

## Toxicological Effects of Paraphenylene-diamine On Skin Of Albino Rats- An In-vivo Study

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### Abstract

**Materials and methods:** This study was undertaken on 24 albino rats, in the department of anatomy in Govt. Medical College Jammu. These rats were divided into four groups of 6 rats each. Group A, Group B, Group C rats were painted with 1mg/kg body wt, 2mg/kg body wt and 3 mg/kg body wt of paraphenylene-diamine respectively while Group D rats were painted with distilled water after shaving their backs.

**Results:** There were gross and histological changes in the liver and skin of the albino rats by the repeated topical application of paraphenylene-diamine on the albino rats.

**Conclusion:** Repeated topical application of paraphenylene-diamine results in systemic absorption of the dye and consequent damage of the internal organs.

**Key words:** paraphenylene-diamine, skin, Acanthosis, Topical

### Introduction

The art of hair dyeing was known as early as 5000 years BC among the Egyptians. Historically, hair has functioned as a social indicator of attractiveness, femininity, masculinity, health and beauty (AL-Shaikh TM et al., 2018).

Paraphenylene-diamine was first described by Hofmann in 1863 (Mukkana KS et al., 2017). Paraphenylene-diamine (PPD) [C<sub>6</sub>H<sub>4</sub>(NH<sub>2</sub>)<sub>2</sub>] is an aromatic amine not found in nature and it is commercially produced by many industrial companies (Hummdi L 2012).

Paraphenylene-diamine (PPD or 1, 4 diamino benzene, CAS: 106: 50:3) is a widely used chemical in almost all hair dye formulation. The major aim of using phosphoric acid as a hair dyeing material is fastening the dyeing process as compared to traditional henna (Bharali MK and Dutta K 2009).

Paraphenylene diamine, regardless of brand, is used in almost every hair dye on the market. The darker the colour, the more concentrated (Devi M 2016).

The introduction of "black henna", which contains the synthetic aniline derivative paraphenylene-diamine has created a significant public health issue due to the risk of hypersensitivity reactions. No natural black henna exists (Cubitt J et al., 2015).

Paraphenylene-diamine, an important constituent of hair dye toxicity of which one could herald fatal complications such as rhabdomyolysis, renal failure, angioneurotic edema, respiratory failure and atrophy (Gude D et al., 2012).

Paraphenylene-diamine is a highly sensitizing oxidizer, and its use on the skin can result in severe allergic contact dermatitis (ACD), post inflammatory pigment alteration, scarring and life-long sensitization ( Goldenberg A and Jacob SE 2014).

Paraphenylene-diamine is an important contact allergen and primarily used in hair dyeing (Schnuch A et al., 2008).

Amusingly, erratic concentrations of paraphenylene-diamine are used in different hair dye formulations from poles apart countries depending on the home maker advice (Abdelraheem M et al., 2010).

The European Union maximum allowable paraphenylene-diamine concentration in hair care

products is 6%, and the use of paraphenylenediamine and its derivatives for dyeing eyelashes, eyebrows, or the skin is prohibited (Ronald RB et al.,2002).

Paraphenylene-diamine was voted Allergen of the year in 2006 by the American Contact Dermatitis Society (Ashraf W et al 1994).

The Dermal exposure study of environmentally-friendly chemicals is a new toxicology research initiative. This is especially relevant for chemicals which enter the human system through the skin.(Mathur AK et al.,2005).

Exposure routes are through skin absorption, inhalation, ingestion, skin and eye This study was undertaken on 24 albino rats, weighing between 60-120 gm in the contact (Tokumoto Y et al., 2003). Paraphenylene-diamine is known to be a potent sensitizer and to cause allergic contact dermatitis. The higher paraphenylene-diamine concentration, combined with the extended period of skin exposure without neutralization, results in potent skin sensitization to paraphenylene-diamine.

Transcutaneous absorption of paraphenylene-diamine is rapid and may lead to systemic side effects including angioedema, gastrointestinal disturbances, tremors, convulsions, acute renal failure, liver atrophy ( Dressler WE and Appelqvist T 2006). Histopathological lesions have been widely used as biomarkers for health evaluation of organisms exposed to chemicals and can be used as warning symptoms for organism health. A thorough scan of existing literature has shown that there is a lack of literature concerning the gross and histological effects of paraphenylene-diamine on the skin. This is why a curious desire developed that the effects of paraphenylene diamine on mammalian skin, the albino rat, be studied based on evidence.

### Materials and Methods

department of anatomy in Govt. Medical College Jammu after obtaining clearance from Institutional Animal Ethics Committee. These rats were divided into four groups of 6 rats each. Group A, Group B, Group C rats were painted with 1mg/kg body wt, 2mg/kg body wt and 3 mg/kg body wt of paraphenylene-diamine respectively while Group D rats were painted with distilled water after shaving their backs. The group A, group B and group C rats were painted for continuous thirty days with paraphenylene-diamine while group D rats were painted with distilled water for continuous thirty days. Albino rats of all groups were sacrificed after thirty days by anesthetizing/euthanasizing them in an inverted jar containing large piece of cotton soaked in halothane as recommended by laboratory animals information service centre. The albino rats were dissected, viscera and skin specimens were sectioned into small pieces and these tissue sections were processed, stained with haematoxylin and eosin and studied under the light microscope.

**The Study conducted is summarized in the following table:**

Group No.	Chemical	Dose	Date of administration	Period and route of administration
A	Paraphenylene-diamine	1mg/kg BW	12-03-2019 to 10-04-2019	30 days, topical application
B	Paraphenylene-diamine	2mg/kgBW	11-04-2019 to 10-05-2019	30 days, topical application
C	Paraphenylene-diamine	3mg/kg BW	11-05-2019 to 09-06-2019	30 days, topical application
D	Distiled water	0.5 ml/day	10-06 -2019 to 09-07-2019	30 days, topical application

### Results:

#### Macroscopic observations of skin:

**Group A (Experimental group):** Grossly the skin of the group A animals was greyish brown in colour and rough in consistency.

**Group B (Experimental group):** Grossly the skin of the group B animals was greyish black in color, rough in consistency and presented crusts.

**Group C (Experimental group):** Grossly the skin of group C animals was blackish brown in color, rough in consistency and presented crusts.

**Group D (Healthy Control group):** Grossly the skin of the control group D animals was silvery white in colour and smooth in consistency.

### **Light Microscopic Examination of Skin**

#### **Group A (Experimental group):**

On histological examination of skin of group A rats reflected the following changes:

The epidermis exhibited acanthosis with mild inflammatory infiltrate. The dermis showed mild edema with mild inflammatory infiltrate. (**Fig. 1**).

#### **Group B (Experimental group):**

The histological sections of group B rats showed epidermal acanthosis with moderate inflammatory infiltrate (**Fig. 2 A**). The dermis exhibited intercellular edema with moderate inflammatory infiltrate. Epidermal cysts were seen (**Fig.2 B**).

#### **Group C (Experimental group):**

The histological sections of group C rats showed epidermal acanthosis There was also hyperkeratosis in the epidermis (**Fig.3 A**). Epidermis also showed vacuolization of cells (**Fig.3 B**). The dermis showed marked edema with moderate dermal infiltrate. Hair follicles (**Fig.3 A**) and sebaceous glands were also surrounded by inflammatory infiltrate.

#### **Group D (Healthy control):**

Light microscopic examination of haematoxylin and eosin stained skin sections of group D (Healthy control) animals revealed the normal basic structure of skin. The epidermis was 2-3 layered thick. Basal layer cells were cubical and upper layer cells were oval in shape. Stratum spinosum was typically one layer thick. Cells of all the layers were closely packed.

The dermis showed dense irregular arrangement of collagen fibres along with presence of fat cells.

### **Discussion:**

The present study showed inflammatory reaction in the form of dermal infiltrate with polymorphonuclear leucocytes. The inflammatory infiltrate was also seen in subcutaneous fat in the paraphenylene-diamine treated animals. There was also crusting and abscess formation. (Zeligman I 1957).

Edema was present in epidermis as well as dermis (Tainter ML and Hall EMI 1925).

There was hyperkeratosis, vacuolization of epidermal cells, swelling of collagen fibres (dermal edema) in paraphenylene-diamine treated animals in this study (Mathur AK et al.,2005).

Spongiosis, exocytosis and acanthosis was seen in the skin of paraphenylenediamine treated animals (Belle ABV et al.,2019).

An tremendously bulky quantity of edema was detected in the surface of paraphenylene-diamine treated animals (Yokozeki H et al.,2013).

Acanthosis and sensitization can result from oxidation products formed with precursors of collagen or keratin in the epidermis themselves and fixed in the basal cells. They act as powerful cell stimulants here. The complex collagen-forming forms antibodies of the delayed type of sensitization (Zeligman I 1957).

Acanthosis may also be produced due to increased proliferation of basal cells in the epidermis after treatment with paraphenylene-diamine. Ifparaphenylene diamine is brought into the skin, the basal layers of the epithel contain clumps of pigment. These clumps are transported far faster than normal pigment to the stratum corneum. At the same time many epithelial atypical cells

that proliferate in every direction are formed.(Mathur R L 1949).

Paraphenylene-diamine is forbidden for use on skin because it is a dangerous chemical, it is permitted for use as a hair dye only.

### Conclusions:

Paraphenylene-diamine is a toxic and hazardous material which is commonly available in each house in the developing countries. There should be a health hazard warning on the packaging of hair dyes containing paraphenylene-diamine. Further studies of chronic paraphenylene-diamine toxicities are advised to be accomplished as well as investigating protective drugs ameliorating such toxicities.

In the present study it was observed that skin tissue exhibited the epidermal acanthosis, epidermal cysts, epidermal hyperkeratosis, vacuolization of epidermal cells, dermal edema with inflammation in all the three experimental groups.

So it is highly advisable that in dermal diseases of idiopathic etiology, the history of hair dye use should be investigated.

**Conflicts of Interest:** None

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