Histological and ultrastructural studies of myocardium lesions produced by hair dye (para-phenylenediamine) in rats

Aisha D. Alalwani

Department of Biology, Faculty of Sciences, University of Jeddah, Saudi Arabia.

ABSTRACT

Para-phenylenediamine (PPD) is an aromatic compound and usually applied in a few commercial and mechanical products. Additionally, ladies apply henna for coloring their hair, which contains PPD as one of the components. Henna is used in East Africa, India, and Middle Eastern countries as a part of their culture. However, it is rarely found in western countries. The aim of this study was to examine the effect of two different doses of PPD topical application on the skin of female rats. The microscopic analysis indicates that the body and heart weights were affected. Histopathological studies on cardiac fibers showed that the treatment of rats with 1.5 ml of PPD resulted in increased cytoplasmic vacuolization and the loss of myocardial cells with a globular nucleus. Additionally, an increased dose of 3 ml PPD showed several signs of cardiotoxic effects on increased cytoplasmic vacuolization and myofibrillar loss. Congestion, focal necrosis, swollen mitochondria, and lymphoid infiltration were also observed. Considering the findings of this study, it is concluded that the chronic usage of PPD promotes histopathological alterations in heart tissue of rats.

Keywords: Para-phenylenediamine, rats, myocardium, cardiac fibers, histopathology.
Introduction
Dark henna is used in India, Africa, and the Middle East to decorate hands and feet, especially during festivals, and weddings. PPD is one of the dark henna components. However, the amount of PPD in henna sometimes leads to toxicity and becomes a dangerous compound. Several studies have reported that the addition of PPD to dark henna led to poisoning in adults and children observed during studies in Morocco [1] Tunisia [2], and India [3], and caused an edema on all of the following: neck, tongue, face, pharynx, larynx, and trachea [4]. Further, users may experience dermal infections, giddiness, spasm, and coma. Acute liver and renal failure necrosis of tubular, bleeding tendency, subconjunctival hemorrhage, and bleeding from the mucous membrane happen in the later stages [5].

A few studies have shown the dermal absorption functionality of PPD in humans and animals. Underexposure conditions are proposed for hair colorations (NIOSH, 2010). Under the proposed use conditions, dermal assimilation of PPD is 0.54% to 2.7% in human and 2.7% in primates [6, 7]. Dermal absorption of 2.7% has been noted on human corpse skin [8]. The level of dermal absorption changed into accounted for as 0.93% in pig skin [9]. [7] reported similar dermal absorption characteristics, 2.44% and 3.39% in vitro, for human and pig skin, respectively. Myocarditis was also reported in 15% of cases, with the death rate of 29%, and connected to coronary heart muscle that has been diagnosed with the amount of hair color which caused inflammations [10].

Histopathological analysis showed swollen, atrophy, and cavitations inside of the mitochondria, resulting from injured cells and functional loss due to the destruction of the mitochondria. Additionally, calcium ions disturbance in the cells, block in the synthesis of ATP and oxidation of phosphorus in the mitochondria, the change in the pH of cellular or mitochondrial membrane permeability could be affected by PPD adsorption [11].

Tattoos are very common in the work of Arab countries, especially in Saudi Arabia. Thus, people expose themselves to the danger of serious toxicity problems as reported in the previous studies [11]. PPD was noted as the poisonous additive of hair color [12]; however, the non-stop exposure of PPD and its effect on the tissue damage have little information. Further few reports are available in the effect of PPD on the heart tissue. Therefore, this study was designed to determine the effects of PPD on the myocardial lesions.

Materials and methods
Chemical
PPD powder is a purple color and obtained from Jeddah in Saudi Arabia. Instalment: 99E483, CAS No: 106-50-3. It has a purity of 98%, and molecular weight (MW), 108; C₆H₆N₂ (unfastened-Qaedra), C₆H₇N₂. 2HCl (hydrochloride), C₆H₈N₂.H₂SO₄ (sulfate) [12].

Experimental protocol
Sixty Wistar female rats (Rattus norvegicus), bodyweight 172.25 ± 1.16 g, were purchased from animal house of King Fahd of Medical Researchers in Jeddah, Saudi Arabia. Animals were maintained under standard animal laboratory conditions of a temperature of 25 ± 2°C, certified humidity of 60 ± 5 % and at 12-h light and 12-h dark cycles.

Experimental procedure
Animals were randomly divided into four groups (n=15 per group). The first and second groups were control rats received pure water, and the third and fourth groups were treated with 10% PPD injected subcutaneously at a dose of 1.5 mg and 3 mg daily per week, for a period of three months, respectively, following the method as described by [13, 14]. Rats were weighed weekly and observed daily for the signs of poisoning. The heart tissue samples were used for histological examination.

Histological study
At the end of the experiment, the rats were sacrificed. The heart tissues were weighed and fixed in 10% formalin saline for histological
analysis. Following dehydration in ascending order of ethanol and xylene, the samples were embedded in paraffin wax. Serial sections of two microns thickness were obtained using a microtome and were stained with hematoxylin and eosin, as previously described [15].

**Electron microscopy**

Tissue sections were fixed in 2.5% glutaraldehyde and 0.25 M sodium cacodylate followed by incubation with 1% osmium tetroxide and then embedded in Spurr's epoxy. Ultra-thin sections were picked up on nickel grids, stained with uranyl acetate/lead citrate [16], and analyzed using the Philips Transmission Electron Microscope.

**Statistical analysis**

Results were expressed as the mean average and standard deviation. Statistical differences were evaluated by one-way analysis of variance (ANOVA) through SPSS software and P< 0.05 was considered significant.

### Results

**Clinical symptoms and mortality**

Mortality and clinical symptoms of every group were significantly associated with administration of PPD in rats as shown in Table 1.

**Body and heart weights**

The initial and final body weights, as well as absolute and relative heart weights of the experimental animals, were determined at the respective time points. Significant differences were observed in the final body weight gained between the treated and control groups. Table 2 indicates that the absolute and relative weights of the heart were significantly increased in PPD-treated group when compared to control group. The PPD treatment also induced an increase in final body weight, as observed in this study, which could be explained by physiological disturbances resulted from exposure to PPD.

### Table 1: Clinical symptoms and the mortality recorded in groups 3 and 4 of PPD-treated rats after 12 h to 3 mon.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>No. of experimental animals =15</th>
<th>%</th>
<th>No. of experimental animals =15</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of individual</td>
<td></td>
<td>No. of individual</td>
<td></td>
</tr>
<tr>
<td>Neck and swelling face</td>
<td>17</td>
<td>85%</td>
<td>19</td>
<td>95%</td>
</tr>
<tr>
<td>Dark urine</td>
<td>17</td>
<td>85%</td>
<td>18</td>
<td>90%</td>
</tr>
<tr>
<td>Ataxia</td>
<td>13</td>
<td>65%</td>
<td>15</td>
<td>75%</td>
</tr>
<tr>
<td>Skin changes (dermatitis)</td>
<td>6</td>
<td>30%</td>
<td>10</td>
<td>50%</td>
</tr>
<tr>
<td>Mortality</td>
<td>2</td>
<td>10%</td>
<td>7</td>
<td>35%</td>
</tr>
</tbody>
</table>

### Table 2: Weights of body and heart of experimental animals treated with PPD for 3 mon.

<table>
<thead>
<tr>
<th></th>
<th>Control (G1)</th>
<th>Control (G2)</th>
<th>Treated (G3)</th>
<th>Treated (G4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight of body (g)</td>
<td>172.0 ± 2.03</td>
<td>171.67 ± 6.10</td>
<td>171.67 ± 6.10</td>
<td>175.21 ± 1.10</td>
</tr>
<tr>
<td>Final weight of body (g)</td>
<td>199.67 ± 6.10</td>
<td>195.54 ± 5.07</td>
<td>207.33 ± 4.57</td>
<td>210.00 ± 3.95</td>
</tr>
<tr>
<td>Absolute weight of heart (g)</td>
<td>0.80 ± 0.21</td>
<td>0.91 ± 0.12</td>
<td>1.20 ± 0.14*</td>
<td>1.60 ± 0.16*</td>
</tr>
<tr>
<td>Relative weight of heart(g)</td>
<td>0.40 ± 0.03</td>
<td>0.46 ± 0.03</td>
<td>0.58 ± 0.49*</td>
<td>0.76 ± 0.08*</td>
</tr>
</tbody>
</table>

*P<0.05
Plate (1a-c): heart longitudinal section (a) and heart cross section (b) of female Wistar rats tissue of control group (H&E, X1000). Showing a normal heart histology with Epicardium(E), normal myocardial cell(MC), nuclei Myocyte (N) and blood capillary(B). (c): Electron microscopy of heart sample from control group showed the normal myocardial cell with nuclei (N) mitochondria (M). Note also the presence of intercalated disk (ID) and myofibrils regularly within the fiber line to repeat a series of dark bands (myosin filament) in the light bands (actin filaments) Z bands (Z). (d-h) heart longitudinal sections (d) and heart cross section (e) of female Wistar rats treated with PPD (1.5ml) (H&E, X1000). Showing histopathology of heart. Note myocardial cell (MC) with nucleus globular (NG), vasodilatation (V), increase of interstitial spaces and desmosome. (f-h) Electron microscopy of heart sample from PPD treated group (1.5ml) showed the malformation of myocardial cell with nuclei (N). Note also the presence deformed of mitochondria (M), mylien figure (MF) marked desmosomes and gap junctions deformed (arrows).
Plate (2a-h): heart cross section (a) and heart longitudinal section (b-e) of female Wistar rats treated with PPD (3ml) (H&E, X1000). Showing histopathology of heart. Note myocardial cell (MC) with globular nucleus, and increase of interstitial spaces with vasodilatation (V). We note also presence of congestion (C) and lymphoid infiltration (LI). Some patchy necrosis was observed (NC). (f-h) Electron microscopy of heart sample from PPD(3ml) treated group. The electronic microscopy study of heart tissue showed the malformation of myocardial cell with nuclei, enlarged swollen mitochondria (M), and inflammatory cells nuclei (N) were observed. Note also the presence lysis of myocardial cell (L), damage mitochondria with lost cristae leaving empty space (head arrow), sarcoplasmic reticulum degeneration(SD), marked desmosomes and gap junctions deformed (arrows) and revealed altered Z bands (Z).
Histological analysis
The histological and ultrastructural examinations of the heart tissue from control rats appeared an intact and homogenous histoarchitecture. These results are consistent with the observations obtained from the light microscopy studies on the myocardium. Myocardial cells were long with slender central nuclei, lying within narrow fusiform cells (Figs. 1 and 2).

Electronic transmission microscopy analysis on myocytes of the control and PPD-treated animals also confirmed the results of the histological studies. Myocytes are bound by intercalated disks and junction complexes that contain fascia adherents, desmosomes, and gap junction to provide connection and communication. Cardiac tissue from control group demonstrated organized myofibrils with clear interstitial areas and myofibrils orderly within the fiber line to recur a sequence of dark bands (myosin filament) within the light bands (actin filaments) (Fig. 3).

In contrast, treatment of female rats with 1.5 ml of PPD resulted in extensive myofibril disorganization. Further, fibrotic damage was indicated by increasing the interstitial spaces (Fig. 5). Myocardial cells become slender with a globular nucleus. There were much fewer signs of cardiotoxic effects observed, such as vasodilatation, and some regions of cardiomyocyte necrosis were also visible (Fig. 4). Thus, electronic transmission microscopy analysis revealed the PPD-induced damage in intercalated disk and malformation of mitochondria (Figs. 6, 7, and 8).

In a group treated with 3 ml of PPD, cumulative histopathology was observed. PPD treatment stimulated cumulative cardiotoxicity and vacuolar degeneration of the myocytes, finally leading to necrosis (Figs. 11 and 13).

Cardiac muscle fibers showed focal degeneration with perivascular and interstitial fibrosis, pyknosis of nuclear, venous congestion, and fatty acid infiltration (Figs. 12 and 13). Electron microscopy of heart samples of the rat treated with PPD (3 ml) showed enlarged and swollen mitochondria, destruction of the intercalated disk and consequently damaged desmosome, thus revealing altered Z bands (Figs. 14, 15 and 16).

Discussion
In this study, PPD-treated animals had several clinical signs including, tissue morphological changes, swelling of the neck, dark urine color, dermatitis and high rate of mortality, which is consistent with a number of previous studies which indicated that the topically applied PPD reaches into the systemic circulation after absorption through the skin [6, 17]. Further, [11] reported that the mortality rate in animals treated with PPD is 21.1%, and 22% in cases of poisoning from hair dye, 10% -35 in female Wistar rats in PPD poisoning cases.

The present results indicate that the doses of PPD on topical exposure caused an increase in absolute and relative heart weights, which may be due to the congestion of blood and result in a gradual change of blood level during chronic diseases or toxic substance [18, 19].

Furthermore, the results indicate that the prolonged exposure of PPD induced the vacuolar degenerative changes into necrotic damage and tissue lysis, which is consistent with the previous report of [20], which suggested that the cellular degeneration might be attributed to the liberation of acid hydrolyzes released from the damaged lysosomes to facilitate the process of autolysis. However, in the current work, pyknosis and karyolysis of cell nuclei may indicate the loss of functional efficiency of the cells. Similar results have been demonstrated by [21]. It appears that the degenerative changes appeared earlier in the cytoplasm than in the nuclei. This result is further consistent with the findings of [22], who reported that the nuclear damage is a consequence of cytoplasmic damage.

Conversely, the physiological mechanisms sick can be a build-up free radicals [23], led to cardiac muscle coagulation and necrosis [10].
but a systemic inflammatory reaction specifically provides support to the toxic cells. Constitute diamine nephrotoxic high degree of oxidative metabolism PPD producer and blockage of the renal tubular result myoglobin cast, acute tubular necrosis [11], cardiac toxicity, inflammation of the liver, lower of blood pressure, coma and sudden death due to heart disease pressure are at the end of the toxic of the spectrum [10].

Previously [22] described the vacuolation of hepatocytes as ballooning degeneration and interpreted it as a kind of cellular defensive mechanism against injurious substances. [24] reported that these vacuoles are responsible for collecting the harmful elements and preventing them from interfering with the biological activities of these cells.

However, the present results are consistent with the report of [25] who indicated that the cell swollen may be due to inhibition of the production of energy as a result of the damaged mitochondrial crista and increased permeability of the cell plasma membrane [26]. And with continued cellular toxicity the smooth endoplasmic reticulum can be seen to prevent the adverse effects by an increase in detoxification enzymes, and proliferation in most of the cell, and dilatation of its cisternae with vesiculated [24]. Moreover, the formation of RER is the compensatory activity during the deterioration of the cellular protein when cells are exposed to various toxins [27].

Acute and chronic hepatitis is pathologically characterized by infiltration of lymphocytes into the liver [28]. Furthermore, [24] reported that the inflammation is accompanied by lethal damage to endothelial cells and the loss of tone leads to a marked expansion of the blood vessels and packing of the lumen with erythrocytes. On the contrary, the current work revealed necrosis of Kupffer and endothelial cells, and these findings are consistent with the findings of [29]. [30] showed that the PPD induces free radicals and subsequently results in lipid peroxidation [29]. Histopathological studies showed that high and low doses of PPD demonstrated a significant reduction in the histological score by reducing inflammation and restoring myocardial cell integrity, which supports the other findings of this study.

Conclusions
Heart tissue from the control group showed normal histology. In contrast, treatment of rats with 1.5 ml of PPD resulted in increased cytoplasmic vacuolization and myocardial cell loss with a globular nucleus. Additionally, increasing the dose of treatment with PPD to 3 ml showed fewer signs of cardiotoxic effects. Increased cytoplasmic vacuolization, myofibrillar loss, enlarged swollen mitochondria, patchy necrosis, and inflammatory cells were observed. The presence of congestion and lymphoid infiltration was also evident.

References