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EVALUATION OF ANTIGENOTOXIC POTENTIAL OF BEET ROOT EXTRACT AGAINST HAIR DYE INDUCED GENOTOXICITY

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Keywords:

p-phenylenediamine (PPD), Hair dye, Genotoxicity, Beet root extract (BRE), Peripheral blood lymphocytes (PBLs)

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ABSTRACT: *In-vitro* investigations toward DNA damage reported that the biological effect of genotoxic exposures could influence the life cycles of different human cells. Sister chromatid exchange (SCE) assay is broadly used, sensitive cytogenetic biomarker of exposure to genotoxic agents. Naturally available products may play a significant role against the cytogenetic damage but available research data is not consistent. The purpose of this study was to evaluate the anti-genotoxic potential of beet root extract (BRE) against hair dye induced genotoxicity in cultured human peripheral blood lymphocytes (PBLs) using SCE assay. For this, PBLs were treated with different doses of hair dye ingredients along with BRE. Significant ($p<0.05$) rise in SCE frequency indicates genotoxicity of hair dye ingredients (p-Phenylenediamine-200 µg/ml, resorcinol-200 µg/ml, and hydrogen peroxide-6.168 µg/ml). Treatment of BRE along with hair dye showed significant ($p<0.05$) reduction in SCE frequency in a dose-dependent manner as compared to hair dye treated samples.

INTRODUCTION: P-phenylenediamine (PPD) is an essential ingredient of oxidative hair dyes. PPD and its metabolites are allergenic, mutagenic, and highly toxic. Some body organs like kidneys and bladder are particularly vulnerable to toxic effects of PPD [1]. Various epidemiological investigations have indicated increase incidences of malignancy like bladder cancer due to the dermal application of PPD containing oxidative hair dyes [2, 3]. Many researches also show carcinogenic, mutagenic, and genotoxic effects of PPD using different assay systems [4, 5].

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Another study in human uroepithelial cells indicates the genotoxic potential of PPD due to induced mutation in p53 and upregulation of COX-2 gene [6]. Beet root extract is a good source of bioactive components like flavonoids, polyphenols, betalains, and others, which decrease oxidative stress and improve antioxidant status in humans [7]. They have significant anti-mutagenic or anti-genotoxic effects against Methylnitro-nitrosoguanidine (MNNG) induced mutations [8].

Sister chromatid exchange (SCE) involve breakage and exchange of genetic material between two sister chromatid at the homologous position during metaphase. These can be induced by different types of DNA-damaging agents. SCE has been accepted as a good biomarker for DNA damage [9]. In present study, potential protective effects of beet root extract against hair dye induced genotoxicity have

been investigated in cultured human peripheral blood lymphocytes.

MATERIALS AND METHODS:

Preparation of Solution: P-Phenylenediamine (Sigma-Aldrich, CAS: 106-50-3) and resorcinol (Sigma-Aldrich, CAS: 108-46-3) and 3% H_2O_2 were prepared in autoclaved double-distilled water. Beet root extract (BRE) (Sigma-Aldrich, CDS000584) was also prepared in double-distilled water. Sterilization of all stock solutions (1mg/ml) was done through 0.22-micron syringe filters. Before storage at -20 °C, all stock solutions were appropriately covered to protect from light. Hair dye was prepared by using an equal volume ratio of PPD, resorcinol and 3% H_2O_2.

Blood Sampling: For lymphocytes culture, about 5 ml of venous blood was collected in heparin (sodium) coated sterile vacutainers from healthy individuals. Consent was taken from each individual along with a questionnaire regarding their health history. Ethical approval for this study has been taken from the Institutional Human Ethical Committee of Kurukshetra University (IHEC/17/422).

Culture Setup: The cultures of human peripheral blood lymphocytes (PBLs) were set by slightly modifying the method of Moorhead *et al.* [10] Briefly, 500 µl of peripheral blood was added in 5 ml of RPMI-1640 medium containing L-glutamine (1%), fetal bovine serum (20%), penicillin (100 U/ml), streptomycin (100 µg/ml) and phytohemagglutinin (2%) (HiMedia). Different concentrations of hair dye ingredients PPD + RE + H_2O_2 (33.33 µg/ml + 33.33 µg/ml + 1.028 µg/ml - 200 µg/ml + 200 µg/ml + 6.17 µg/ml) were added to check their genotoxicity by SCE assay. The anti-genotoxic potential of beet root extract was assessed by pre-treating the above culture containing different ingredients of hair dye with different doses of beet root extract (2-12 µg/ml). All cultures were incubated in a CO_2 incubator (5%) at 37 °C. Beet root extract was also checked for its possible genotoxic effect if any. Ethyl methanesulfonate was used as the positive control.

Sister Chromatid Exchange (SCE) Assay: For SCE analysis, the culture of PBLs was set up as stated above for 72 h at 37 °C using the methodology of Perry & Wolff [11]. After 24 h of incubation, 10 µg/ml of 5-bromo-2-deoxyuridine (Sigma-Aldrich) was added in culture and again transferred to the incubator for another 48 h at the same conditions. Two-three drops of colchicines were added in a final concentration of 0.2 µg/ml about 45 min before harvesting the cells. After centrifugation harvesting, hypotonic solution (0.075 M KCl) treatment was given and then fixed in methanol: acetic acid (3:1). Using these fixed cell suspension slides were prepared by putting cells form a height followed by air drying. Subsequently, slides were stained with Hoechst 33258 and 4% Giemsa stain. SCE frequency per cell was calculated by analyzing fifty metaphases.

Statistical Analysis: SPSS software was used for carrying out statistical analysis. The values correspond to $P \leq 0.05$ were considered to be statistically significant. All experiments were performed in duplicates. ANOVA (one way) was used for multiple samples, and Student's t-test was used for comparing paired sample tests, and final outcomes were described as Mean ± SD.

RESULTS: In this current study, the antigenotoxic effects of beet root extract against hair dye induced genotoxicity were studied using sister chromatid exchange (SCE), a classic cytomolecular method which has been considered as an easy and accurate method to monitor genetic damage in DNA.

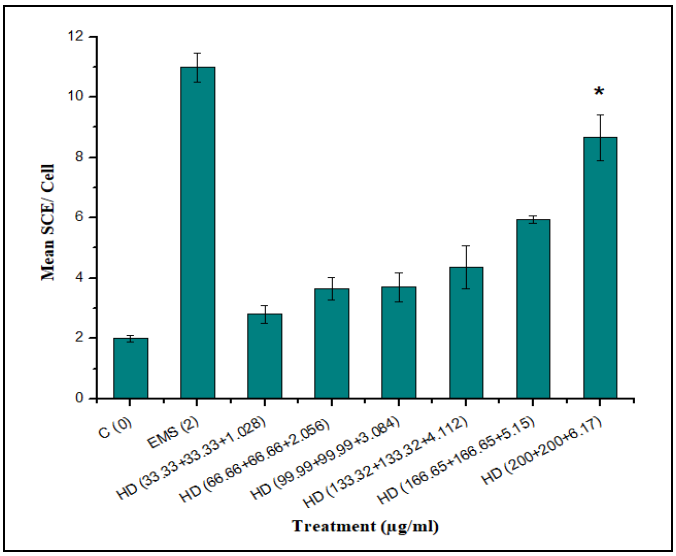

FIG. 1: EFFECT OF DIFFERENT TREATMENTS OF HAIR DYE ON DNA DAMAGE IN PBLS AS EVALUATED BY USING SISTER CHROMATID EXCHANGE (SCE) ASSAY. *p<0.05 (significant as compared to untreated sample). c-control; ems-ethyl methanesulfonate; hd- hair dye (PPD + RE + H_2O_2)

A significant increase in the SCE frequency was observed in dose-dependent manner when cultures were treated with different concentration of hair dye ranging from PPD (33.33 µg/ml) + RE (33.33 µg/ml) + H_2O_2 (1.028 µg/ml) to PPD (200 µg/ml) + RE (200 µg/ml) + H_2O_2 (6.17 µg/ml). Significant higher SCE frequency (8.66 ± 0.76) was observed at a concentration of PPD (200 µg/ml) + RE (200 µg/ml) + H_2O_2 (6.17 µg/ml) treated culture as compared to control (2 ± 0.11) indicating increased genotoxicity in the presence of hair dye **Fig. 1** and **3**. Beet root extract showed anti-genotoxic potential against hair dye induced genotoxicity in a dose-dependent manner as evident by a decrease in SCE frequency. The maximum decrease in SCE frequency (P≤0.05) (3.676 ± 0.37) was observed at a concentration of 12 µg/ml of beet root extract **Fig. 2** and **3**. A separate culture along with beet root extract only (12 µg/ml) showed to genotoxic effects in PBLs as evident by no significant difference in SCE frequency (2.332 ± 0.27) as compared to control sample.

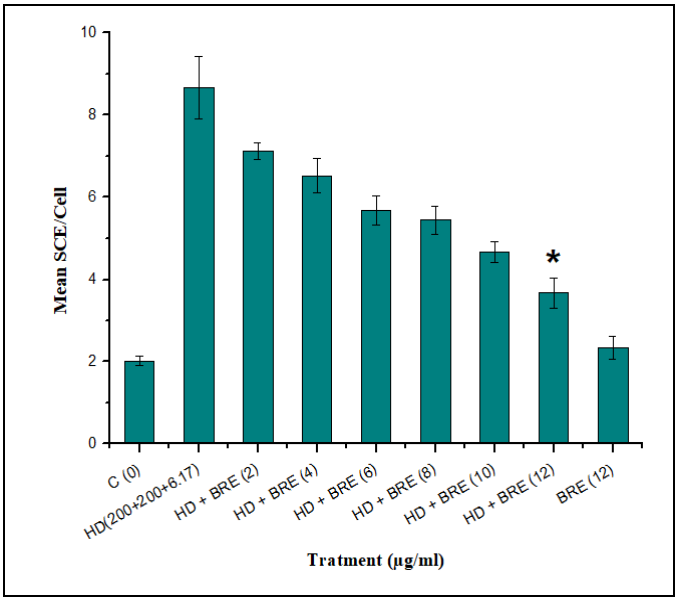

FIG. 2: EFFECT OF DIFFERENT TREATMENTS OF BRE ON DNA DAMAGE IN PBLS WAS EVALUATED BY USING SISTER CHROMATID EXCHANGE (SCE) ASSAY. *p<0.05 (significant as compared to hair dye alone treated sample). c-control; hd- hair dye (PPD + RE + H_2O_2); bre-beet root extract

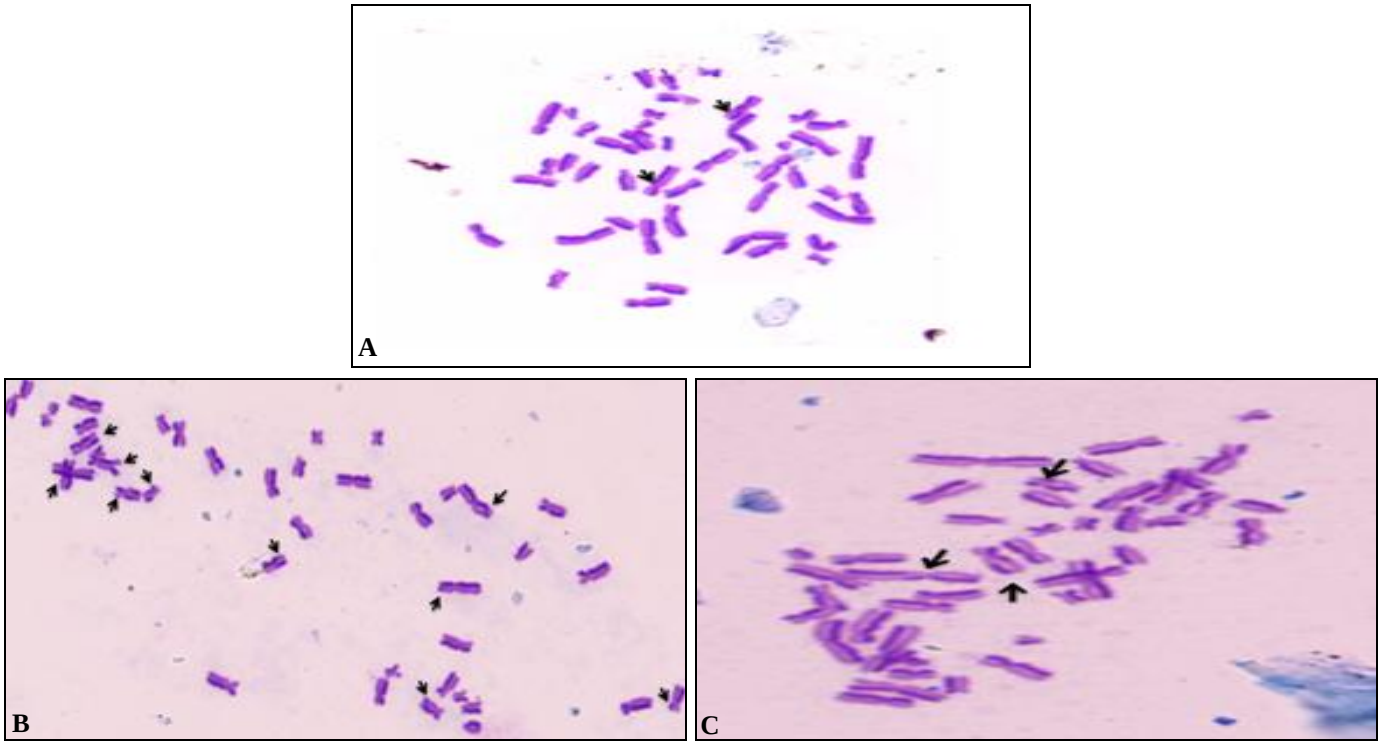

FIG. 3: SISTER CHROMATID EXCHANGE (SCES) IN PBLS (A) CONTROL; (B) HAIR DYE TREATED (C) HAIR DYE ALONG WITH BRE TREATED (ARROW SHOWS SISTER CHROMATID EXCHANGES)

DISCUSSION: In our study, it has been observed that an increase in BRE concentration leads to a significant decrease in SCE frequency. Due to some novel findings, beet root extract catches the attention of researchers towards its health benefits. Beet root extract exhibit powerful free radical scavenging property and act as an antioxidant that helps in oxidative stress-related disease, including cancer [12, 13]. In this regard, the anti-tumour activity of beet root extract was demonstrated in laboratory

animal [14, 15]. Short and prolonged treatment of beet root extract using a mouse model has shown a delay in the NDEA-induced tumor [16]. This delay in tumor onset could involve the protection against DNA damage caused by NDEA-derived electrophiles or ROS.

Beet roots are a good source of phytonutrients called betalains. Two best-studied betalains are betanin and vulgaxanthin, which exhibit free radical scavenging antioxidant activity, anti-inflammatory, and detoxification potential [17]. These natural compounds present in the beet root extract may interact directly with the genotoxicant and inhibit them chemically. Possibly, these compounds protect DNA from damage by altering the binding of mutagen by competitively interacting with the nucleophilic sites in DNA. But, mutagenesis inhibition can act through different complex molecular mechanisms [18].

Esatbeyoglu *et al.,* found that beet root extract reduced DNA damage in cultured human liver cells (hepatoma) induced by H_2O_2 [19]. It also considerably inactivated pathway for the production of ROS in cultured human neutrophils, and reduced DNA damage [20, 21, 22].

Chyau *et al.,* evaluated the ability of betanin (a component of beet root extract) to cause any cytotoxicity effect in cell survival and found no cytotoxicity in the range from 10-100 µM [23].

Lee *et al.,* reported that pre-treatment of beet root extract significantly decreased MDA level and increased GSH and SOD level against ethanol mediated toxicity in rats [24]. Kapadia *et al.,* studied the effect of beet root extract on multiorgan tumors in experimental animals and found it significantly effective in suppression of tumors [12].

CONCLUSION: Our results concluded that PPD, a component of oxidative hair dyes, can cause genetic damage in peripheral blood lymphocytes. Beet root extract may be used as natural dietary supplement against genotoxicity of hair dye.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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