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A Review On Toxicological Hazards Of P-Phenylenediamines: A Primary Ingredient Of Hair Dye And Potential Biomarker-Based Risk Assessment

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Abstract

In modern time, the use of cosmetic, beauty and personal care products (PCPs) has increased all over the world. Peoples use hair dye to change their appearance so that they seem attractive. An increasing amount of different compounds are used to make these PCPs effective in performance and quality. Numerous chemical additives have toxic effects to humans and can cause mild hypersensitivity to lethal intoxication. These compounds may have adverse effects after dermal penetration and absorption by other routes as these can act as mutagens, toxin to cells and cellular system, carcinogen and endocrine disruptors. The pathways of toxicity and mechanism of action are still underway for hair dyes. The evaluation of potential toxicity of hair dye is very difficult due to involvement of primary molecules, their intermediates and final product in reaction at a single point. Therefore, hair dye toxicity should be examined and tested individually and with different combinations in order to determine any toxic effects of reaction products produced. Since toxicity of hair dye is of concern, a brief discussion about biomarker based toxicological assessment of hair dye and its ingredients.

Keywords: Hair dye, Genotoxicity, p-phenylenediamines (PPD), Oxidative stress, Biomarkers

INTRODUCTION

Genotoxicity is a crucial element of hazard assessment for hair dye and their ingredients. Since the exposure of hair dye not only limited to those who dye their hair but also to the workers who are occupationally exposed such as hairdressers and persons working in the industry of manufacturing and packaging of the hair dyes, it leads to broad human systematic exposure of hair dye ingredients which

ultimately cause several cancer types. Different types of hair dye products have been used by humans to color hair ultimately leading their skin to broad exposure to its components. As we know that the human scalp is not a resistant barrier always. Intrinsic components such as primary intermediate (p-phenylenediamines) and coupler (resorcinol) may lead to potential toxicity in the human body. In vitro data proposes the adverse effects of carcinogenicity, mutagenicity and human systematic risk including acute, reproductive and genetic toxicity. So, it is necessary to check any potential hazard that might be due to hair dye exposure.

Oxidative hair dyes are mainly composed of two components- one is "primary intermediates" including p-phenylenediamine (PPD), p-toluenediamine (PTD), substituted p-diamines, ortho- or p-aminophenols which on coupling with modifiers and successive oxidation result in coloured reaction products. Another one is "couplers" including broad range of aromatic derivatives like resorcinol, m-aminophenols and m-phenylenediamines which destine the ultimate stain by encountering previousoxidised intermediates. In the presence of peroxides (hydrogen peroxide, sodium percarbonate or perborate and urea peroxide) primary intermediates and couplers give a coloured product formation. During this chemical reaction in hair it is believed that origin of Bandrowski's base occurs which is a possible carcinogenic and absolute mutagenic product of PPD and H_2O_2 reaction[1]. Some hair dyes also have alkalinising agents such as ammonia, mono-ethanolamine or amino-methylpropanol. Temporary hair dyes constitute anthraquinone, triphenylmethane and azo dyes although semi-permanent hair dyes constitute N-phenylenediamines, N-aminophenols and a few azo dyes. Ames et al., found that about 85-90 % hair dye components were carcinogenic or mutagenic in Salmonella typhimuriumthat means they may lead to risk of cancer in consumers[2]. Ames et al., in Salmonella typhimurium mutagenicity assay, revealed mutagenicity of 10 out of 18 amine derivative of stable hair dyes, along with 4-nitro-o-phenylenediamine, 2,4-diaminotoluene, 2-nitro-p-phenylenediamine, 2,4-diaminoanisole, 2-amino-5-nitrophenol, 2-amino-4-nitrophenol, 2,4-diaminoanisone, o-phenylenediamine, 2,5- diaminotoluene and m-phenylenediamine[2]. The process of monitoring for carcinogenicity by National Cancer Institute (NCI) U.S, for some hair dye ingredients provided indications of carcinogenicity after oral administration.

Traced back in 1883, when Erdmann and Monnet applied p-phenylenediamine and diaminotoluene with an oxidizing component was the first oxidative hair dyeing patent confirmed by Charles and Sag[3]. Because of no other substitute the technique was used extensively in hair dyeing for many years. The Scientific Committee on Consumer Safety (SCCS) released a notion for 46 ingredients in which 10 were supreme allergen (PPD included), 13 were vigorous and 4 recognized as mild skin irritant. Above mentioned particular dyes contain aromatic amines that can form probable carcinogenic derivatives and reactive molecules during exposure to humans. Various toxicological experimental concerns endorsed the existence of aromatic amines in different hair dyes. These numerous experimental findings collectively suggested that active reaction products of hair dye have potential to cause genotoxicity.

1.HAIR DYE EXPOSURE

The main exposure route of hair dye and its ingredients is dermal due to personal use of hair dye on the scalp**(Figure 1)**. Various studies demonstrated and explored dermal and systemic exposure of hair dye ingredients. A study showed percutaneous absorption of PPD (hair dye ingredient) due to hair dying procedure through monitoring PPD metabolites in urine after 24 and 48 hours[4]. For direct dyes, scalp penetration was marginally greater. Hueber-Becker et al.evaluated systemic exposure dose by

application of hair dye containing [^{14}C]-PPD for 30 min[5]. The mean systemic dose was nearly 0.09±0.04 mg/Kg body weight. An in vitro human skin study demonstrated that around 2.4±1.6% of applied dose was absorbed with a rate of 10.6±6.7 µg/cm^2[6].

In addition, hairdressers and customers can be exposed to hair dye and its ingredients through a route of air in hairdressing salons. During mixing and application of hair dye the exposure to hair dye and ingredients is generally high. Secondly, repetitive exposure during working hours may put hairdressers toward higher risk [7]. Several case reports of human poisoning and deaths from consumption of PPD have been reported. Depending upon the dose and time after oralexposure, different symptoms start developing in patients such as vomiting and dyspnea [8].

Consistent with the above described reports, there isevidence of renal toxicity in humans from chronic exposure to PPD [9].Populations working in hair dye manufacturing and packaging industries are also toward greater risk.

Figure 1: Exposure ways and routes of hair dye

2. CHEMISTRY AND CLASSIFICATION OF HAIR DYES

Oxidative hair dyes and their ingredients mainly belong to arylamines, the supreme chemically reactive ingredients of personal care products. Zviak and Millequant (2005) studied the chemical structure, reactions and categories of hair dyes[10]. Following categories have been described:

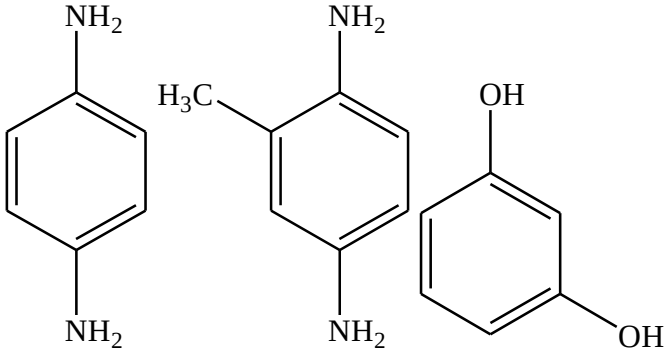
- Permanent/ Oxidative hair dyes
- Semi-permanent hair dyes

- Temporary hair dyes
- Natural hair dyes
- Metal system
- Enzymes

2.1 Permanent/ Oxidative hair dyes: It contributes maximum share (70%) amongst hair dye products because of having protean nature, long lasting efficacy and easy to apply which makes free to achieve any color**.** These are exclusively used dye to generate a permanent colour that lasts until the hair expands.

2.1.1 Primary intermediates: Simple and essential molecules which include para-substituted molecules of phenylenediamine (PPD), toluenediamine (PTD),diamines, and aminophenol are being shown in figure 1. Colour composition based on oxidation followed by reaction with modifiers that produces coloured and reactive reaction products on the hair shaft.

2.1.2 Couplers: The second necessity for formulation of hair dyes covers meta-substituted derivatives of aromatic amines of phenylenediamines, aminophenols and resorcinol. These lay down the decided formulation by counteraction of oxidised state of essential intermediates and many oxidative reactions. Present day dyes for hair comprise of couplers and essential primary intermediates around a molar ratio of 1:1.



PPD (1,4-diaminobenzene) PTD (1-methyl-2,5-diaminobenzene) Resorcinol (1,3-dihydroxybenzene)

Figure 2: Hair dye primary intermediates i.e 1,4-diaminobenzene (PPD), 1-methyl-2,5-diaminobenzene (PTD) and resorcinol as coupler

The formation of these dyes requires essential intermediates such as p-phenylenediamine i.e an amino-phenyl precursor and coupler such as resorcinol **(Figure 2)**. Under alkaline conditions i.e. in the presence of peroxide, oxidation reaction gives coloured product. **Figure 3** represents the scheme of formation of coloured product from primary intermediate and couplers.

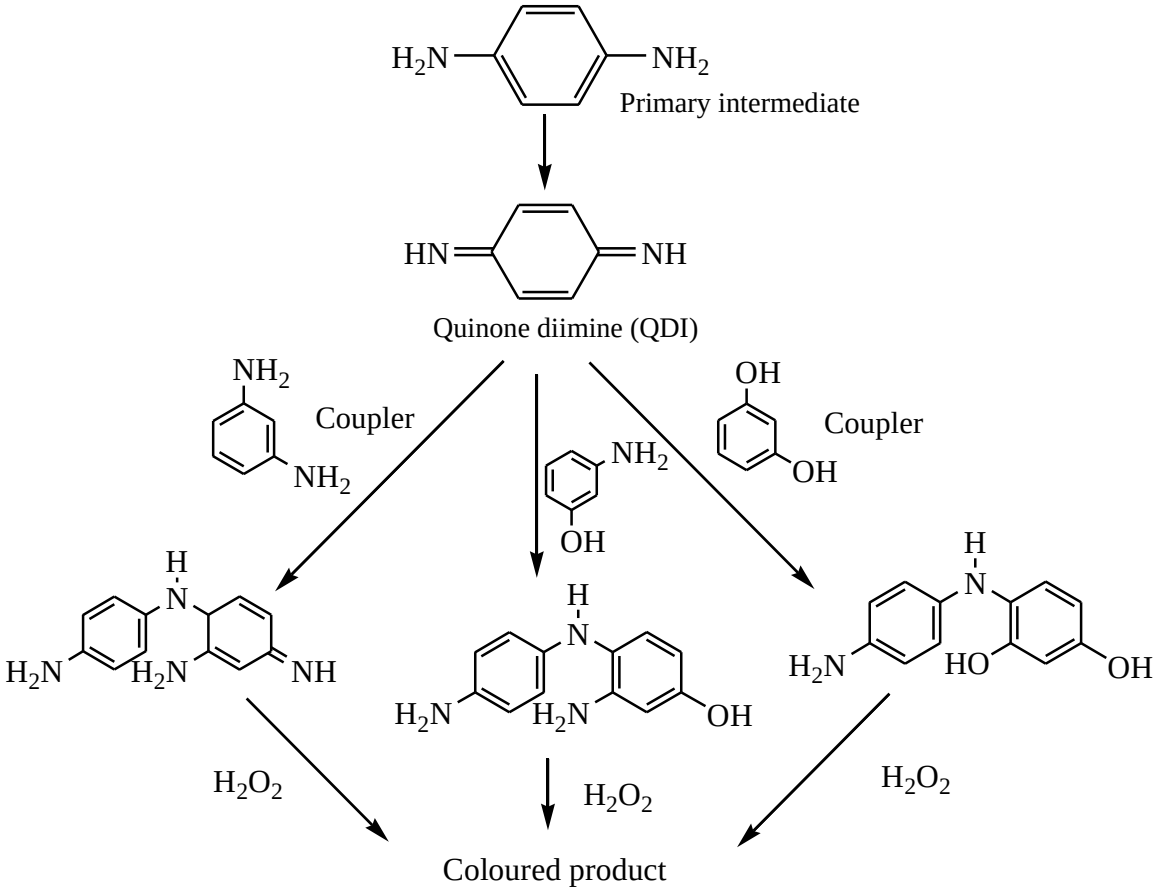


Figure 3: Chemical reaction network route of oxidative hair dye formation by primary intermediate (PPD), Coupler (resorcinol) and H_2O_2[11]**.**

Hair dye formulation involves oxidation of a precursor that generates QDI intermediate by H_2O_2 peroxide or by any other peroxide. Newly short lived QDI intermediate immediately reacts with coupler and producescolourlessleuco dyes. After oxidation, it produces colouredproducts**(Figure 4)**. The accumulation of both QDI and leuco dyes does not occur because both are transitory. The intensity ofcolour depends upon dimerization, trimerization and oligomerization of precursor compounds with coupler. Ratio between dimer and trimer mainly depends upon the structure of primary precursor and coupler and the kinetics of the reaction. Steric-hinderance of precursor occurs when couplers with ortho substituents are used because that stops reaction at dimer stage. Otherwise further reactions take place and lead to formation of trimers and many more possible large polymers inside the hair thread. The accumulation of dimers and oligomers is confirmed in an industry test that is also acknowledged by the Scientific Committee on Consumer Safety (SSCS).

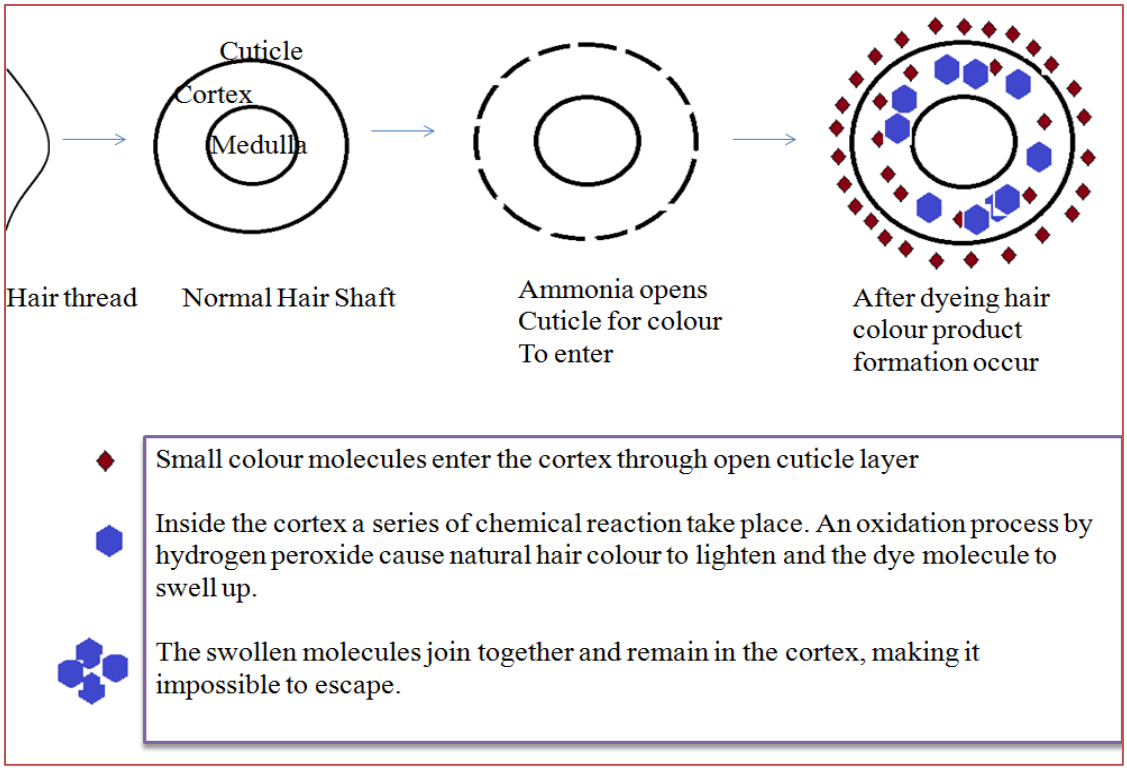


Figure 4: Pictorial representation of mechanism of oxidative permanent hair dye working on normal hair shaft

Primary intermediate p-phenylenediamine in Salmonella typhimurium test was less mutagenic [12] but in the presence of H_2O_2 formation of Bandrowiski's base **(Figure 5)** occurred that is openly mutagenic [2].

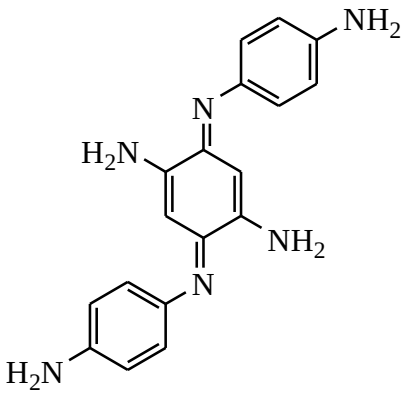


Figure 5: Structure of Bandrowiski's base which forms after auto-oxidation of primary intermediates.

The most important class of hair dye is the permanent hair dyes though their bleaching effect degrades the hair fiber. To nullify these side effects various alternatives with improved colouring effect were developed, for example- azo dyes, methine and azomethine dyes, leuco vat dyes and latent permanent technology.

2.2 Semi-permanent hair dyes: The hair dyeing process uses a direct dye which doesn't need any oxidant. These are not chemically reactive and there is no sensitization and irritation observed. These are of mainly two types one is nitrobenzene dyes (2-nitro-p-phenylenediamine) and another is disperse dyes (Disperse Blue 1).

2.3 Temporary hair dyes: These dyes cannot penetrate into the hair follicle, making them adherent to hairs which can be removed in 2-3 washing of hairs. These are easier to apply and don't cause any hair damage. Basic red 76, basic brown 57, D & C orange 4, Tartrazine, brilliant blue FCF and orange II are some examples of these types of dyes.

2.4 Natural hair dyes: Natural herbs like henna can be used to produce a certain type of colour. Natural ingredients of black walnut or indigo powder produce black colour; chamomile and calendula leads to darker blond. Natural ways to dye the hair with hibiscus flower and black tea leaves is used especially in South East Asia.

2.5 Metal system: The decomposition of hydrogen peroxide is catalysed by metals. The reaction chemistry revolves around transition state of lower oxidation stage tohigher and then back to lower oxidation state. The crucial conclusion of the reaction is formation of new oxidizing spices which are more powerful reactive substitutes than parental. Metal salts such as Cu, Co, Zn, Ag, Ni and Fe with non-toxic anions like sulphate, phosphate, carbonate, nitrate, chloride are preferred catalysts.

2.6 Enzymes: An economically and environmentally attractive process is enzymatic dyeing that does not damage hair keratinous fabric when carried out at low temperature and mild pH. Strong oxidation by peroxide under alkaline conditions causes damage to hair and phenylenediamines cause allergic reactions. So, there is a need to develop any alternative to these problems. Laccases and peroxidases can be used to produce less hair damaging dyes but toxicity by phenylenediamine still exists.

3. RISK ASSESSMENT THROUGH BIOLOGICAL MONITORING

In occupational health studies, risk assessment of exposure is a very critical component. To determine health effects due to exposure, biomonitoring can be an effective and essential tool. According to the National Research Council (NRC) report, biomonitoring can give efficient data to measure exposure rate when combined with other relevant information from toxicological, epidemiological and modelling studies [13]. Using above information, we can estimate rate of absorption, amount that absorb (dose), influence of exposure and potential health risks **(Figure 6)**. Human biological monitoring provides disposition of all exposures [14]. Biomarker can be defined as any function, structure, process or substance that can be monitored or measurable in biological systems or samples (tissue, fluids) to predict the influences on health [13].Biomarkers define the correlation between exposure and health effects. Biomarkers have been divided into three major categories- biomarkers of exposure, effect and susceptibility based on pharmacokinetic relevance[15].

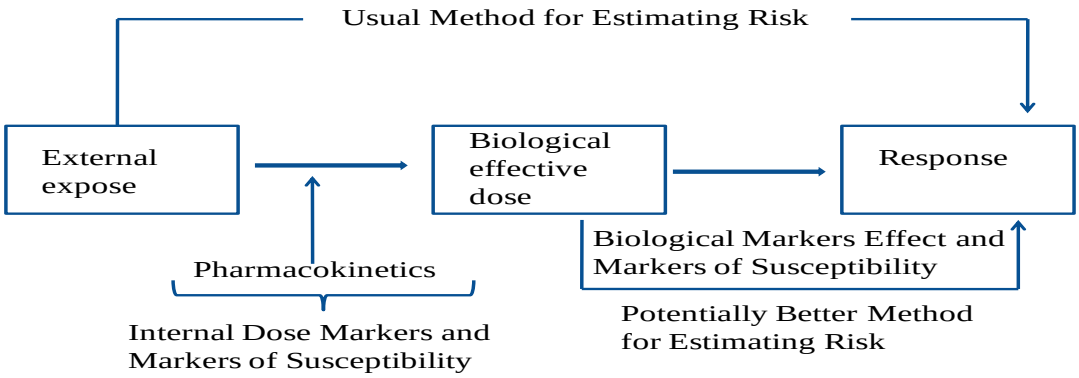


Figure 6: Rationale for using biomarker to assess risk (from Schulte and Waters, 1999)[16]

3.1 Biomarkersof exposure

Exposure biomarkers are known as effective and valuable tools to measure the level of exposure at which compounds are absorbed in biological samples by quantifying the toxic molecules or metabolites [14, 17]. Blood, serum, saliva and urine are known as reliable, reflecting exposure load and have been used to assess toxic effects due to external exposures [18]. Exposure to hair dye in occupational and personal are known to be related to risks of adverse effects on skin, liver, kidney, bladder and other organs along with immune system. Several cases of toxicity and mortality were recorded due to hair dye ingestion [19]. Biomarkers of exposure reflect the distribution of chemical or its metabolites throughout the organism. These biomarkers can be used as important tools for measurement and understanding the underlying mechanism of toxicity and adverse health effects reflecting towards disease susceptibility [20].

3.2 Toxicokinetic studies of hair dye

PPD is the key ingredient of hair dye that causes toxicity. PPD was found to cause angioneurotic edema, renal dysfunction[21]and almost every body system is affected with different degrees[22]. While preparing and applying process, hair dye can be penetrated to the blood through skin [20]**(Figure 7)**. Nohynekaet al. (2004) also mentioned that hair dye and constituents have mild to low acute toxicity[23].

Study also mentioned that PPD caused histopathological distortions in tissues such as vascular obstructions and white blood cells infiltration, hepatocytes degeneration along with renal tubules destruction [19]. Studies also mentioned association between myocardial damage and hair dye use due to increased death rate [22, 24]. Study has reported adverse effects of hair dyes on the liver by causing hepatitis[23]. Bharali and Dutta (2009) reported hepatotoxicity through increased accumulation of neutrophils[26]. .

Al-Shaikh et al. (2018) also have shown health risk of PPD on rat kidney through disruption of tubular shape, dilation and degeneration of cellular nucleus[20]. They also observed that hair dye containing PPD is a potential nephrotoxic agent. One another study also showed intense neurotoxic effects in humans and histopathological defects of severe neutrophilia[23].

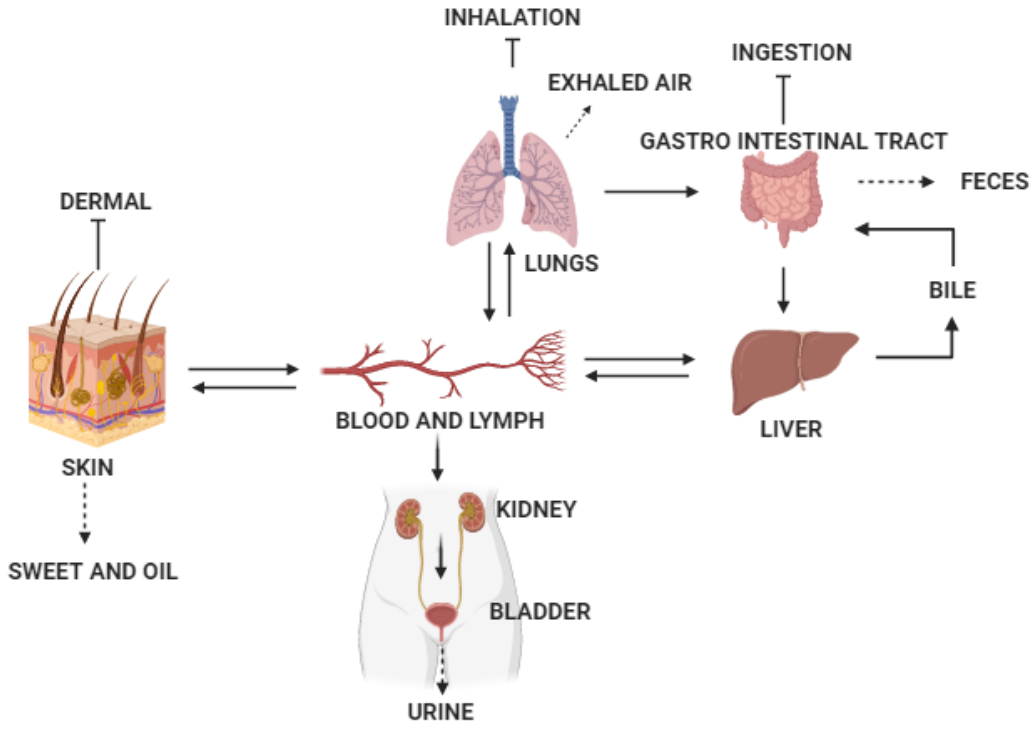


Figure 7: Route of absorption, distribution and excretion of hair dye and its ingredients and metabolites in the human body (https://www.britannica.com/science/distribution-physiology)

3.3 Biomarkers of effect

The existence of toxic molecules or reactive agents induces physiological or biochemical changes in an individual and measurement of these alterations can be correlated with adverse effects of exposure. These changes in response to exposure are manifested as regulation, change in expression or suppression of important biological, metabolic and molecular mechanism [28].

Biomarker of effect assesses damage in genetic material or DNA, enzyme activity alterations, gene mutation, gene transcription and epigenetic modifications [29]. Genetic toxicological biomarker elucidates the impact of physiochemical compounds on chromosomes or DNA inducing distortions or damage in genome structure. Damage in genetic material is a random process but somehow this damage is being repaired by DNA repair mechanisms in the cells. Genetic damage due to external agents disrupts the function of cellular repair systems that cause the accumulation of DNA lesions that could lead to adverse effects such as cell death, apoptosis, alteration in cell division or proliferation and replication of DNA. Most of the genotoxicity biomarkers included in molecular epidemiology studies are related to DNA damage and oxidative stress [30].

Biomarkers of effectprovide scientific confirmation of exposure-disease pathway which occurred due to biological interactions of chemical agents in human populations [31]. Blood lymphocytes are used frequently for evaluating genotoxic effects in human biomonitoring studies. Lymphocytes circulate

throughout the body and can be damaged in any organ or tissue due to toxic exposure [32]. The most frequently used end points in genotoxicity assessment are comet assay, sister chromatid exchange assay, micronucleus, chromosomal aberrations (CA) etc.

The comet assay (single-cell gel electrophoresis—SCGE) is a fast, easy, and sensitive approach for detecting single-strand breaks in DNA. The tail moment measures the potency of the genotoxic compound to induce DNA damage [33, 34, 35]. DNA strand breaks may result from alterations in DNA by chemical agents, replication, DNA repairs, recombination or apoptosis. Interaction of DNA with ROS directly generates breaks in DNA strands[36]. This method has become a standard assay for genotoxicity assessment with a broad range of applications.

Sister chromatid exchange is defined as mutual exchange of chromatin among homologous sister chromatids. Although little is known about its molecular basis, SCE probably occur during DNA replication and synthesis both due to breakage or error and also because of replication inhibition. An increase in SCE frequency is the marker of chromosomal damage against genotoxicant response when compared to the control samples. Subsequent differential staining of the chromatid is required to visualize that can be achieved by Hoechst plus Giemsa staining [37].

Oxidative stress has a significant role in initiation and growth of adverse health effects or disease. It is a condition where excessive reactive oxygen species (ROS) produce or antioxidative enzymes are not able to eliminate them. This elevated level of ROS damages the cells by oxidizing lipids, proteins and nucleic acid **(Figure 8)** and thus leading to loss or change of their function and finally causes cell death. Various antioxidant enzymes produced in the body like SOD, GPx and CAT which act as scavenger of free radicals [38].

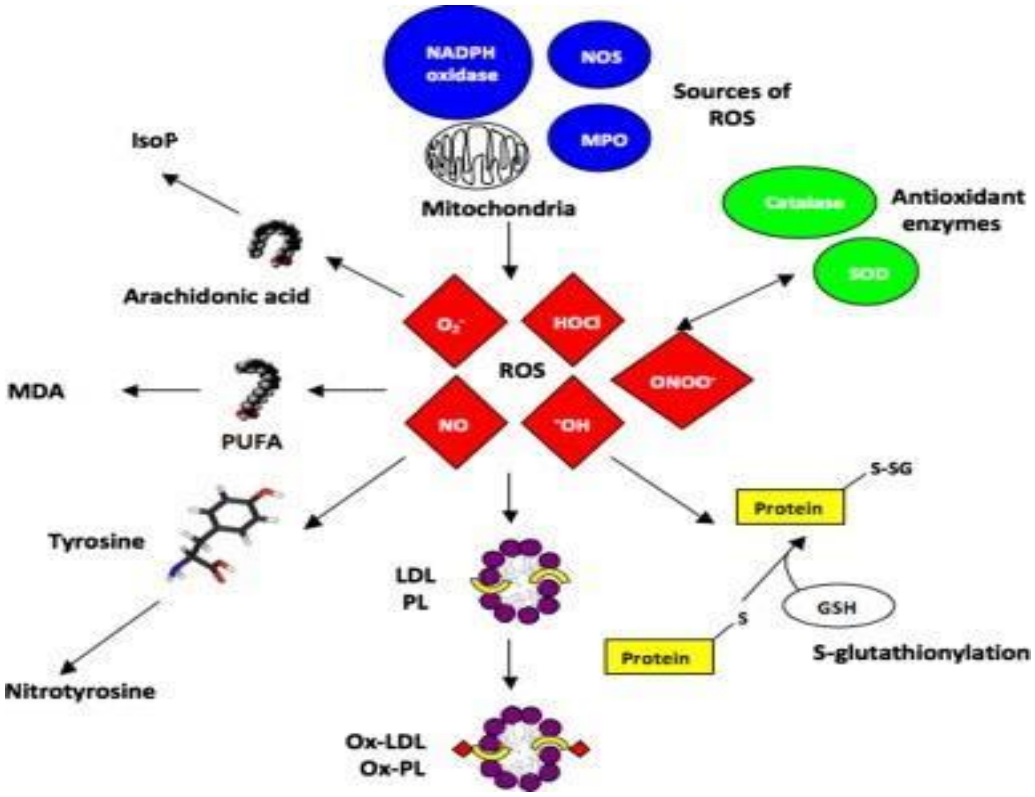


Figure 8: Biological markers of oxidative stress [39].

Biomarkers of oxidative stress can be defined as biomolecules that are modified by interaction with ROS and change the redox status of cellular system or microenvironment. Reactive oxygen species directly damage DNA, lipids and proteins or indirectly through recruitment of inflammatory molecules that generate secondary oxidative response. The enzymatic and non-enzymatic antioxidant defense systems regulate ROS generation and protect cellular system from oxidative damage[40]. SOD catalyzes the dismutation of superoxide into O_2 and water [41], GPx utilizes reduced glutathione (GSH) as substrate to convert oxidized glutathione (GSSG) [42]; then glutathione reductase converts it back to reduced glutathione [43]. ROS can induce cellular lipid oxidation which distorts the structure and function of cell membrane. Thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), and hydroxynonenal (HNE) are the most studied biomarkers for lipid peroxidation [44]. Cell viability, cytotoxicity and proliferations are the important indicators for biological evaluations. Toxic compounds can influence cellular health and metabolism that leads to change in viability and proliferation of cells [45]. Different assays are there to determine cell cytotoxicity and viability in the cell culture system. The mostly used and simplest method is dye exclusion assay where viable cells exclude dyes while dead cells take up the dye. A variety of dyes are used for cell viability and cellular integrity. Trypan blue is the most extensively used one method [46]. Trypan blue dye exclusion method is based on the concept that a cell with intact membrane exclude dye means viable cells do not uptake dye, whereas dead cells do not exclude dye uptake [47, 48]. Some colorimetric assays such as MTT or XTT are also used for measurement of cell viability for adherent or suspended cells using spectrophotometer [49]. MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) protocol determines viability of a cell through measuring mitochondrial functional enzymes activity [50]. Viable cells reduced MTT to a formazan product (purple) that can be estimated by spectrophotometer using specific wavelength. The intensity of purple color indicates viability of cells and inversely proportional to the extent of cytotoxicity [51].

The activation of gene expression by different stimuli represents the cellular response of the stimulus. The transcriptional profile of exposed cells indicates response dose, cellular defense system, gene induction or repression and signaling cascades associated with the process [52].

The current techniques to analyze gene expression profile for specific gene of interest includes cDNA microarrays and real-time PCR. Lateritinvolves extracting total RNA, preparing cDNA and then analyzing gene expression using real-time PCR [53].

Apoptosis is a major biochemical mechanism of cell death which is intended to proceed without the release of cellular contents and successively initiation of an inflammatory reaction. This supports embryo development, regulation of immune functions and response to DNA damage. Imbalance in apoptosis leads to carcinogenesis, cell proliferation and malignancy[54].

. Apoptosis is activated through ligand-receptor mediated extrinsic pathway or mitochondrial-directed intrinsic pathway. The extrinsic pathway activated by ligands binding to the receptor that leads to trigger initiator and effectors caspase 9 and caspase 3 [55].Then it is regulated through pro- and anti-apoptotic proteins (bak, bax, bcl-2, bcl-xl etc.) at mitochondrial surface where the change in intra-family protein interactions determines the Cytc release which again activates initiator and effector caspases [56].

3.4 Biomarker of susceptibility

Biomarkers of susceptibility determine the susceptibility of individuals towards the progression of health risk through exposure to toxic agents. The ratio of dose and duration with susceptibility correlations regulates biological response and the risk for disease development.

The specific genes encode metabolic or detoxification enzymes against the effects of chemical toxicant on human health. These enzymes play a vital function in genetic susceptibility to xenobiotics[57].Two major classes of metabolic enzymes: phase I and phase II are most frequently used as susceptibility biomarkers.

A broad range of xenobiotics are metabolized by phase I cytochrome P450 enzymes into reactive intermediates that can affect cellular function and integrity. Phase I enzymes are involved in oxidation, reduction, hydrolysis, activation and detoxification reactions. On the other side, phase II antioxidants play a role in neutralizing the electrophilic sites and increasing its solubility, stimulating the cells for removal and executing the process of detoxification [58].Phase II involved many conjugation reactions for detoxification of xenobiotics compounds such as glucuronidation, sulfation, acetylation, methylation etc**(Figure 9)**[59].

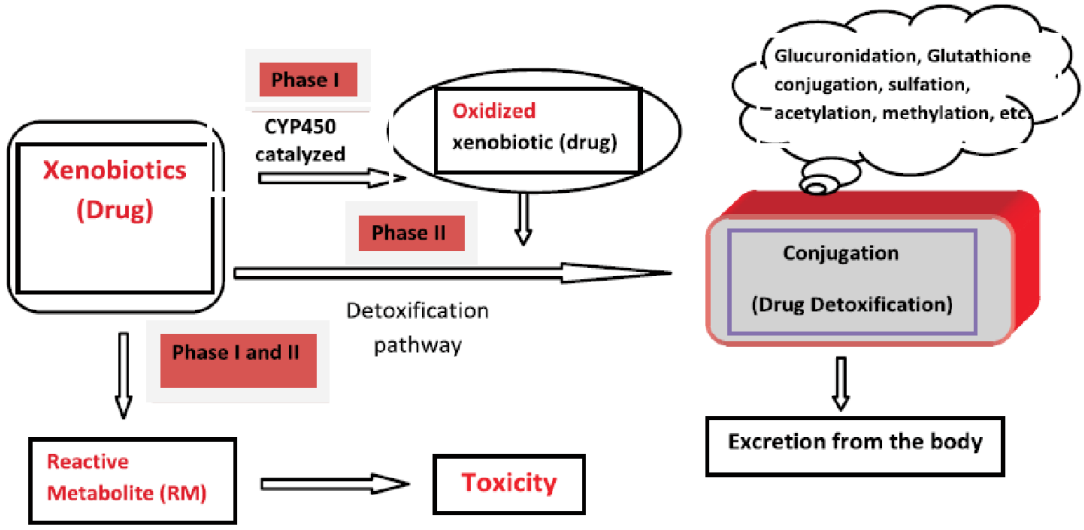


Figure 9: General pathway of xenobiotic metabolism[59].

3.5 Cell survival and apoptosis related genes

Cells respond to stress in different ways ranging from activation of a variety of cellular pathways that promote survival to eliciting apoptosis or programmed cell death to eliminate damaged cells. Cell survival critically depends upon the appropriate response toward the exposure or stress stimuli such as activation of cellular defence system or activation of antioxidative enzymes[60].The mechanism by which a cell dies are apoptosis, necrosis, pyroptosis and programmed cell death depend upon the exposure or environmental conditions.

Apoptosis is mainly initiated or controlled by damage in DNA, viral infection, loss of cellular signalling, other cellular damages, disturbance in gene regulation and other developmental or environmental

factors. The regulatory genes are mainly caspases (3, 9), bcl-2 family genes **(Figure 10)** (bcl-2, bcl-xl, bak, bax) and intermediate regulator genes (p53) which along with other regulatory factors control the process of apoptosis.

bcl-2 gene: This gene encodes Bcl-2 protein in humans that regulate cell death or apoptosis by inhibiting apoptosis. Bcl-2 protein is localized on mitochondrial outer membranes and acts as anti-apoptotic protein through promoting cell survival and inhibiting pro-apoptotic proteins [61].

bcl-xl gene: It encodes B-cell lymphoma-extra large (Bcl-xl) protein that is locatedon the mitochondrial membrane. This is also a member of Bcl-2 family proteins and act as anti-apoptotic through blocking the release of mitochondrial contents like cytochrome c or regulating the action of pro-apoptotic proteins [62]. The combination of Bcl-xl/Bcl-xl, Bcl-2/Bcl-xl and Bcl-2/Bcl-2 inhibit cellular apoptosis and promote cell survival.

bak gene: The bak gene encodesBak protein that acts as pro-apoptotic in the cellular system. Bak is a single-pass transmembrane protein that localises to the mitochondrial outer membrane in cells. This protein in combination with Bax protein activates an apoptotic pathway through permeabilization of mitochondrial outer membrane. This protein also interacts with the suppressor p53 after exposure to cell stress [63].

bax gene: This is a member of the bcl-2 family and encodes Bcl-2-like protein 4 that is pro-apoptotic in nature. The bax gene has been recognised as a pro-apoptotic member of the bcl-2 family [64]. The relative ratio of Bax and Bcl-2 proteins determines cell susceptibility to apoptosis **(Figure 11)**.The functional Bax protein activates the cascade of deleterious events inside and outside the cell through conformational changes, permeabilization of membrane, release of mitochondrial contents and trigger the cascade of caspases [65, 66, 67].

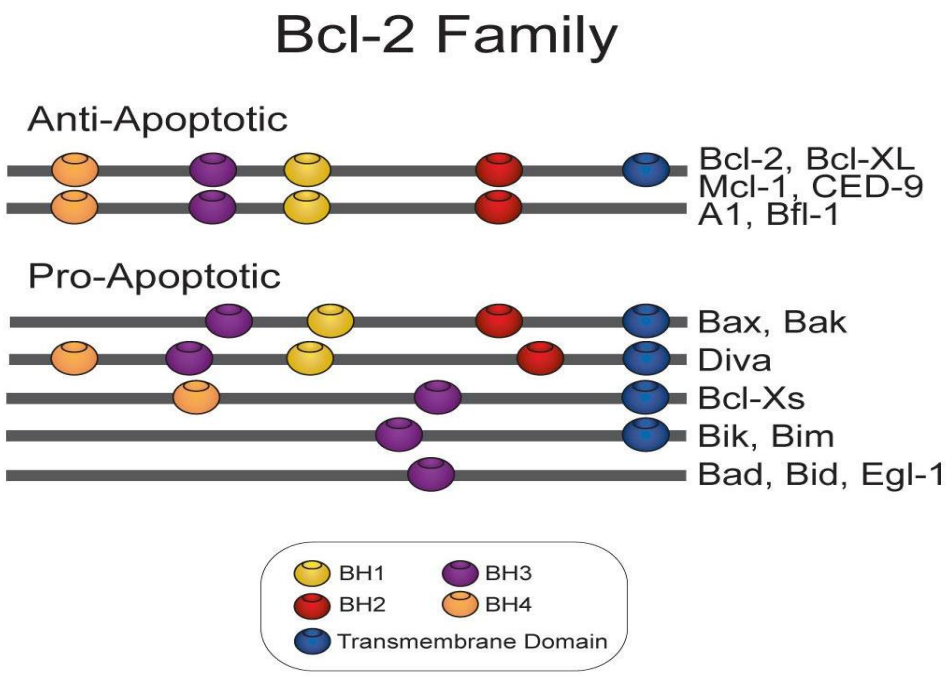


Figure 10: Bcl-2 family genes with domains and nature of action (pro- or anti-apoptotic) (https://en.wikipedia.org/wiki/Bcl-2_family#/media/File:Bcl-2_Family.jpg)

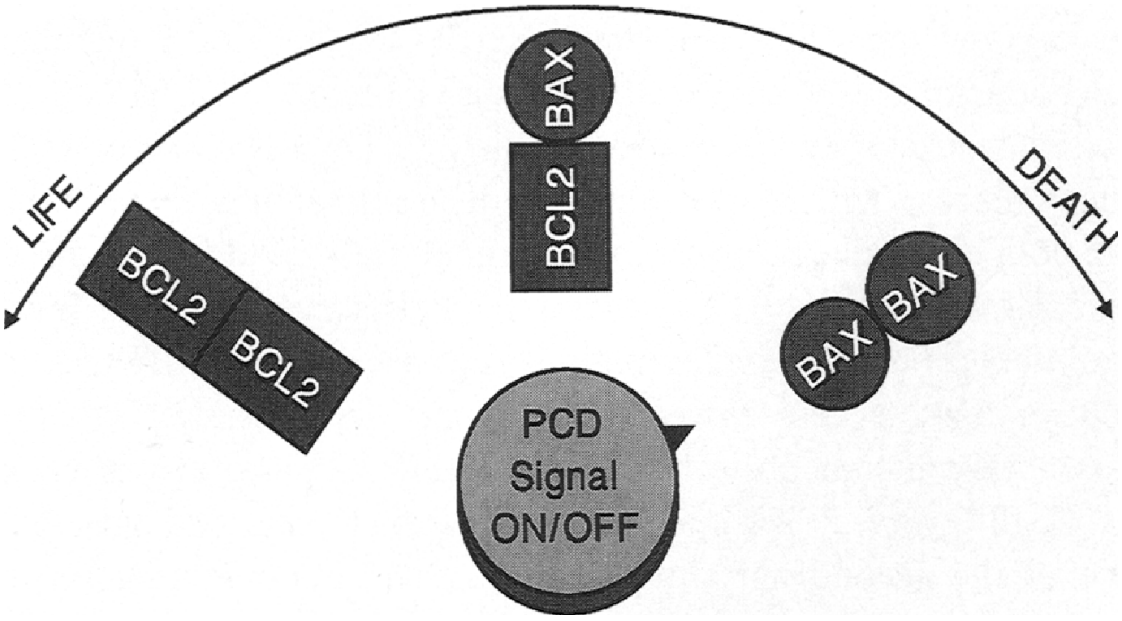


Figure 11: Susceptibility to programmed cell death or apoptosis through relative ratio of bcl-2 to bak[68]

p53 gene: This gene also known as TP53 encodes for a protein p53 which regulates the cell cycle and apoptosis. p53 also described as “the guardian of the genome” or tumor suppressor which have three major functions as growth arrest, DNA repair and apoptosis (cell death) on DNA damage and trigger of other stress signals [69].

caspase 3: It encodes caspase 3 protein which is cysteine-aspartic acid protease and acts as catalyst during apoptosis. Caspases act as proenzymes on proteolytic processing. The caspase 3 activates apoptosis through both intrinsic and extrinsic pathways[70]. Activation of bak and bax results in transfer of cytochrome c which again triggers caspase 9 and caspase 3 [71].

caspase 9: It encodes the protein which acts as an enzyme in humans as initiator caspase. It plays a major role in apoptotic pathways. A protein molecule Apaf-1 interacts with cytochrome c and caspase 9 and activates it to recruit at apoptosome[72]. The caspase 9 also activates caspase 3 and caspase 7 through proteolytic action that finally leads to apoptosis[73].

4. HAIR DYE SAFETY ASPECTS

4.1 Allergic reactions

The contact sensitization epidemiology of p-phenylenediamine (PPD) and hair dyes intermediates have been evaluated lately [74,75]. PPD, the absolute ordinary “primary intermediate” of permanent hair dyes and others like p-toluenediamine sustain the ability to develop contact allergy by inducing different pathways in the human body **(Figure 12)**. Many research studies on animals and humans confirmed the allergic potential of these intermediates [76]. Potential towards the development of allergic reactions by PPD and other respective intermediates are because of generation of oxidized products [77]. It has been reported that the elevated level of PPD in human and other mammals causes Quinke’s disease which results in intense problems in respiration, breakdown of skeletal muscles which leads to kidney failure, muscles pain and weakness and 7 g of para-phenylenediamine may cause death of retinal ganglion cells that can adversely affect vision in human [78].

Labels of warning phrases like “Able to elicit allergic reactions”; “Consist of phenylenediamines”; “Avoid to apply on eyelashes or eyebrows” on oxidative hair dyes were decided in EU to advise consumers and salon workers to check a test for allergy before applying the dyeing material [79]and to wear gloves to cover skin at the time of hair dyeing. Patch test reported that rise in skin allergic reactions because of PPD is nearly 5% of 36,000 randomly chosen patients suffering from eczema that means after a few major allergens PPD was listed as most sustained allergen[75, 80]. PPD displays rates of 0.2–0.5% in healthy individuals. Another study demonstrated that 0.2% population of 83000 patients suffering from contact allergy was probably because of hair dye compound i.e. PPD [80]. PPD was listed as 5thto 16thand most eternal to supreme or frequent allergen throughout 1985 to 1996 in various studies[81]. A novelanalysis for cosmetics confirmed a slow and constant rate of allergy because of hair dye. Other studies found association between hair dye allergy and black henna tattoos [82].

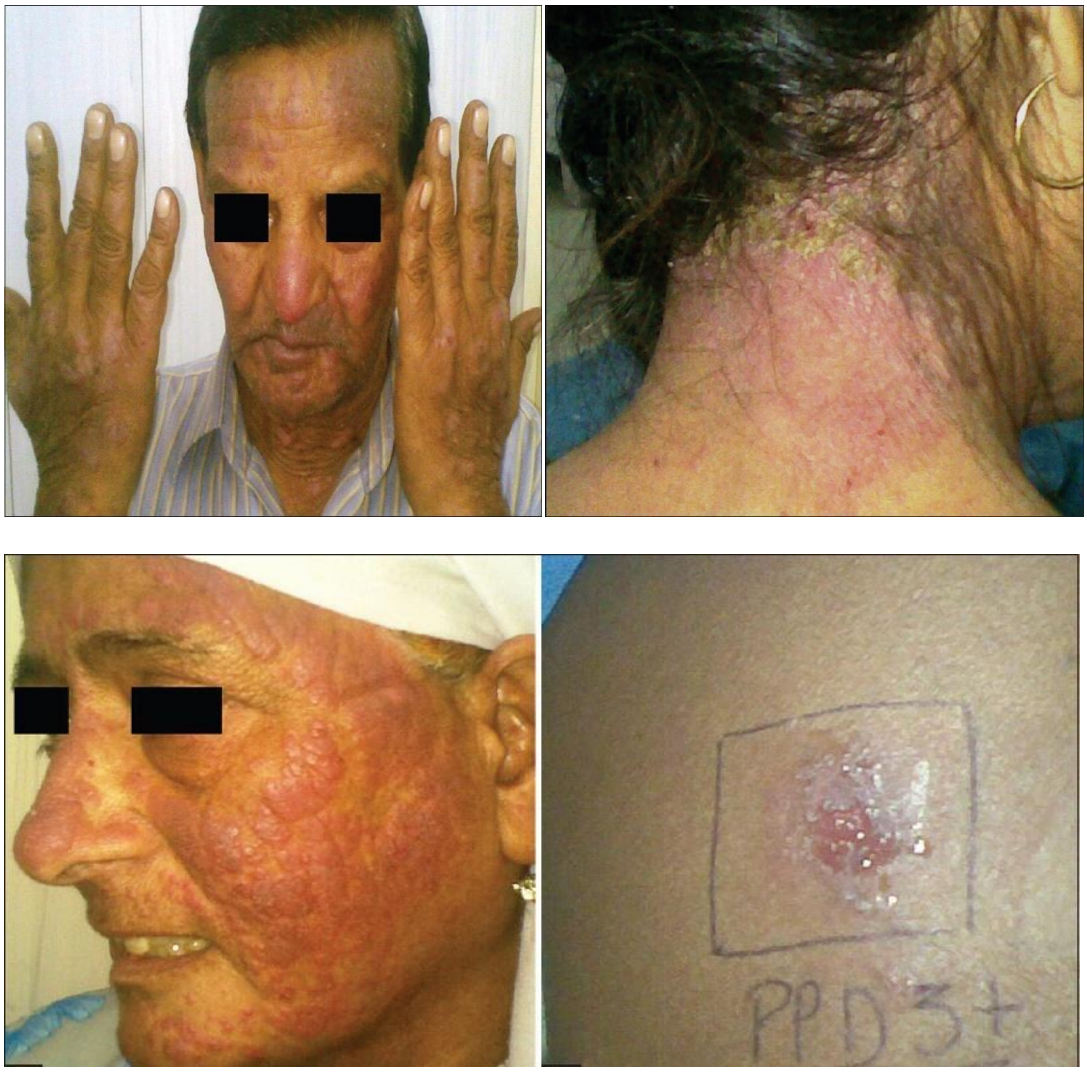


Figure 12: Allergic reactions due to p-phenylenediamine[83]

The accessible familiarization of risk management measures involve using some protective means such as using some protective apparel to reduce hair dye contact with scalp and skin during application of hair dye. Another one is precaution labelling which increases threat awareness among consumers and hairdressers [84]. Occupational hairdresser's exposure to hair dye ingredients may put them at a threat of dermal allergy [85].

In brief, ingredients of oxidative hair dyes have allergic potential that may cause adverse effects to the exposed consumer population which can be minimized by using precautionary measures.

4.2 Genotoxicity of hair dyes

For prediction of genotoxic and carcinogenic potential of compounds/substances, genotoxic short-screening studies are carried out. On the basis of experiments performed in biological entities and

outside to their natural environment, genotoxic hazard and pathways were put forwarded by many scientists [86, 87, 88]

Salmonella typhimurium assay (STA) by Ames, detecting the events of reversal mutation in deficient mutants of histidine synthesis is a prime test to measure genotoxicity. The results of STA provoked the dispute for the genotoxicity and carcinogenicity by many colouring products for hair in 1975. Ames manifested that about 85-90% constituents of hair dyes were able to cause mutations in STA that may put consumers at high risk of carcinogenicity [2]. Modern scrutiny of Ames test informed 77% correlation between 368 rodent cancer causing and non-cancer causing substances and many other studies informed that about 50% rodent cancer causing substances are positive by this assay[89].

According to US National Toxicological program 33 arylamine were found invariably positive by Ames test on mice and rats but only 16 were found carcinogenic to rodents (Ashby and Tennant, 1991). Different in vitro mutagenicity assays like thioguanine resistant (HPRT) and others demonstrated low potential of carcinogenicity by aromatic amines on mammals of the order rodentia[90].

All these tests require incubation time of many hours with test substances of hair dye whereas colouring of hair requires just 30 minutes, so it is unable to correlate all the chemical reactions within hair and development of carcinogenicity by hair dye ingredients. Peroxide of hydrogen is a key ingredient of hair dyes which is cytotoxic as well as genotoxic but many in vitro genotoxic studies do not include this ingredient. For example PPD alone was found non-toxic but when it was left in air or with oxidant it showed positive results, thus indicating the importance of exposure time and oxidation by peroxide.

Different studies found that hair dye are mutagenic and carcinogenic in nature [91]. There are reports that indicate hair dye and their ingredients induce free radical formation and can exert site directed DNA damage [92]. A study determined chromosomal aberration after repeated exposure of hair dyes with an increase in chromosomal aberration rate[93].

Oxidative hair dye component p-aminophenol showed an increased DNA damage in mice splenocytes and bone marrow using micronucleus assay [94]. P-aminophenol also shows significant impacts in several in vitro assays like sister chromatid exchange and chromosome aberration assays [95].

So, it is a prime requirement to build up data first from all the chemical, genotoxic and other biological reactions of oxidative hair dyes, their ingredients and derivatives effects, type of human exposure, significant quantity and genotoxic assessment for human risk so that meaningful results can be obtained.

5. CARCINOGENICITY OF HAIR DYE

Probable carcinogenicity potential of oxidative hair dyes, its ingredients and their derivatives draw the attention of toxicologist and epidemiologist for many years because these oxidative hair dyes belongs to large family of aromatic amines which cover known carcinogens like 4-aminobiphenyl, benzidine and 2-naphthylamine.

The ability to cause cancer by oxidative hair dye and their ingredients was detected by both industries and different researchers. The IARC of WHO and U.S. National Toxicology Program also explored the potent carcinogenicity of hair dyes[96, 97].

6. EPIDEMIOLOGY

The evidence for cancer hazards to industries workers, hairdressers and the consumer which come in contact regularly to hair dyes were reviewed and summarized by Rollisonet al. (2005)[98]. Study revealed that some other cancers showed small increases as compared to urinary bladder cancer in females in the specific hairdresser population[99].

Increased incidences of cancer in the urinary bladder in female consumers were reported in an observational study [100]. Another research study indicated the elevated cancer risk in bladder within the same population having slow acetylation response of N-acetyltransferase 2 (NAT2) that functions as a major detoxification enzyme in liver for arylamines [101]. So it was considered that populations with NAT2 slow acetylation activity might have higher risk for developing bladder cancer [102].

Collectively, epidemiological studies on hair dyes correlated with bladder cancer conducted in the US [103], Europe [104] and similarly two other studies on carcinogenicity of dye products [105, 106]demonstrated that there were no evidences between bladder cancer and hair dyes. Finally, a very recent investigation considering nearly 70,000 women for almost 7 years concluded that there were no enhanced events of bladder cancer by using hair dyes[107]. Data suggested that there are no incidental demonstrations related to bladder cancer by hair dyes and other constitutes[108]. A study based on observational incidences of acute myeloid leukemia in hospitals with 722 cases and 1444 controls with respect to hair dye exposure found no increased risk of leukemia due to personal use of hair dye[109]. Another observational study based on use of hair dye with respect to occurrence of bladder cancer in general population was performed in Netherland[110] and combined data using statistical procedure of 15 case control and 2 cohort studies [111] also indicated no relative risk of occurrence of bladder cancer.

Though, there are so many studies which did not have enough data and strong evidence to demonstrate relativity between cancer and use of hair dye among the exposed population[105, 111]. Many other studies also didn’t detect any link with occurrence of cancer in mammary gland in breast, respiratory organs, bladder, digestive organs, colon or rectum and hematopoietic cancer in hair dye consumers [112].

Although, all the measures were taken during these studies but also have some limitations like population size, time duration and exposure intensity. So, more studies should have been evaluated carefully for occurrence of cancer because of hair dyes [113].

7. SUB-ACUTE TOXICITY OF HAIR DYES AND ITS INGREDIENTS

Some crucial genes regulate division, proliferation and cell death programme known as programmed cell death that plays a critical role in apoptosis. Apoptosis execution ensures that damaged and irreparable cells with weird division cycle are removed from the generative cell pool and stop cell transformation.

Two main pathways, receptor mediated extrinsic and cysteine rich caspases activated mitochondrial intrinsic pathway are known to date for apoptosis. Error at any stage including cell pathways make cells unable to undergo apoptosis and this leads to cancer progression [114, 115].

Different studies demonstrated increased incidences of tumour in rats after PPD treatment. Two in vitro studies on different cell lines investigated apoptosis pathway. One study on PPD induced apoptosis usingMDCK cells [114]observed a declineinviability of cells in dose dependent manner. Several observations like DNA fragmentation in TUNEL assay, sub G1 peak and Go/G1-phase arrest and positive Anenexin-v staining indicated presence of apoptosis. Systemic reactive oxygen species and stress make membrane permeable to generate reduction potential that may lead to activation of p53 gene and also the cell death pathways which may be involved in apoptosis [114,116]. Another study carried out within rat kidney cells (NRK-52E) demonstrated that dye ingredient (PPD) initiated cell death by up-regulating the expression of protein kinase of stress activated phosphorylate (SAPK)/N- terminal kinase of c-Jun (JNK) protein and downregulation of Raf and Ras proteins but somehow level of Akt, Bad, Bc-xl and Bcl-2 protein were remain unchanged. This indicated that PPD induced apoptosis through any of Ras/JNK/Raf/ PTK dependent pathways and not dependent upon Akt/PI3K pathway [115]. In human urothelial cells, PPD induced apoptosis occurs via blocking NF-kB activation and m-TOR and Wntsignalling[117].

PPD mediated apoptosis in Chang liver cells demonstrated activation of MAP kinase pathway through activation of p38, MAPK, ERK and SAPK/JNK [118]. In another study, Chyeet al. (2008) also demonstrated that PPD has the potential to induce DNA strand breaks in human lymphocytes[119].

8. METABOLISM OF HAIR DYES

In various research studies metabolism of arylamines especially from dyes and body care products have been studied on a large scale and reported publicly. N-acetylation of hair dyes and its ingredients such as PPD (arylamine) occur in skin and the key enzyme is NAT1[120, 121, 122, 123]. N-acetylation represents a detoxification reaction which leads to make compound non-genotoxic and non-carcinogenic metabolites[123, 124, 125, 126]. N-acetyltransferase-2 (NAT-2)is found in liver and gut which catalyzes the conversion of PPD in to N-mono and N,N-diacetylated (MAPPD and DAPPD respectively) metabolites [127, 128]. Systematic exposure of PPD to the human body releases metabolites through the liver which usually excreted in urine.

It was found that PPD and hair dye metabolites are excreted in urine through renal clearance [129]. In human study, subjects treated with oxidative hair dye containing C_{14}PPD excreted five different types of metabolites in urine having a majority of MAPPD and DAPPD metabolites. About 80-90% of absolute metabolites were found along with glucuoronic acid conjugates [122]. Because of slow acetylation activity of NAT2 the individual may have a high cancer threat for bladder which was proven by a study among cigarette smokers with slow NAT2 acetylators[121].

Another key step in activation of arylamines is CyP450 oxidation which converts aromatic amines to N-hydroxylamines which finally leads to formation of nitrenium ions by O-acetylation or sulfation that can damage DNA and trigger a carcinogenic effect in bladder[130].

CONCLUSION

The growing worldwide demand for hair dye among adults and adolescents has created an understanding of the safety concerns associated with their use. The main objective of this review is to highlight the toxicological effects of chemicals in hair dye and highlight their long-term use associated with possible health risks. Hair dyes are a kind of silent enemy for humans, and continuous exposure can cause serious health problems for users. As reported in the past, many hair dyes and theirconstituents have been classified as possible human carcinogens. This chemical has a penetration stimulator that facilitates skin penetration and toxicity.

Overall, this review highlights the negative health effects for hair dye users, especially when exposed systematic and dermal route that induce oxidative stress-mediated cytotoxicity; therefore, some safer alternatives should be selected and considered for the total safety of mankind.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

Conflict of interest

The authors report no financial or any other conflicts of interest in this work.

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