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HEMATOLOGICAL ALTERATIONS, OXIDATIVE STRESS, AND APOPTOSIS ASSOCIATED WITH HAIR DYE USE IN JORDANIAN WOMEN

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ABSTRACT

Many people both male and female use hair dye to change their hair colour, but theses dyes induce several deteriorating effects like DNA damage, apoptosis and cancers. This study investigated the association of hair dye use and hematological alterations, oxidative damage, and cell death by measuring blood indices and genes expression involved in DNA damage and repair, and apoptotic pathways (XRCC1, p53, cytochrome C, and caspase-9) in women. A total of 50 women, comprising of 25 regular hair dye users and 25 age-matched controls, were enrolled in this case-control study and venous blood samples were collected to evaluate hematological indices and oxidative stress biomarkers (TAC and MDA). Gene expression levels of DNA damage and repair markers (*XRCC1* and *p53*) and apoptosis-related markers (*Caspase*-9 and *Cytochrome C*) were quantified in peripheral blood lymphocytes using reverse transcription quantitative PCR. Our findings showed that both Hb levels and RBC count were significantly reduced in hair-dyed group, along with significant elevation in WBC count suggesting the inflammatory response against the chemicals used in hair dyes. In terms of oxidative damage, total antioxidant capacity was significantly depleted along with significant rise in malondialdehyde (MDA) and Caspase-3 levels in women using hair-dyes suggesting the oxidative stress and cell apoptosis mediated by hair dyes. The increased expression of XRCC1, p53, Caspase-9, and cytochrome C further substantiated the opinion that hair dye chemicals induce DNA damage and apoptosis. The findings emphasise on the need for more long-term monitoring of the impacts of exposure to hair colour and raise alarms on the risk of prolonged use of hair dyes.

Keywords: Apoptosis, DNA damage, hair dye, oxidative stress

INTRODUCTION

Hair has long been a primary marker of beautify and attractiveness. Consequently, the use of hair dyes has extensively expanded all over the world. Not only elderly people colour their white hair to mask their age but it has now become a fashion for adults. Conservative estimates reveal that 10% of men over 40 and 35% of women of over 18 age regularly use hair dyes (He *et al*., 2022). Due to the widespread use of hair dyes, it is vital to evaluate the potential carcinogenicity and toxicity of dyes and their chemical constituents.

Hair dyes rely on a complex chemical reaction to attain long lasting colour. The primary precursors include aromatic amines, which may increase the risk of cancer (Ali *et al.*, 2022; Nandini, 2023), and p-phenylenediamine (PPD), toluene-2,5-diamine (PTD), modified para-diamines (Jairoun *et al.*, 2024). These substances undergo a sequence of oxidative reactions with a developer, typically hydrogen peroxide, to form large pigmented molecules (Kumar *et al.*, 2021),

and get polymerized within the hair cortex to dye the hair permanently (Fernandes *et al.*, 2023). The precursors used in dye formulations, especially PPD and other aromatic amines, induce significant toxicological impact (Venkatesan *et al.,* 2022). PPD increases reactive oxygen species, which may trigger apoptosis (Salami *et al.,* 2023). Further, PTD and 2-methoxymethyl-p-phenylenediamine (MEPPD), are classified as potent skin sensitizers. These are capable of eliciting dermal allergic reactions in people, when in contact with skin, so are acutely hazardous (He *et al.*, 2022).

Hair colours frequently contain the aromatic amines which are linked to the physiological disruptions. For instance, N-phenyl-p-phenylenediamine 4 induces the degradation of seminiferous tubules, skeletal deformities, skin hypersensitivity, and body weight loss (Acharya *et al.,* 2025). Similarly, 2-chloro-p-phenylenediamine 7 and its sulphate salt, are linked to skin irritation, reduced body weight, and ocular irritation (Khan *et al.,* 2022). Exposure to hydroxypropyl bis (N-hydroxyethyl-p-phenylenediamine) hydrochloride salt 6 is associated with decrease in body weight, reduction in serum glucose level, attenuated total protein levels, as well as reproductive and developmental issues (Burnett *et al.,* 2022). Due to the reported carcinogenicity in rats and mice, 4-methoxy-m-phenylenediamine 8 and its hydrochloride and sulphate salts are not recommended for use in hair dye products (Nandini *et al*., 2023). The rate of chromosome aberration often increase with hair dyeing duration (Kumar *et al.,* 2021).

Although the genotoxic and oxidative effects of hair dye constituents such as PPD are well established in keratinocytes and experimental models, evidence regarding systemic hematological and molecular responses in chronically exposed women remains scarce. Little is known about how regular hair dye use influences the inflammatory status in blood, oxidative balance in peripheral lymphocytes, and the activation of DNA repair and apoptotic pathways. The present study hypothesizes that repeated exposure to hair dye chemicals promotes systemic oxidative stress that triggers inflammation, activates DNA damage response pathways, and enhances apoptosis in circulating blood cells. Therefore, the present study was aimed to evaluate the impact of hair dye (e.g. Herbatint 4N Chestnut, Garnier Olia 6.3, and Wella Koleston 2/0) on the molecular pathways of genotoxicity by examining changes the changes in the expression of genes regulating DNA damage, DNA repair, and apoptosis (XRCC1, p53, Cytochrome C, and Caspase-9) in women.

MATERIALS AND METHODS

The present study was carried out at the Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Mutah University, Al-Karak, Jordan, from January 2024 to October 2024. This case-control study was designed to evaluate the association between chronic hair dye use and systemic hematological alterations, oxidative stress, DNA damage response activation, and apoptosis-related molecular changes in Jordanian women.

Study population and data collection

The study's control group featured 25 women who have never dyed their hair. These women were of 25 to 50 years age. The test group comprised of 25 women (aged between 30 to 60 years) who regularly dyed their hair (most common dye use in Jordan is Garnier colour naturals). It was confirmed from them that they have been using hair dye for a sustained period. Every participant filled out a questionnaire and consented to participate in the study. The study was restricted to female participants because hair dye use is considerably more prevalent among women in Jordan, whereas its use among males remains limited due to cultural and traditional considerations. Including males would have resulted in inadequate sample size and reduced statistical power. Regarding age distribution, the slightly different age ranges between the dyed (30-60 years) and non-dyed (25-50 years) groups reflect the real-world pattern of hair dye use, which is more common among women above 30 years of age. Complete age homogeneity was difficult to achieve during

recruitment; however, participants were selected to ensure reasonable overlap between groups and to minimize age-related bias. All the assays in this study were conducted with prior ethical approval from the Mutah University Scientific Research Ethics Committee (vide ethical No. 3/2024 dated 3 January, 2024).

Blood sample collection

Whole blood (10 mL) was drawn from each volunteer in the morning after an overnight fast. For hematological parameters, 4 mL was transferred to EDTA tubes. The rest 7 mL was put in a plain vial and centrifuged at 2500 rpm for 20 min at 2°C to isolate plasma. The plasma was used for the measurement of biomarkers of oxidative damage including total antioxidant capacity (TAC), and malondialdehyde (MDA). To evaluate molecular responses, *XRCC1* and *p53* were selected to represent DNA damage and repair pathways, Caspase-9 and Cytochrome C to assess apoptosis.

Hematological parameters

The blood parameters including hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and platelets for both women with and without dyed hair was measured using BC-5300 Auto hematology analyser.

Determination of oxidative stress biomarkers and DNA damage

The total antioxidant capacity (TAC) of blood was estimated by the methods of Onikanni *et al.* (2021). MDA was conducted as per the method of Ghonemy *et al.* (2021) for both group of women with and without dyed hair.

Reverse transcription quantitative PCR (RT-q)

Peripheral blood lymphocytes (1×10^6 cells $well^{-1}$) were isolated from the whole blood of women with dyed and undyed hair as per method of Alhawamdeh *et al.* (2024) and seeded into six-well plates. Total RNA (2 µg) was reverse transcribed into cDNA using iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc.) as per the manufacturer's instructions. Quantitative RT-PCR reactions were performed in triplicate in a final reaction volume (10 µL). Primer sequences (Millipore Sigma; Merck KGaA) were validated using Primer-BLAST and the NCBI database. The primers sequences used for RT qPCR were as under:

Genes	Primers (5'- 3')		Annealing temperature (˚C)
	Forward	Reverse	
XRCC1	CTGGGACCGGGTCAAAAT;	CAAGCCAAAGGGGGAGTC	56
Cytochrome C	TGCAGCTAGGGATGTGAATCTTC;	GGAGCCCAGTCCATCAGAACT	59
Caspase-9	GGTGGCCCTAAAGGACTCTC;	AAGGTGCTTGGGGAATTTCT	57
P53	GGATCCTAATACGACTCACTA;	GGCAGTGACCCGGAAGGCA	59
GAPDH	GGAGCGAGATCCCTCCAAAAT;	GGCTGTTGTCATACTTCTCATGG	60

The gene expression fold change was calculated according to the following formulas, respectively:

Formula 1: Mean Ct values of each sample.

Formula 2: ΔCt = mean Ct of target gene (formula 1) – mean Ct value of GAPDH (housekeeping gene)

Formula 3: ΔΔCt = ΔCt value of sample – mean ΔCt of control.

Fold change = 2^- ΔΔCt

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal housekeeping gene to normalize gene expression data in RT-qPCR analysis (Shen *et al.*, 2022). GAPDH is a constitutively expressed glycolytic enzyme involved in cellular energy metabolism, and widely used as a reference gene due to its relatively stable expression across different cell types and experimental conditions (Bustin, 2000). In present study, GAPDH was selected to control for variations in RNA quantity, cDNA synthesis efficiency, and amplification performance. The relative expression levels of target genes (XRCC1, p53, Caspase-9, and Cytochrome C) were calculated using 2^-ΔΔCt method after

normalization to GAPDH expression to ensure accurate and reliable quantification of gene expression changes between dyed and non-dyed groups.

Statistical analysis

All the results were analysed using two-tailed T-test. Also normality was assessed with the Shapiro-Wilk test in GraphPad Prism.

RESULTS AND DISCUSSION

To assess the possible effects of hair dye exposure on blood components, hematological parameters were measured in women with and without dyed hair. A potential immunological or inflammatory reaction to hair dye chemicals was observed (Fig. 1), as indicated by a substantial rise in WBC count in the coloured hair group compared to the undyed hair women ($p < 0.05$). However, both RBC and Hb levels significantly decreased in hair-dyed women group compared to the non-hair dyed group. There was no significant difference in the platelet counts between the two groups (Fig. 1). The level of TAC, MDA, and Caspase-3 were measured in this study to determine the oxidative damage and cell apoptosis induced by hair dyes. TAC level significantly decreased, along with significant increase in MDA and Caspase-3 level in hair-dyed women group compared to the non-hair dyed group suggesting the oxidative damage and cell apoptosis of hair dyes (Fig. 2).

For further study, the expression of XRCC1, Caspase-9, p53, and Cytochrome C genes was measured. The results demonstrated significantly higher expression levels of these genes in the hair- dyed women group compared with non-dyed group (Fig. 3). XRCC1 is a scaffold protein that plays a central role in the base excision repair pathway, coordinating the repair of single-strand DNA breaks. Therefore, its upregulation reflects the activation of DNA repair machinery in response to the genotoxic stress rather than direct induction of damage. Similarly, p53 functions as a key regulator of cellular stress response, promoting cell-cycle arrest, DNA repair, or apoptosis when DNA lesions are detected (London, 2020; Vaddavalli and Schumacher, 2022). The elevated expression of XRCC1 and p53

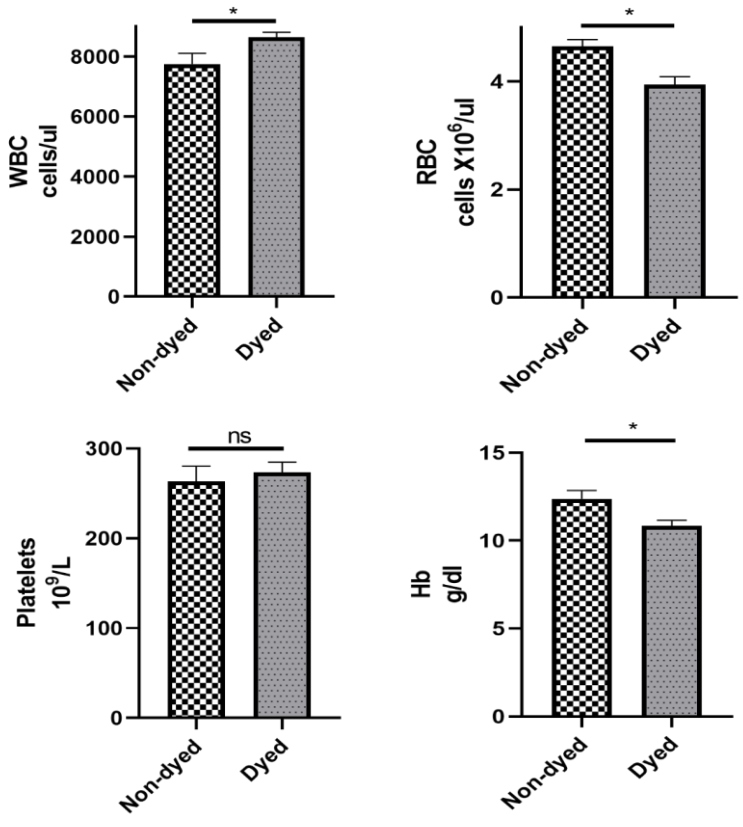

Fig. 1: WBC and RBC counts, haemoglobin (Hb) levels in hair dyed and non-hair dyed women groups.
Data presented as mean ± SEM. * indicates $p \leq 0.05$, ns: not significant

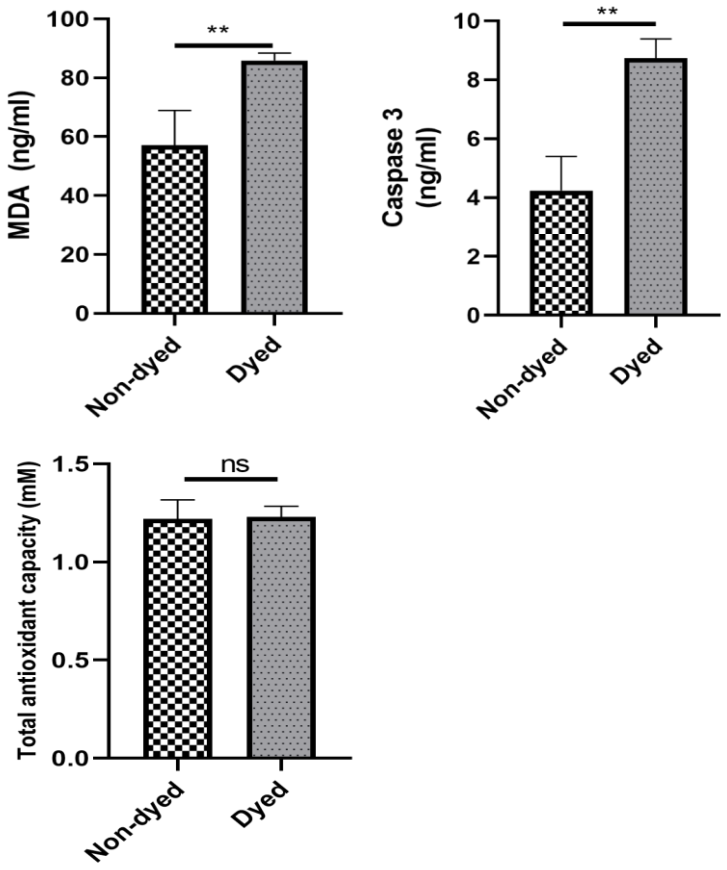

Fig. 2: The oxidative damage and cell apoptosis induced by hair dyes. MDA: malondialdehyde, non-dyed: non-hair dyed women group, dyed: hair dyed women group. Data are presented as mean ± SEM. *, and ** indicates P value ≤0.05, P value ≤0.0021 respectively.

in the dyed group suggests the presence of increased DNA damage requiring the enhanced repair and checkpoint activation. In addition, the increased expression of Caspase-9 and cytochrome C, which are critical mediators of the intrinsic mitochondrial apoptotic pathway, indicates activation of programmed cell death mechanisms (Jiang and Wang, 2004; Unnisa *et al.*, 2023).

In this study, the effect of oxidative stress, apoptosis, and inflammation induced by hair dye were determined. It is clear from our findings (an increase in WBC count) that the chemicals in hair dyes induced inflammatory and immune-logical response (Germolec *et al.*, 2022). The observed leucocytosis reflect the activation of immune system in response to chemical-induced oxidative stress. Reactive oxygen species are known to stimulate inflammatory signalling pathways and cytokine production, which promote the proliferation and mobilization of WBC.

The reduction in RBC count and hemoglobin may result from oxidative injury to erythrocyte membranes, leading to the reduced survival or increased clearance of damaged cells.(Ju et al., 2024). The maintenance of a steady platelet count suggests that the chemicals agents used in hair dye may not directly affect the coagulation pathway. In terms of oxidative damage induced by hair dye, MDA levels which are a biomarker for lipid peroxidation and oxidative stress (Apak, 2019) are significantly increased, same as Caspase-3 level which indicates cell apoptosis due to oxidative stress and accumulated DNA damage. The oxidative stress mediated by hair dye is supported our TAC results the showed significantly decreased of antioxidant defense in the hair-dyed women.

Unlike earlier studies that mainly focused on isolated skin cells or experimental models, the present study provides evidence of systemic molecular alterations in peripheral blood of women habitually using hair dyes. This real-life exposure model strengthens the clinical relevance of our findings. This study differs in that significant differential expression levels of XRCC1, p53, Caspase-9, and Cytochrome C were observed in dyed and undyed hair, suggesting exposure to hair dye increases DNA damage and apoptosis. An increase in XRCC1 indicates that the DNA repair response mechanism is initiated, as a result of oxidative stress and genotoxic chemicals within hair dyes (Haronikova *et al.*, 2019). The increased expression of *p53* is another indication of the activation of stress response pathways that may lead to apoptosis (Kashyap *et al.*, 2021). However,

the expression of Cytochrome C and Caspase-9 genes are implicated in the activation of programmed cell death (Alhawamdeh *et al.*, 2024).

In summary, it is clear from our results that hair dyes have several damaged effects on DNA, and cells. These hair dyes mediate DNA damage, cell apoptosis, inflammation and reduced RBC counts and Hb levels. However, this work has limitations that all samples were obtained from a single geographic area (Jordan), and the sample size is relatively low. Further, the assessment of dye exposure largely relied on participant's information through consent form, and the questionnaire were made, data about dyes frequency, duration, cumulative exposure, and exact chemical composition of each product were not available. Also other potential factors like environmental exposures, nutritional status, lifestyle habits, and occupational risks were not controlled. The future studies are desired to include larger sample size from different regions, ethnicities and gender. Precise exposure characterization, including chemical analysis of dye constituents is also desired. Additional assays like proteomic assays, enzyme activity measurements, and pathway analyses would provide deeper mechanistic insight into how these compounds modulate DNA repair and apoptotic signalling.

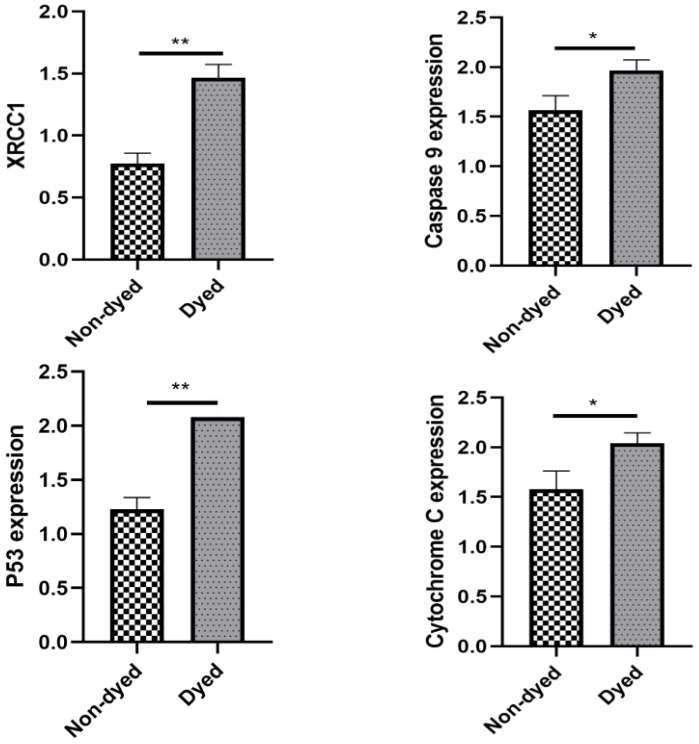

Fig. 3: XRCC1, Caspase-9, P53, and Cytochrome C genes expression. Non-dyed: non-hair dyed women group, dyed: hair dyed women group. Data are presented as mean ± SEM. *, and ** indicates P value ≤0.05, P value ≤0.0021 respectively.

Ethical statement: All the procedures carried out in the research with participation of humans were in compliance with the ethical standards of the National Research Ethics Committee and with the Helsinki Declaration of 1964 and its subsequent changes or with comparable ethics standards. Informed voluntary consent was obtained from each participant. I declare no conflict of interest.

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REFERENCES

Acharya, B., Shalini, S., Shalini, M., Maneesha, R., Rajesh Kumar, M., Satyendra Kumar, R., *et al.*, 2025. Ayurvedic approaches to managing premature greying of hair: A comprehensive review. *Current Cosmetic Science,* **4:** 24-34.

Alhawamdeh, M., Almajali, B., Hourani, W., Al-Jamal, H.A.N., Al-Wajeeh, A.S., Mwafi, N.R., *et al.*, 2024. Effect of IFN-γ encapsulated liposomes on major signal transduction pathways in the lymphocytes of patients with lung cancer. *Oncology Letters*, **27:** 8. [*DOI: 10.3892/ol.2023.14141*].

Ali, A., Moinuddin Allarakha, S., Fatima, S., Ali, S.A. and Habib, S.J.I.J.O.C.B. 2022. Risk of carcinogenicity associated with synthetic hair dyeing formulations: A biochemical view on action mechanisms, genetic variation and prevention. *Indian Journal of Clinical Biochemistry*, **37:** 399-409.

Apak, R. 2019. Current Issues in Antioxidant Measurement. *Journal of Agricultural and Food Chemistry*, **67:** 9187-9202.

Burnett, C.L., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., *et al.*, 2022. Safety assessment of 1-hydroxyethyl 4, 5-diamino pyrazole sulfate as used in cosmetics. *International Journal of Toxicology*, **41:** 69S-79S.

Bustin, S.A. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology*, **25:** 169-193.

Fernandes, B., Cavaco-Paulo, A. and Matamá, T.J.B. 2023. A comprehensive review of mammalian pigmentation: Paving the way for innovative hair colour-changing cosmetics. *Biology (Basel)*, **12**(2): 290. [*https://doi.org.10.3390/biology/1220290*].

Germolec, D.R., Lebrec, H., Anderson, S.E., Burleson, G.R., Cardenas, A., Corsini, E., *et al.*, 2022. Consensus on the key characteristics of immunotoxic agents as a basis for hazard identification. *Environmental Health Perspectives*, **130:** 105001. [*https://doi.org/10.1289/EHP10800*].

Ghonemy, H., Farahat, F., Hassen, E. and Omran, B. 2021. Paraphenylenediamine hepatotoxic effects in adult female albino rats. *Zagazig Journal of Forensic Medicine and Toxicology*, **19:** 21-41.

Haronikova, L., Olivares-Illana, V., Wang, L., Karakostis, K., Chen, S. and Fåhraeus, R. 2019. The p53 mRNA: An integral part of the cellular stress response. *Nucleic Acids Research*, **47:** 3257-3271.

He, L., Michailidou, F., Gahlon, H.L. and Zeng, W. 2022. Hair dye ingredients and potential health risks from exposure to hair dyeing. *Chemical Research in Toxicology*, **35:** 901-915.

Jairoun, A.A., Al-Hemyari, S.S., Shahwan, M., Jairoun, O., and Zyoud, S.H. 2024. Quantification of p-phenylenediamine in hair dyes and health risk implications in the UAE: Describing discordances between regulations and real-life practices. *Risk Management and Healthcare Policy*, **17**: 663-675.

Jiang, X. and Wang, X. 2004. Cytochrome C-mediated apoptosis. *Annual Review of Biochemistry*, **73:** 87-106.

Ju, S., Singh, M.K., Han, S., Ranbhise, J., Ha, J., Choe, W., *etal.*, 2024. Oxidative stress and cancer therapy: Controlling cancer cells using reactive oxygen species. *International Journal of Molecular Sciences*, **25**(22): 12387 [*https://doi.org/103390/ijms252212387*].

Kashyap, D., Garg, V.K., Goel, N. 2021. Intrinsic and extrinsic pathways of apoptosis: Role in cancer development and prognosis. *Advances in Protein Chemistry and Structural Biology*, **125:** 73-120.

Khan, S., Ali, A., Moinuddin, Mir, A.R., Khan, R.H., Alhumaydhi, F.A. and Habib, S. 2022. 4-Chloro-orthophenylenediamine alters DNA integrity and affects cell survival: Inferences from a computational, biophysical/biochemical, microscopic and cell-based study. *Journal of Biomolecular Structure and Dynamics*, **40:** 14176-14187.

Kumar, S., Yadavb, S., Vishwakarmaa, V., Yadava, B., Guptac, R., Aggarwald, N., *et al.*, 2021. A review on toxicological hazards of p-phenylenediamines: A primary ingredient of hair dye and potential biomarker-based risk assessment. *Natural Volatiles and Essential Oils* **8:** 754-781.

London, R.E. 2020. XRCC1 - Strategies for coordinating and assembling a versatile DNA damage response. *DNA Repair (Amst)*, **93:** 102917. [*doi:10.1016/j.dnarep.2020.102917*].

Nandini, M.S., Kumar, S.K. and Narayanan, R.S. 2023. Components in hair dye and possible health concerns from using straighteners, including cancer risks: Oncology-influence of hair dyes for cancer. *International Journal of Trends in OncoScience*, **1:** 44-52.

Onikanni, A.S., Lawal, B., Olusola, A.O., Olugbodi, J.O., Sani, S., Ajiboye, B.O., *et al.,* 2021. Sterculia tragacantha lindl leaf extract ameliorates STZ-induced diabetes, oxidative stress, inflammation and neuronal impairment. *Journal of Inflammation Research,* **14:** 6749-6764.

Salami, M., Pourahmad, J. and Zarei, M.H. 2023. Toxicity of para-phenylenediamine (PPD; 1, 4 diaminobenzene) on isolated human lymphocytes: The key role of intracellular calcium enhancement in PPD-induced apoptosis. *Toxicology and Industrial Health,* **39:** 388-397.

Shen, C.H., Peng, L.J., Zhang, Y.X., Zeng, H.R., Yu, H.F., Jin, L., *et al.,* 2022. Reference genes for expression analyses by qRT-PCR in *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Insects,* **13**(2): 140. [*https://doi.org/10.3390/Insects13020140*].

Unnisa, A., Greig, N.H. and Kamal, M.A. 2023. Inhibition of caspase 3 and caspase 9 mediated apoptosis: A multimodal therapeutic target in traumatic brain injury. *Current Neuropharmacology,* **21:** 1001-1012.

Vaddavalli, P.L. and Schumacher, B. 2022. The p53 network: Cellular and systemic DNA damage responses in cancer and aging. *Trends in Genetics,* **38:** 598-612.

Venkatesan, G., Dancik, Y., Neupane, Y.R., Karkhanis, A.V., Bigliardi, P. and Pastorin, G. 2022. Synthesis and assessment of non-allergenic aromatic amine hair dyes as efficient alternatives to paraphenylenediamine. *ACS Sustainable Chemistry & Engineering,* **10:** 838-849.