Original Article

PARAPHENYLENE DIAMINE INDUCED HISTOMORPHOLOGICAL CHANGES IN RAT OVARY

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ABSTRACT

Objective: To evaluate histomorphological changes in ovary of *Sprague Dawley* rat induced by topically applied paraphenylene diamine (PPD).

Study Design: Laboratory based randomized control trial.

Place and Duration: The study was carried out in the Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad from November 2015 to November 2016.

Material and Methods: Thirty adult Sprague Dawley female rats, weighing 200-300grams, were used and divided into 3 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 and C were applied with 1mg and 3mg per kg body weight of paraphenylene diamine dissolved in distilled water on dorsal surface clipped free of hair. The solution was applied daily for 30 minutes per day for 60 days. All animals were sacrificed on 60th day of study project. Right ovary of each rat was removed, fixed in 10% formalin, processed, sectioned and stained with H&E. SPSS 21 was used for data analysis.

Results: It was observed on microscopic examination that topically applied paraphenylene diamine solution effects histology of rat ovary by formation of cystic follicles, appearance of interstitial vacuoles and atresia of large follicles.

Conclusion: It was concluded from results that topically applied paraphenylene diamine solution induces histomorphological changes in ovary of rat with 3mg/kg dose inducing statistically significant changes than 1mg/kg dose.

Keywords: Paraphenylene diamine (PPD), Ovary, Sprague Dawley rats, Topical application.

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INTRODUCTION

Paraphenylene-diamine (PPD) is available in the form of white crystals, and rapidly turns brown when exposed to air. It is used in industrial products such as textile or fur dyes, dark colored cosmetics, temporary tattoos and painting inks. Paraphenylene diamine is a chemical compound used now days as a constituent of almost all hair dye formulations [1]. The use of chemicals as hair dyes can be traced back to Egyptian times when mummies were dyed with henna. It is human nature to enhance ones appearance to create great on personality and build up confidence. Human skin is permeable to chemicals which are applied topically leading to systemic effects [2].

PPD diamine is used to intensify color produced by henna (lawsonia intermis) and to reduce time required for dyeing and decorating hands and feet with henna [3]. Black henna powder contains paraphenylene diamine which stains the skin black quickly but can cause severe allergic reactions. PPD is mainly used to impart a dark brown color to enhance the process of dyeing [1]. Paraphenylene diamine is main component of all permanent hair dyes, the darker the color higher is the concentration of paraphenylene diamine [4]. PPD is used as primary intermediate that diffuse rapidly into hair shaft where they undergo oxidative chemical reaction to impart colors [3].

Topically applied PPD is metabolized in skin, and its acetylated metabolites that are responsible for systemic effects [5]. PPD undergoes spontaneous oxidation to generate short lived free radicals. Long term exposure cause production of hydrogen peroxide and superoxide. Free radical formation is

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responsible of tissue damage observed in animal studies [4].

Exposure to PPD occurs through skin, accidental ingestion or inhalation of particles from hair dye formulation during dyeing. Acute exposure to high levels of paraphenylene diamine may cause severe dermatitis, asthma, renal failure, edema of face, neck and larynx. Cases of rhabdomyosis, acute tubular necrosis with acute renal failure and hepatic failure have been reported [6]. Decrease in hemoglobin leading to anemia due to hemolytic effect of paraphenylene diamine on red blood cells has been noticed in rats that received sub lethal doses of paraphenylene diamine [7].

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

MATERIALS AND METHODS

This study of 1 year duration (from November 2015 to November 2016) was a randomized control trial carried out in Department of Anatomy, Army Medical College, Rawalpindi in collaboration with National Institute of Health Islamabad and Army Medical College (Pathology Department). The study was conducted following ethical consideration of authorities of Army Medical College. The experimental chemical paraphenylene diamine was purchased from Sigma Enrich.

Thirty Sprague Dawley female rats weighing 200-300gms housed in a animal house of NIH Islamabad were used. All animals were kept in ventilated room following 12 hourly cycle of light and dark and temperature range of 20 to 26 degree[8]. They were fed with standard lab diet of animal house for 2 months with water *ad-libitum*. Rats were randomly divided into 3 groups each with ten rats. Each rat was kept in separate cage

2 days before dosing, the dorsal surface of all animals were clipped free of hair by using electrical clipper. An area of 5×6mm^3 was exposed for application of solution [3]. Group A served as control with ten rats. Each rat was applied on the dorsal surface with distilled water daily for 2 months. Group B and C were applied on the dorsal surface with 1mg and 3mg per kg body weight of PPD solution based on previous studies [9].

Solution of PPD was prepared every day in distilled water. 0.1ml of this solution was applied on the exposed dorsal surface of experimental animals of group Band C. Control group was applied with 0.1ml of distilled water alone. The solution was applied with plastic syringe and spread with spatula over the dorsal surface. The solution was left on skin for 30 minutes and then washed with tap water [3].

All animals were dissected and sacrificed using chloroform anesthesia. Ovaries were localized and 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 analysis was done by light microscope. study was done at 10X and 40X objective magnification under light microscope.

SPSS version 21 was used to analyze the data. Quantitative data was expressed as mean ± SD and using 1 way ANOVA the means were compared. Qualitative variables were expressed in frequency and percentage and compared by Pearson Chi-square test. *P*-value of < 0.05 was considered statistically significant.

RESULTS

The slides of specimen of all groups were observed under microscope for paraphenylene diamine induced histomorphological changes in ovary. The cystic follicles were counted at 10X objective. At 40X vacuolation in the interstitium was observed as presence or absence of cells with clear or pale staining cytoplasm. Atresia of large follicles was marked as absent (< 50% large follicles were atretic), slight (nearly 50% of large follicles are atretic), moderate (> 50% of large follicles are atretic) and severe (all large follicles are atretic) [10].

The slides of specimen in control group A showed no cystic follicles, vacuolation or atresia of large follicles. The slides of specimen in group B showed mean ± SD of cystic follicles 0.6 ± 0.5. Specimen of 1 out of 10 rats showed vacuolation (10%) while specimen of 5 rats (50%) showed absence of atresia and specimen of 5 rats (50%) showed slight atresia of large follicles. The slides of specimen in group C showed mean ± SD of cystic follicles 3.6 ± 1.4. Specimen of 8 out of 10 rats showed vacuolation of interstitium (80%) while no atresia in 1 specimen, slight atresia in 3 (30%) and moderate atresia in 4 (40%) specimen of rats.

Statistically significant difference was observed on comparing group A with B for atresia of large follicles (p =0.03) and no significant difference regarding cystic follicular count and interstitial vacuolation (p >0.05). Group A when compared with C showed great statistical significance for all parameters (p=0.000). Group B and C when compared showed statistically significant difference (p< 0.05).

Table-1: Frequency and percentagesof interstitial vacuolation and atresia of large follicles among the control and experimental groups.

Parameters	Findings	Group A	Group B	Group C
Atresia of large follicles	Absent	10 (100%)	5 (50.0%)	1 (10%)
	Slight	0 (0%)	5 (50%)	4 (40%)
	Moderate	0 (0%)	0 (0%)	5 (50%)
	Marked	0 (0%)	0 (0%)	0 (0%)
Interstitial vacuolation	Absent	10 (100%)	9 (90%)	0 (0.0%)
	Present	0 (0.0%)	1(10%)	10(100%)

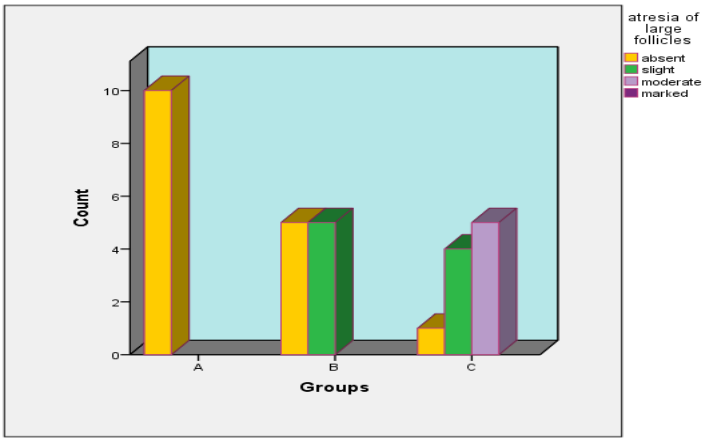

Figure-1: Cluster bar chart showing comparison of frequency of atresia of large follicles among the control group A and experimental groups B and C.

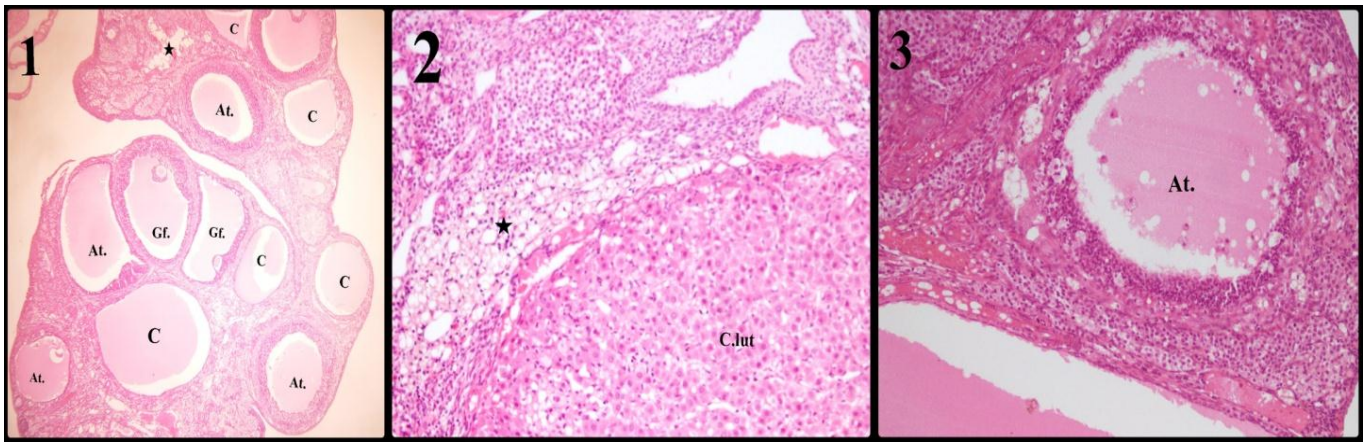

Figure-2: Simple bar chart showing comparison of mean value of alveolar septal thickness among the control group A and experimental groups B, C and D.

DISCUSSION

Substances used as cosmetics have gained great interest in the past decays as their effects are found to be local as well as systemic. PPD used in hair dyes accelerates coloring process [11]. Effects of paraphenylene diamine on organs like liver, spleen, pancreas, kidneys and testes have been documented however less data is available regarding its effect on female reproductive system. The objective of this study was to evaluate the effects of topically applied paraphenylene diamine on rat ovary.

PPD causes oxidative stress [12]. Paraphenylene diamine is soluble in distilled water and topical application as hair dye compound penetrate skin and is detected in plasma, urine and faeces [13]. In this study cystic changes are seen in experimental groups B and C, with average of 3.6 cysts in group C which was more than in group B. Oxidative stress induces cystic changes in ovary [14]. Subjects exposed to oxidative stress in experimental trials develop polycystic changes in ovary [15].

In this study interstitium was observed at 40X magnification for vacuolated cells. Cells in the form of groups were seen in experimental groups B and C, more in later group with increase in dose of paraphenylene diamine solution. The presence of vacuolation represents hyper cellular stroma with luteinizing changes [16]. Vacuolar changes also correlate with lipid accumulation in ovarian interstitium as seen in other studies [17].

This study also revealed that topically applied paraphenylene diamine solution causes atresia of large follicles in ovary. All animals in control group showed no atresia while group B and C showed atretic large follicles with maximum atretic large follicles in group C. Such changes correlate with previous studies on pesticides induced oxidative stress [18]. Atresia is normally present but is increased as a result of oxidative stress as seen in previous study [19].

CONCLUSION

It was observed in present study that topically applied paraphenylene diamine solution induces cystic and atretic follicular changes and interstitial vacuolation in rat ovary.

Moreover, it is concluded that 3mg/ kg of paraphenylene diamine has statistically significant effect than 1mg/kg. Further investigation is required to explore the mechanism of action of paraphenylene diamine

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AUTHORS CONTRIBUTION

Sana Malik: Conceived the idea, analysed the data and drafted the manuscript.

Khadija Qamar: Critically viewed the findings and analysis and also revised the manuscript.

Sabah Rehman: Gave her input in data interpretation and improving the script.

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