic parenchyma, sinusoidal and central vein endothelial cells. Abd-ElZaher et al. (2012) added that the increase of hemoglobin content in the liver clarifies that PPD causes damage to sinusoidal or central vein endothelial cells and causes massive hemorrhage and subsequent fibrin accumulation in central vein and sinusoidal spaces which in turn results in impaired sinusoidal blood flow that eventually contributes to ischemic/hypoxic hepatic injury.

Severe necrosis of some hepatocytes which was detected after 8 weeks of PPD exposure in the present study, are corroborated with Saad et al. (2000), Alalwani (2013) & Al-Shaikh et al. (2018) who informed that PPD produces provokes free radicals, resulting in lipid peroxidation, which in turn causes necrosis of liver cells and renal tissue.

As regard improvement of histopathological changes of liver of follow up group, confirmed by Popescu et al. (2012) who stated that the liver has the extraordinary capacity to regenerate

after traumas or various injuries (toxic or infectious). Also, Fisher et al. (2015) demonstrated that most instances of drug-induced liver injury consist of a slight increase of serum transaminase levels can be resolved after removal of the offending chemical agent.

Comet assay is a widely used assay in basic DNA damage and repair studies, genotoxicity testing of new chemicals and drugs, environmental biomonitoring and human population surveillance (Arora et al., 2012). In this study, comet assay presented the genotoxic effect of the PPD on the liver cells through significant DNA damage indicated by the damaged nuclei.

In agreement with the current results, Bharali and Dutta (2010) & AlSeigini et al. (2014) reported that PPD is genotoxic in nature and caused DNA fragmentation of peripheral blood lymphocytes in dose and time dependent manner as was evaluated with comet assay after subchronic dermal exposure to PPD in adult albino rats.

Coulter et al. (2008) and Chen et al. (2010) observed cells death with PPD treatment of cell culture. Chen et al. (2010) noticed that PPD-treated cells displayed up to 3-fold damage to DNA relative to untreated cells. DNA damage was dose and duration dependent and was evaluated by comet assay.

Similar toxic effects were observed by Elyoussoufi et al. (2013) who spotted that PPD is a suspected carcinogen that can induce apoptosis. The aforementioned studies showed that PPD induced both dose-dependent and time-dependent cytotoxicity and cell death. Apoptosis was assessed by light microscopy revealing nuclear pyknosis, chromatin condensation and further confirmed by DNA fragmentation analysis in agarose gel electrophoresis.

Consistent with other human studies, Denli et al. (2002) detected DNA damage in women after long years of using hair dyes. Damage was duration dependent and was evaluated by comet assay in peripheral blood lymphocytes. Cho et al. (2003) also reported that the acute exposure to hair dyes induced DNA damage in peripheral lymphocytes from 20 human volunteers manifested by higher tail moment.

In addition, Galiotte et al. (2008) noticed a higher frequency of DNA damage of female hairdressers exposed to oxidative hair dyes as compared to control group in a study by which DNA was evaluated by comet assay. In the same context, Chye et al. (2008) recorded a significant increase in mean tail moment in 20 female volunteers after using oxidative hair dyes.

In Brazil, a study by Tafurt-Cardona et al. (2015) explored the toxic impact of chemical substances used in black hair dyes and found that they were capable of causing marked cytotoxic and genotoxic effects in human cells and in vitro experiments at concentrations much lower than those used commercially.

A study performed by Hammam et al. (2014) to detect genotoxicity among hairdressers in Zagazig city, Egypt, the results of total comet score showed a highly significant difference in DNA damage between the studied hairdressers and their controls.

In contrast, a review of biomonitoring studies assessing genotoxicity in humans exposed to hair dyes, revealed that there is no strong evidence of genotoxicity in humans exposed to hair dyes on an occupational or individual basis (Preston et al., 2009).

To justify negative results, Dressler and Appelqvist (2006) conducted in vivo experiments in two dissimilar animal models revealing that topical application of PPD resulted in negligible blood concentrations, and no parent compounds were found in the blood, though the studies were performed under full exposure conditions.

According to Skare et al. (2007), humans are not systemically exposed to the parent compound, after dermal contact with PPD-containing products but rather to their N-acetylated metabolites, which exist in human skin and is catalyzed by NAT1. N-acetylation is a detoxifying reaction, that results in non-sensitizing, non-genotoxic and non-carcinogenic metabolites (Skare et al., 2007 & Aeby et al., 2009).

Concurrently, explanation of positive results was suggested by Bharali and Dutta (2012a) who stated that repeated exposure of the organism to PPD may reduce the detoxifying capabilities of skin and thus exposing it to the parent PPD molecule which is genotoxic.

However, Kawakubo et al. (2000) offered another explanation that large amount of PPD could be oxidized at skin surface by series of enzymes, such as ceruloplasmin, myeloperoxidase and cytochrome p-450 to benzoquinone diamine which in turn might form Bandrowski's base. It was thought that the reactivity and mutagenicity of topical PPD increased by the development of such base.

From another point of view, Mathur et al. (2005) recorded that the DNA damage observed was most likely due to PPD induced oxidative stress and

production of ROS. Likewise, Filipič et al. (2007) reported that ROS are known to induce DNA strand breaks which can be easily detected by comet assay.

Sutrapu et al. (2010) reported that PPD induced a depletion of glutathione in addition to an elevation of MDA marker of lipid suggesting a decrease in antioxidant status and an overwhelming oxidative stress supports that DNA damage was mediated by oxidative stress.

PPD mediated intracellular ROS generation, according to Chen et al. (2010) initiating both intrinsic (caspase 9) and extrinsic (caspase 8) pathways that converge to activate caspase 3/7 resulting in loss of cell viability and apoptosis. By using vitamin C and E, well-known antioxidants, which inhibited ROS generation, the involvement of ROS in DNA damage was validated (Bharali and Dutta, 2012b).

In the same context, Waggas (2011) stated that ROS can cause DNA damage of cells by oxidizing lipids in cell membranes or directly attacking DNA. Lipid peroxidation disturbs the physical stability of the membranes, resulting in altered calcium homeostasis, activation of endonucleases, sulfhydryl modification of proteins and oxidative DNA damage (Elyoussoufi et al., 2013).

However, as stated by the International Agency for Research on Cancer (IARC), certain hair dyes are known to be mutagenic and carcinogenic in experimental studies and to the exposed human populations (Tafurt-Cardona et al., 2015).

Results of follow up group revealed minor recovery in DNA damage and slight decrease in unit tail moment as an important parameter of

DNA damage following discontinuation of oral PPD.

Repair of DNA damage is a very difficult process which needs long time and optimum withdrawal time for the tissue to fix strand breaks and chromosomal abnormalities and sometimes, the damage become irreversible with persistent effects (Sliwinska et al., 2015).

However, Persistence of DNA damage for long time can be explained by oxidative stress and ROS production. As, ROS can damage DNA and cause cell division with unrepaired or misrepaired damage leading to mutations. Also, this permanent damage may result from apoptic cells (Oh et al., 2019).

V. CONCLUSION

From these observations, it can be inferred that: oral administration of PPD in adult female albino rats caused toxic effects and DNA damage in the liver which may be due to oxidative stress, while cessation of PPD treatment for 2 weeks partially alleviates its damaging effects.

VI. RECOMMENDATIONS

Considering the findings of the current work, the followings are recommended:

- Public education programs and the collaboration of different

health authorities are required especially in developing countries to raise public awareness of PPD related hazards.

- Avoidance of long term and continuous usage of hair dyes containing PPD and discourage the use of TBHT for fashion especially among children and adolescents to prevent PPD sensitization.
- It is important to consider replacement of the current hair dye materials with less hazardous or natural ones.
- Paraphenylenediamine handlers such as hair dressers, textile manufacturing workers, and chronic tattoo users should be subjected to serial biochemical and genetic assay follow up.
- Further studies about chronic PPD systemic toxicities were advised to be accomplished to identify the exact mechanism by which PPD induced toxicity, which might be helpful in improving the therapeutic strategies.
- The effectiveness of protective agents such as antioxidants (natural or synthetic) in treatment of PPD poisoning needs to be more evaluated.

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تأثيرات البارافينيلينديامين السمية الكبدية في إناث الجرذان البيضاء البالغة

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مادة البارافينيلينديامين هي إحدى الأمينات العطرية الغير موجودة في الطبيعة ويتم انتاجها تجاريا من قبل العديد من الشركات الصناعية. وتستخدم على نطاق واسع في العديد من المنتجات مثل أصباغ المنسوجات والفراء ومستحضرات التجميل ذات الألوان الداكنة والوشوم المؤقتة والتصوير الفوتوغرافي والمطاط الأسود والزيوت والشحوم والبنزين. وتعتبر مكون أساسي تقريبا في جميع صبغات الشعر التي يتم تسويقها في جميع أنحاء العالم. وقد أجريت هذه الدراسة لتقييم الآثار السامة لمادة البارافينيلينديامين على كبد إناث الجرذان البيضاء البالغة. تم تقسيم ٣٢ من إناث الجرذان البيضاء البالغة إلى ثلاث مجموعات رئيسية: المجموعة الضابطة (٦ جرذ) - مجموعة البارافينيلينديامين (٨ جرذ) - مجموعة المتابعة (٨ جرذ).

بعد ٨ أسابيع في المجموعة الأولى والثانية وبعد أسبوعين من المتابعة في المجموعة الثالثة، تم تخدير الجرذان بالإيثير ثم جمعت عينات الدم واستخدمت لقياس المؤشرات الحيوية الكبدية (نسبة أنزيم الأسبرتات ترانسامينيز والألانين ترانسامينيز والألكالين فوسفاتيز) - وأخذت عينات من أنسجة الكبد لقياس دلالات الإجهاد التأكسدي (المالونداكسيد و الجلوتاثيون المختزل) - تم استخدام أجزاء من أنسجة الكبد وتجهيزها وفحصها تحت المجهر الضوئي باستخدام صبغة الهيماتوكسلين والأيويسين - وأخيرا تم إجراء فحص المذنب لخلايا الكبد لتحديد مدى الضرر الذي حدث في الحمض النووي.

أسفرت النتائج عن ارتفاع ذي دلالة في وظائف الكبد؛ وارتفاع متوسط المالونداكسيد وانخفاض في متوسط الجلوتاثيون المختزل المستخلصان من نسيج الكبد بعد ثمانية أسابيع مقارنة بالمجموعة الضابطة السالبة. كما أسفر فحص المذنب لخلايا الكبد عن حدوث تلف للحمض النووي. كما أظهر الفحص المجهر لشرائح الكبد العديد من التغيرات الهستوباثولوجية الواضحة. كما أثبتت الدراسة أن التوقف عن العلاج ومتابعة الجرذان لمدة أسبوعين أدى إلى تحسن جزئي في هذه الآثار السامة. الخلاصة: التعرض للبارافينيلينديامين يؤدي إلى حدوث آثار سمية وتغيرات هستوباثولوجية قد تكون عائدة إلى إنتاج شوارد الأكسجين الحرة كما أن له تأثير جيني سام وذلك من خلال أحداث تلف بالغ في الحمض النووي لخلايا الكبد. لذا يجب رفع مستوى الوعي العام بالمخاطر المرتبطة بمادة البارافينيلينديامين وتجنب الاستخدام المستمر لأصباغ الشعر المحتوية على البارافينيلينديامين لفترات طويلة والحد من موضة استخدام وشم الحناء السوداء وخاصةً بين الأطفال والمراهقين.