CODEN [USA]: IAJPBB **ISSN: 2349-7750**

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
http://doi.org/10.5281/zenodo.3243277

Available online at: http://www.iajps.com ***Research Article***

TOXICITY EFFECTS OF HAIR DYE APPLICATION ON RESPIRATORY SYSTEM IN EXPERIMENTAL ANIMALS

Dr. Salah Eldeen Dafalla[1], Dr. Shaik Rasheed Ahemad[2], Dr. Ehab Ibrahim Salih El-Amin[3]
[1]Assistant Professor Department of Human Anatomy. Ibn Sina National College for Medical studies., [2]Assistant Professor Department Clinical Pharmacy and Pharmacology, Ibn Sina National College for medical studies., [3]Assistant Professor of Human Anatomy, Buraydah Private Colleges, Faculty of Applied Medical Sciences, Kingdom of Saudi Arabia.

Article Received: April 2019 **Accepted:** May 2019 **Published:** June 2019

Abstract:
Hair dye poisoning has been emerging as one of the important causes of intentional self-harm in the developing world. Hair dyes contain Para-Phenylenediamine and a host of other chemicals that can cause Rhabdomyolysis, laryngeal edema. This study aims to determine the toxicity effects of hair dye application on respiratory system in rats.
In this study, Albino Westar Rats were obtained from the Faculty of Pharmacy, University of Khartoum – Sudan in October 2016. The rats were randomly divided into two batches on the basis of using commercial Para-Phenylenediamine as oral or subcutaneous administration respectively; each batch has four groups [control and three test groups] each comprising six rats. Batch-1 [group-2, 3, and 4 orally administered with 10, 20, and 30mg/kg body weight of PPD, respectively]; and Batch-2 [group-2, 3, and 4 subcutaneously administered with 10, 20, and 30mg/kg body weight of PPD, respectively]. The study was conducted in the period from July 2013 to February 2014.
The laboratory features in this study indicated tapering decrease in the arterial blood gasses parameters among the different route [subcutaneous and oral] particularly the partial pressure oxygen and the bicarbonate accompanied by significant decrease in the pH and hemoglobin. This results may indicated the outcome of the respiratory distress and the relevant complications such as dyspnea, tachypnea, and asphyxia that reached over 85% as a clinical sign between the different groups using different route of administration.
All experimental protocol was approved by the Ethical Approval Committee for Animal Experimental of National Research Institute, Ministry of Science and Technology in November 2016. Ethical clearance was obtained from the Faculty of Laboratory Sciences at Omdurman Islamic University-Sudan in 15 December 2016 prior to enrollment in the study.
Statistical analyses were performed using statistical package for social sciences [SPSS] version 20 and excel 2007 statistical program. The study highlighted the major toxicity of commercial PDD and its association with respiratory system.

Key words: *Hair dye, Paraphenylenediamine, Asphyxia, Respiratory distress.*

Corresponding author:
Dr. Salah Eldeen Dafalla,
Assistant Professor Department of Human Anatomy,
Ibn Sina National College for Medical studies.

QR code

Please cite this article in press Salah Eldeen Dafalla et al., ***Toxicity Effects of Hair Dye Application on Respiratory System in Experimental Animals.,*** *Indo Am. J. P. Sci, 2019; 06[06].*

INTRODUCTION:

Para-Phenylenediamine [PPD] is an aromatic amine compound; its chemical formula is C6H8N2 [1]. This derivative of aniline is a white solid, but samples can darken due to air oxidation. It is also an ingredient used in Sudan and other countries in combination with henna "lawasonia Alba" for tattooing to give black color in a short time in traditional and during local and social festival. It was found to be toxic and there are some reports from these countries showing its toxicity on different systems of the body. The consumers use this product because its price is 20-30 times less expensive than pharmaceutical hair dye preparations [2].

The first artificial dye was synthesized in the laboratory in 1856, and permanent hair colorants have been in commercial use for over 100 years [3]. There are many studies showed effects on respiratory, renal, muscular system, but no study determines the effects on all these systems together, and no study describes the correlation of PPD toxicity to body's biochemical alterations in liver [4]. Paraphenylenediamine [PPD] is used in Sudan and other countries in combination with henna "lawasonia Alba" for tattooing to give black color in a short time in traditional and during local and social festival. It was found to be toxic and there some reports from these countries showing its toxicity on different system of the body but it still used because, its price is 20-30 times less expensive than pharmaceutical hair dye preparations [5].

Many accidental cases of toxicity and mortality have been reported in Sudan, Egypt and other countries in cases of suicidal and homicidal due to oral ingestion or subcutaneous mistaken of hair dyes [6]. There was a continuous inflow of suicidal and homicidal cases in Sudanese hospitals while the causes of poisoning with PPD are much conflicting in the determination of clinical order of PPD Patients [7]. Systemic toxicity is manifested as dyspnea, hypothermia, tachypnea, pallor, profuse sweating and respiratory failure [8]. This study aimed to determine the respiratory abnormalities associated with major toxicity of commercial PDD in experimental animals.

MATERIALS AND METHODS:

This study was conducted at National Research Center-University of Khartoum. The commercial PPD was collected from local markets [Libya – Omdurman]. Albino Westar male rats at age of 11 weeks, weighting 140-160g were obtained from the Faculty of Pharmacy, University of Khartoum – Sudan. The animals were housed in cages provided with rice husk as bedding materials and kept under ambient temperature of 23±2℃. The animals were kept in the laboratory condition for 1 week to adapt the climate condition and for the commencement of treatment protocol. The rats were randomly divided into two batches on the basis of using commercial PDD as oral or subcutaneous administration respectively; each batch has four groups [control and three test groups] each comprising six rats. Batch-1 [group-2, 3, and 4 orally administered with 10, 20, and 30mg/kg body weight of PPD, respectively]; and Batch-2 [group-2, 3, and 4 subcutaneously administered with 10, 20, and 30mg/kg body weight of PPD, respectively]. The animals were killed after 3 - 6 days after the administration. The lethal dose of PPD for rates was determined as 80mg/kg body weight [9] and the lethal subcutaneous dose was determined as 37mg/kg body weight [10]. Hence, we tested the toxicity of various sub lethal doses through different routes. The study was conducted in the period from July 2013 to February 2014.

2 ml of blood samples were collected from eye blood vessels of each rat in heparinized containers for blood gasses analysis.

Determination of pH, PO_2, CO_2, HCO_3, and Hb were analyzed by a ABGs analyzer. The clinical manifestations for respiratory were observed and recorded. The features observed on rats were dyspnea, tachypnea, asphyxia, respiratory distress and asphyxia death.

Our protocol was permitted by the Ethical Approval Committee for Animal Experimental of National Research Institute, in Addition of that the Ethical clearance was achieved from the Faculty of Laboratory Medicine Science in Omdurman Islamic University-Sudan prior to enrollment in the study.

Statistical analyses were performed using statistical package for social sciences [SPSS] version 20 and excel 2007 statistical program. Continuous and categorical variables were analyzed using student's *t*-test and Chi-square test respectively. *P* value was considered significant if it was less than 0.05

RESULTS:

Blood gasses changes were shown in all rates administered orally or subcutaneously with the commercial hair dye, however, the blood gasses changes such as pH was noticed in group 3 and group 4 which dropped significantly. The respiratory difficulty prior to death which occurred at about four hours post oral ingestion of the commercial hair dye in group-4 especially when administered subcutaneously

in group 2, 3 and 4, due to decrease pO2 levels to [88.6%, 80.5%, and 73.7% respectively], while it is only noticed in group 4 when administered orally due to decrease pO2 level to 71.8%. The laboratory features in this study indicated gradual decrease in the arterial blood gasses parameters among the different routes [subcutaneous and oral] especially in the partial pressure oxygen and the bicarbonate accompanied by significant decrease in the pH and hemoglobin. [Table 1, and 2].

The symptoms of respiratory distress, dyspnea, tachypnea, asphyxia, and Asphyxial death were observed in all experimental groups for both administration routes. It ranged between the three experimental groups [group 1, 2, and 3]. The dyspnea was reported significantly among group 2, 3, and 4 in the two batches. These results showed that the respiratory distress and the relevant complications such as dyspnea, tachypnea, and asphyxia that reached over 50% as a clinical sign between the different groups using different route of administration. [Table 3, and 4]

Table [1]: Showing the mean differences of arterial blood gasses parameters between the study groups when received different subcutaneous doses [10- 20- 30 mg/kg b.w.] using commercial PPD.

Groups / Parameters	**Group 1** Control	**Group 2** 10 mg/kg	**Group 3** 20 mg/kg	**Group 4** 30 mg/kg
Ph	7.35±0.13	7.30±0.29	6.40±0.41*	6.43±0.37*
pO_2 [mmHg]	89.7±2.70	88.6±0.11*	80.5±1*	73.7±2*
HCO_3 [meq/l]	20±3.20	19.7±2.72	18.3±2.30*	16.5±1.81
pCO_2 [mmHg]	49.1±2.12	48.4±1.83	51.1±1.25	50.9±1.12*
Hb [g/dl]	12.85±0.67	10.38±0.73	9.59±0.68*	8.67±0.82*

*P<0.05 compared between control group and experimental group by ANOVA test

Table [2]: Showing the mean differences of arterial blood gasses parameters between the study groups when received different oral doses [10- 20- 30 mg/kg b.w.] using commercial PPD.

Groups / Parameters	**Group 1** Control	**Group 2** 10 mg/kg	**Group 3** 20 mg/kg	**Group 4** 30 mg/kg
pH	7.36±0.74	7.36±0.05	5.88±0.31*	5.39±0.15*
pO_2 [mmHg]	88.4±0.30	87.7±0.01	80.1±0.25	71.8±0.49*
HCO_3 [meq/l]	19±1.20	18.3±1.27	16.1±3.00*	17.5±0.21
pCO_2 [mmHg]	48.5±1.43	47.2±1.36*	53.4±1.80	57.3±1.71*
Hb [g/dl]	12.80±0.67	10.23±0.64	9.32±0.50*	8.60±0.82*

*P < 0.05 compared between control group and experimental group by ANOVA test

Table [3]: Showing the percentage of death due to respiratory manifestations between the study groups when received different subcutaneous doses [10- 20- 30 mg/kg b.w.] using commercial PPD.

Groups / Manifestations	**Group 1** Control	**Group 2** 10 mg/kg	**Group 3** 20 mg/kg	**Group 4** 30 mg/kg
Dyspnea	0	83.3%*	66.7%*	83.3%*
Tachypnea	0	66.7%*	66.7%*	83.3%*
Respiratory distress	0	66.7%*	83.3%*	83.3%*
Asphyxia	0	100%*	100%*	100%*
Asphyxial death	0	50%*	66.7%*	100%*

*P<0.05 compared between control group and experimental group by ANOVA test

Table [4]: Showing the percentage of death due to respiratory manifestations between the study groups when received different oral doses [10- 20- 30 mg/kg b.w.] using commercial PPD.

Groups / **Manifestations**	**Group 1** Control	**Group 2** 10 mg/kg	**Group 3** 20 mg/kg	**Group 4** 30 mg/kg
Dyspnea	0	66.7%*	66.7%*	83.3%*
Tachypnea	0	50%*	66.7%*	83.3%*
Asphyxia	0	50%*	66.7%*	83.3%*
Respiratory distress	0	100%*	100%*	100%*
Asphyxial death	0	50%*	50%*	100%*

*P<0.05 compared between control group and experimental group by ANOVA

accompanied by significant decrease in the pH and hemoglobin. This results may indicated the outcome of the respiratory distress and the relevant complications such as dyspnea, tachypnea, and asphyxia that reached over 50% as a clinical sign between the different groups using different route of administration. The effect of the hair dye on the respiratory dysfunction could be slightly high among the group administered orally as compared to the subcutaneous group, and this is most likely indicated the strong and hazardous effect of the administration of hair dye orally. The low pH in the blood shown in groups 3 and 4 is strong indication to acidosis that could be as a result of high dose of hair dye. In this study and in previous study [12], the direct toxic effect of the hair dye poisoning is the low hemoglobin levels among different groups due to hemoglobinuria that contribute to renal failure. Respiratory distress together with myocarditis and cardiac arrhythmias are found to be the major early challenges, which requires cautious monitoring to prevent early death and intubation and ventilation support is required for asphyxia [13], which was the major cause of deaths in our study. Renal and hepatic failure due to hair dye

DISCUSSION:

PPD is the main constituent in hair dye and is an organic derivative of paranitroaniline, when ingested in a dose-dependent manner, results in multisystem involvement and can cause Rhabdomyolysis and acute kidney injury [AKI], flaccid paralysis, severe gastro-intestinal manifestations, cardio toxicity and arrhythmias. In addition respiratory distress characterized by severe angio-edema of the upper airway manifested with a hard swollen protruding tongue, which is indicated as one of sever clinical manifestations and the main cause of death often requiring tracheostomy [11]. Human exposure to PPD mainly occurs through skin contact during a hair dyeing or tattooing process and due to accidental or deliberate oral ingestion. For this reason we applied the hair dye by using the different routes [orally and subcutaneously] in order to verify the toxicity effect of different exposure on renal efficacy.

The laboratory features in this study indicated tapering decrease in the arterial blood gasses parameters among the different route [subcutaneous and oral] particularly the partial pressure oxygen and the bicarbonate

6. Delmar R. F. In Jiro J. Kameko [1980]. Clinical Biochemistry of Domestic Animals 3th edition Academic press New York.
7. Mogens G. Simesen in Jiro J. Kaneko [1980]. Clinical Biochemistry of Domestic Animals, 3th ed. Academic press New York, 576-635.
8. Mizrahi I. J. and Emmelot P. [1962]. The effect of cysteine on the metabolic changes produced by two carcinogenic n-nitro sodia kylamine in rat liver. Cancer Res. 22: 339-351.
9. Hamed L. A. and linderman R. D. [1978]. Dysphasia and vertical nystagmus in magnesium deficiency. Am. Int. Med. 89: 228.
10. Rogar P.S. Toxic response of the blood. In Curtis D K., Many O. A. and John Doull M. D [Ed] [1986]. Casarett and Doulls Toxicology the Basic Science of poison, Macmillan publishing Co. 223-244.
11. Chaudhary SC, Sawlani KK, Singh K. Phenylenediamine poisoning. Nigerian Journal of Clinical Practice 2013; 16[2]: 258-259.
12. Singla S, Miglani S, Lal AK, Gupta P, Agarwal AK. Para- Phenylenediamine [PPD] poisoning. J Acad Clin Care Med 2005;6:236–8.
13. Sampathkumar K, Yesudas S. Hair dye poisoning and the developing world. J Emerg Trauma Shock 2009; 2:129-31.

administration has been described, but very view reports in the literature have mentioned the respiratory distress complications and associated parameters. Awareness about early interventions to secure and airway by tracheostomy and /or endotracheal intubation is a key management strategy to save patient who is otherwise likely to die.

REFERENCES:

1. Scientific Committee on Consumer Products [SCCP] Opinion on P-Phenylenediamine. Public Health and Risk Assessment; 9th plenary meeting; Brussels, Belgium. 10 October 2006.
2. European Commission Health and Consumer Protection Directorate-General. Opinion on p-Phenylenediamine. Scientific Committee on Consumer Products. SCCP/0989/06.
3. Crawford JM, Boyer JL. Clinicopathology conference Inflammation-induced cholestasis. Hematology 1998; 28: 253-260.
4. Kim YC, Matthews HB. Comparative metabolism and excretion of resorcinol in male and female in F344 rats. Fundamental Applied Toxicol 1987; 9[3]:409 - 414.
5. Hume H. D., Weinberg J. M. and Knauss T. C. [1982]. Clinical and pathological aspects of amino glycoside nephrotoxicity. Am. J. Kidney Dis. 11: 5-29.

Photos for experimental rats with Asphyxial death: