

MEDULLARY CONGESTION IN OVARY OF TOPICALLY APPLIED PARAPHENYLENE DIAMINE EFFECTED RAT

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ABSTRACT

Objective: To evaluate the effect of topically applied paraphenylene diamine (PPD) on medulla of ovary in Sprague dawley rat.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: The study was carried out in the Anatomy Department, Army Medical College, Rawalpindi; in collaboration with animal house, National Institute of Health, Islamabad. The study duration was one year, from Jan 2016 to Dec 2016.

Material and Methods: Forty adult Sprague Dawley female rats, weighing 200-300 grams, were used and divided into 4 groups with 10 rats in each group. Group A served as control group and animals were applied with distilled water on dorsal surface clipped free of hair. Group B, C and D were painted with 1mg, 2mg and 3mg per kg body weight of paraphenylene diamine in addition to distilled water on dorsal surface clipped free of hair. The solution was applied for 30 minutes daily for a continued duration of 60 days. All animals were sacrificed on day 60 and right ovary of each rat was removed, fixed in 10% formalin, processed and sectioned. For histological study haematoxylin and eosin (H&E) stains were used.

Results: On microscopic examination it was observed that topically applied paraphenylene diamine solution caused vascular congestion in the medulla of ovary.

Conclusion: It was concluded from results that topically applied paraphenylene diamine solution induces medullary congestion in ovary of rat.

Keywords: Congestion, Ovary, Paraphenylene diamine (PPD), Topical application, Vessels.

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INTRODUCTION

Paraphenylene diamine (PPD) exists in the form of white colored crystals when pure, and rapidly turns brown when exposed to air. It is used in products such as textile or fur dyes, dark colored cosmetics, temporary tattoos and painting inks use in industries. Paraphenylene diamine is a chemical compound used currently as an essential component of almost all hair dye formulations¹. The use of chemicals as hair dyes can be traced back to ancient days when mummies were dyed with henna. Human skin is permeable to chemicals when applied topically and can it lead to systemic effects².

Paraphenylenediamine is a common ingredient used in hair and leather dyes. PPD

poisonings have been reported in developing countries due to its widespread industrial application. PPD is an allergen as declared by the American Centers for Disease Control and Prevention that can induce renal, hepatic, and cardiac injury, angioneurotic edema, and delayed hypersensitivity. According to International Agency for Research on Cancer (IARC) there is an evidence that use of hair dye may cause carcinogen exposure leading to higher incidence of bladder cancer, non-Hodgkin's lymphoma, multiple myeloma, and hematopoietic cancers³. People receiving tattoos have a higher risk of PPD absorption depending upon the duration of exposure⁴. The significant impact on public health of widespread applications of PPD and the associated health hazards should be considered.

PPD is used to intensify color produced by henna (*lawsonia intermis*) and to decrease the time span for dying⁵. Black henna powder

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contains PPD which stains the skin black almost instantly but can cause severe allergic reactions¹. PPD is an essential component of all permanent hair dyes, the higher the concentration of PPD, the stronger will be the color with higher concentration of PPD⁶. PPD as primary intermediate diffuse rapidly into shaft of hair and undergoes oxidative chemical reaction to impart colors⁵.

PPD when applied topically is metabolized in skin, and its metabolites exerts its systemic effects⁶. PPD undergoes oxidation and give rise to short lived free radicals. Long term exposure causes production of hydrogen peroxide and superoxide radicals as metabolites. Free radical formation is mainly responsible for oxidative injury observed in animal studies⁷.

Exposure to PPD occurs through skin contact, accidental ingestion or inhalation of particles from hair dye formulation during dyeing. Acute exposure to PPD can cause severe dermatitis, asthma, and renal failure, edema of face, neck and larynx. Cases of rhabdomyolysis, acute tubular necrosis with acute renal failure and hepatic failure may occur due to PPD exposure⁸. Drop in hemoglobin noticed in rats that received sub lethal doses of PPD was seen as PPD causes anemia due to hemolytic effect on red blood cells⁹.

The potential effect of paraphenylene diamine on reproductive system should be investigated in view of extensive hair dyes used all over the globe. Apart from regular hair dyeing, human exposure to PPD may occur during tattooing or inhalation in case of industrial workers. The rationale of present study is to evaluate the effect of repeated dermal exposure of aqueous solution of paraphenylene diamine on ovary of rat.

MATERIAL AND METHODS

This study was randomized control trial carried out in department of Anatomy and Department of Pathology, Army Medical College, Rawalpindi in collaboration with National Institute of Health, from January 2016 to

December 2016. The study was conducted following ethical approval from authorities of Army Medical College. The experimental chemical paraphenylene diamine was purchased from Sigma Enrich.

Forty Sprague dawley rats weighing 200-300gms were housed in animal house of NIH Islamabad. All animals were selected by non probability convenient sampling and divided into four groups by lottery method. All animals were kept in well ventilated room following 12 hourly light and dark cycle and temperature 20 to 26 degree¹⁰. All animals were fed with standard lab diet of animal house for 2 months with water ad libitum. Rats were randomly divided into 4 groups each with ten rats. Each rat was kept in separate cage.

Before administration of the dose, the dorsal surface of all animals were clipped free of hair by using electrical clipper. An area of 5×6mm³ was exposed and selected for application of solution⁵. Group A served as control with ten rats. Each rat was applied on the dorsal surface with distilled water daily for 2 months. Groups B, C and D were applied on the dorsal surface with 1mg, 2mg and 3mg per kg body weight of PPD solution based on mode of preparation in previous studies¹¹.

Solution of PPD was prepared in distilled water daily and 0.1ml of this solution was applied on the exposed dorsal surface of experimental animals of group B, C and D. Control group was applied with 0.1ml of distilled water alone. PPD solution was applied with plastic syringe and spread with spatula over the dorsal surface. The solution was applied daily and was left on skin for 30 minutes and then washed afterwards⁵.

Animals of all four groups were dissected and sacrificed using chloroform anesthesia. Both ovaries were localized and examined. Right ovary of each rat was removed. The specimen was fixed in 10% formalin solution. After processing the tissue was embedded in paraffin wax. Cross sections of each specimen

5µm thick were obtained from tissue block by means of microtome. Haematoxylin and eosin staining was done and microscopic study was done at 10X and 40X magnification under light microscope.

SPSS version 21 was used to analyze the data. Qualitative variables were expressed in

magnification for absence and presence of congested blood vessels.

In control group A no section of the specimen showed vascular congestion in medulla of ovary. Sections of the ovaries of group B had medullary congestion absent in 50% of the specimen and present in rest of the 50% (table-I).

Table-I: Frequencies and percentages of vascular congestion in medulla between the control and experimental groups.

Parameters	Findings	Group A	Group B	Group C	Group D
Medullary congestion	Absent	10 (100%)	5 (50%)	4 (40%)	0 (0%)
	Present	0 (0%)	5 (50%)	6 (60%)	10 (100%)

Table-II: Comparison of p-values of vascular congestion in medulla of control group A and experimental groups B, C and D.

	Group A vs. B	Group A vs. C	Group A vs. D	Group B vs. C	Group B vs. D	Group C vs. D
Vascular congestion in medulla	0.03	0.01	<0.001*	1.0	0.03	0.087

frequency and percentage and compared by Pearson Chi-square test and Fisher’s exact test. A p-value of <0.05 was considered significant.

RESULTS

The slides of specimen of all groups were observed under microscope for vascular congestion in medulla of ovary. Blood vessels

Intergroup comparison between group A and B showed statistically significant difference with p-value of 0.03 (table-II).

In group C 40% of the specimen had no congestion of ovary and 60% of the specimen had congestion (table-I). Intergroup comparison of group C with group A was statistically significant (p=0.01) whereas comparison of group C with B

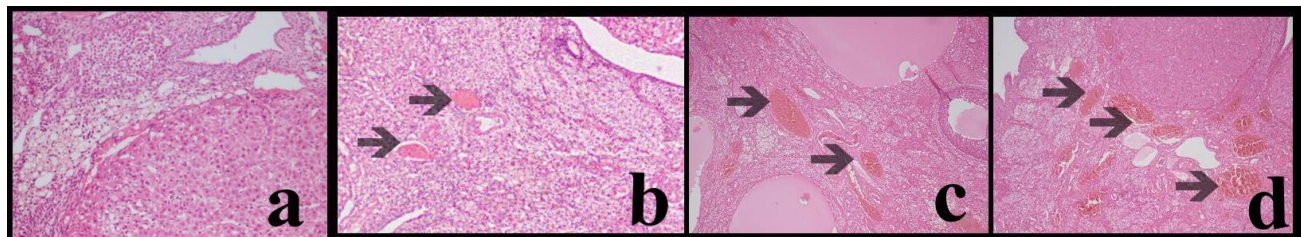


Figure-1: Photomicrograph showing medullary congestion of ovary(a), absent congestion in animal no.4 of group A (b) congestion seen in animal no. 7 of group B (c), congestion seen in animal no. 7 of group C (d), congestion seen in animal no. 5 of group D at 40X magnification, H&E.

within the central medullary area were observed. Congestion was noted as excess of blood contained in the blood vessels with thickening of vessel wall¹² (fig-1a, 1b & 1c). Vessels were considered filled when the entire vessel was filled with blood, absent congestion was taken as normal vessel while positive congestion when >10% of the vessels show congestion or filling¹³. The section of ovary was observed at 40X

showed no statistical significant difference (p=1.0) (table-II).

In group D 100% of the specimen had congestion in medulla of ovary. Intergroup comparison of group D with A and B was statistically significant with p-value of 0.000 and 0.03. Intergroup comparison of group C with D was statistically insignificant with p-value of 0.08

(table-II). The results were expressed in frequency chart (fig-II).

DISCUSSION

Substances used for the purpose of cosmetics have gained great recognition in the past decade as their effects are found to be local as well as systemic. PPD used in hair dyes accelerates coloring process¹⁴. Effects of PPD on organs like liver, spleen, pancreas, kidneys and testes have been documented however scarce data is available evaluating its effect on female reproductive system. The objective of this study was to evaluate the effects of topically applied

artery stiffness¹⁸. PPD is soluble in distilled water and topical application as hair dye compound penetrate skin and is detected in plasma, urine and feces¹⁹. Previously a study was conducted for the percutaneous penetration of [14C]-labelled PPD, and an attempt was made to measure plasma kinetics of [14C]-PPD-equivalents, radioactivity present in plasma represented metabolites of PPD and parent compound PPD was also present²⁰.

Congestion in group D was statistically significant when compared with other groups. Same observations were seen in ovary when

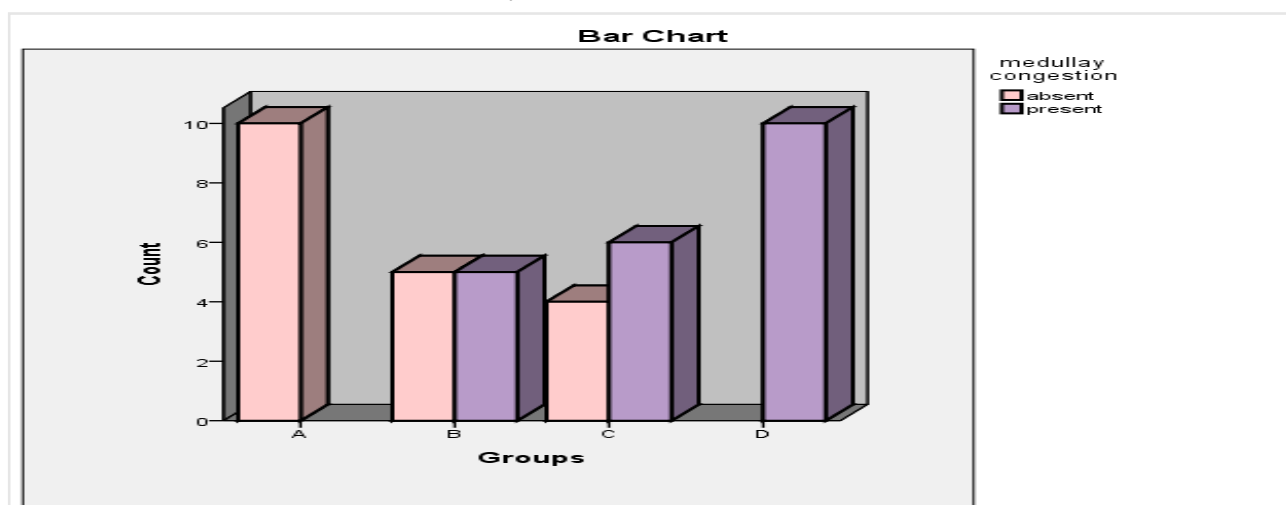


Figure-2: Cluster Bar chart showing comparison of frequency of vascular congestion in ovary with scoring between the control group A and experimental groups B, C and D.

PPD on rat ovary.

The present study was conducted at maximum dose of 3mg/kg/day, which is lower than no toxic adverse effect level (NOAEL) for oral dose which is 4 mg/kg/day, prescribed for this chemical (SCCNFP/0129/99)¹⁵. PPD causes oxidative stress¹⁶. Exposure to PPD results in peroxidative damage in the fatty acid pattern of membrane phospholipids. Depending on the concentration of PPD and duration of exposure, PPD with a significant decrease in membrane polyunsaturated fatty acids occurs. Oxidative stress is an essential element in the preimmunological period of contact sensitization¹⁷. Oxidative stress is responsible for impairment in vascular function leading to vascular dilatation and large

effect of monosodium glutamate for 2 months was conducted^{21,22}. Congestive changes in ovary were also seen in studies conducted on inhaled metals like mercury²³. Industrial chemicals like lead cause congestion of ovary by formation of free radicals and lipid per oxidation²⁴. Congestion indicates damage to ovary resulting from the oxidative stress caused by metabolites of PPD. Prostaglandins are responsible for regulation of blood flow. PPD antagonizes prostaglandin receptors¹⁵. Inhibition of prostaglandin synthesis may have a role in vascular congestion of ovary²⁵.

CONCLUSION

It was concluded from results that topically applied paraphenylene diamine solution induces medullary congestion in ovary of rat.

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CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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