Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2010; 3(1):1-5

ISSN: 0975 4873

Research Article

Oxidative Stress and Anti-oxidant Status in Hair Dye Poisoning

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ABSTRACT:
Para phenylenediamine (PPD) is a very common ingredient in most of the hair dye preparations. It accelerates the dyeing process and may produce local as well as systemic toxic effects when applied topically or ingested. It is highly toxic when taken by mouth and the outcome depends mainly on the dose taken. The present study is planed to estimate of this PPD in the dye poisoning patents using a sensitive RP-HPLC method using UV-Visible detection (486nm). The serum proteins were precipitated by a single step liquid-liquid extraction using Ethyl aceto acetate (EAA). Chromatographic separation was achieved with a combination of Ammonium acetate buffer and acetonitrile at 80:20 v/v ratios was run isocratically through a C_{18} (250mmX4.6mm, 5µm) reverse phase analytical column. Glutathione, Malondialdehyde (MDA), and Creatine phosphokinase (CPK) were estimated and corelated with PPD levels.

Key words: Para phenylenediamine (PPD), Creatine phosphokinase (CPK), MDA, Glutathione, hair dye poisoning.

INTRODUCTION

Hair dye poisoning occurs when someone swallow dye or tint used to color the hair. Para Phenylenediamine (PPD) is a very common ingredient in most of the hair dye preparations. It accelerates the dyeing process and may produce local as well as systemic toxic effects when applied topically or ingested. It is highly toxic when taken by mouth and the outcome depends mainly on the dose taken. Unfortunately they have also been commonly misused for self poisoning (suicide). Accidental consumption of hair dye is rare, the lethal dose of PPD is exactly not known and it varies from 7-10grams.

Para phenylenediamine ingestion produces a typical edema of the head and neck[1,2,3], rhabdomyolysis and acute renal failure[3,4,5,6,7], acute tubular necrosis[8]. PPD also contribute factor for non- Hodgkin's lymphoma, multiple myeloma, or other cancers[9]. PPD induces COX-2 mRNA, protein and enhanced PGE_2 and $PGF_{2\alpha}$ formation; it also induces COX-1 mRNA and protein[10]. PPD breaks single strand DNA in human lymphocytes[11]. PPD causes systemic toxicity[12]. Dyes contain resorcinol in addition to PPD causes nephrotoxicity[13].

MATERIALS AND METHODS:

Para Phenylenediamine (PPD), methanol (HPLC grade), acetonitrile (HPLC grade), disodium hydrogen phosphate DTNB reagent, glutathione, sodium dihydrogen phosphate, thiobarbituric acid, ammonium acetate, trichloroacetic acid and 1, 1, 3, 3-tetra ethoxy propane.

Estimation of Lipid Peroxides: In this method the amount of lipid peroxidation products present in the serum samples were estimated by the thiobarbituric acid reactive substances (TBARS) method. To 0.5ml of serum 0.5 ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30sec. Clear supernatant was taken after centrifuging at 3000rpm for 10min. To the supernatant 100µl of 1%TBA solution was added and the solution was heated for 1hr at 98^0C. It was kept in ice for 10-15mins. Then the supernatant was collected. The mixture which was in pink in color and absorbance was read at 532nm.

Estimation of Glutathione: Glutathione forms a colored complex with DTNB, which is measured spectrophotometrically. To 0.5ml of citrated blood, 0.5ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000rpm for 20mins. To 0.1ml of supernatant, 1ml of sodium phosphate buffer and 0.5ml of DTNB reagent were added. The absorbance of the yellow color developed was measured at 412nm. The glutathione content was determined from standard graph by using pure glutathione.

Estimation of Creatine phosphokinase (CPK): Serum creatine phosphokinase was estimated by using XL system packs from ERBA-Mannheim diagnostic kit (Creatine-kinase reagent DGKC method).

Estimation of Para Phenylenediamine (PPD) by HPLC method

HPLC description: A Cyber lab HPLC system used in the study consisted of a pump (Model LC-P100, Cyber lab corporation, USA) operating at 1ml/min, a syringe

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loading sample injector of 20µl capacity (Model 7725i), a C_{18} reverse phase column of 250 X 4.6mm dimension and 4µ particle size and a dual wavelength UV-Visible detector (Model LC-100).

Table 1: Standard graph of PPD

PPD conc. (µg/ml)	Peak area
2	0.2122
6	0.4179
10	1.0791
14	1.6589
40	2.6879
60	7.0052
100	9.8198

Chromatographic conditions: The mobile phase consisted of Ammonium acetate buffer and Acetonitrile (80:20) v/v. The mobile phase was filtered through 0.22µm membrane filter. The flow rate was 1 ml/min and the effluent was monitored at 486nm. Retention time is 20.25min.

Table 2: Standard graph of glutathione

S. No	Conc.	Abs (µ. mol/ml)
1	5	0.008
2	10	0.077
3	30	0.295
4	50	0.512
5	70	0.708

Extraction of PPD from serum samples: PPD was extracted from serum samples by using Ethyl aceto acetate (EAA). T0 100µl of serum 250µl of ethyl aceto acetate was added, samples were thoroughly mixed by vortex mixer, then the samples were centrifuged at 3000rpm. After 15min. supernatant was separated in to other tubes, these were subjected to evaporation. Then dried tubes were reconstituted by 150µl of mobile phase. After thorough mixing these samples were injected in to HPLC system.

Preparation of calibration curve of PPD: 1mg of PPD is weighed and dissolved in 10ml of methanol that will give 100µg/ml solution and this was further diluted to get 2, 6, 10, 14, 20, 60µg/ml solutions

Table 3: Standard graph of 1, 1, 3, 3-tetraethoxy propane (MDA)

S.No	Concentration (nmol/ml)	Absorbance
1	10	0.124
2	30	0.389
3	50	0.568
4	70	0.756
5	100	0.998

RESULTS AND DISCUSSION:

Glutathione: Glutathione (GSH) a tripeptide comprised of glutamate, cysteine and glycine. GSH is present in mast cells, where it functions as an antioxidant protecting cells from toxic effects of ROS. In addition to its antioxidant role GSH plays a vital role in maintenance of cell viability, DNA replication. GSH has also been reported to regulate immune cell functions. GSH has shown to play an important role in apoptosis and to regulate antigen-presenting cell functions[19]. Glutathione peroxidase catalyses the conversion of reduced glutathione present in the peroxysomes of cytoplasm to oxidized glutathione which is toxic to the cells. PPD induces over production of free radicals and subsequently results in lipid peroxidation. The glutathione levels were depleted with increase of time suggesting decrease of anti-oxidant status in PPD poisoning. The normal value of glutathione is 9.593±7.00 (n=20).

Malondialdehyde (MDA): MDA is one of the aldehyde products of lipid peroxidation. The lipid peroxidation is initiated by an existing free radical ($X^{\bullet}$), by light, metal ions or by toxins. Malondialdehyde is only formed by fatty acids used as a measure of lipid peroxidation. The normal values of MDA is 16.29±7.85 (n=20).

Table 4: Glutathione and MDA levels in PPD patients at various time periods

Time	Glutathione (µ.mol/ml)	MDA (n.mol/ml)
3rd hr (n=30)	7.00±2.53	21.71±9.58
6th hr (n=28)	6.42±2.37	32.44±13.12
12th hr (n=27)	5.50±1.37	35.99±13.22
24th hr (n=31)	5.66±2.214	41.73±14.78

Depletion of glutathione levels and elevation of MDA levels suggesting decrease in antioxidant status and an overwhelming the oxidative stress respectively. With the symptomatic therapy there is improvement.

Table 5: CPK levels (Mean ± SD) in healthy volunteers and PPD patients at various time Periods in men and women

Women	CPK (IU/L) (n=28)	Men	CPK (IU/L) (n=10)
Normal(n=6)	115.±30.383	normal(n=6)	126.5±26.95
3 hr	329.049±354.77	3 hr	197.465±54.62
6 hr	641.9595±930.6	6 hr	389.7±261.89
12 hr	791.788±801.57	12 hr	733.45±439.7
24 hr	928.898±871.53	24 hr	879.06±419.77

There is a steep increase in serum levels of CPK at various time points in hair dye poisoning

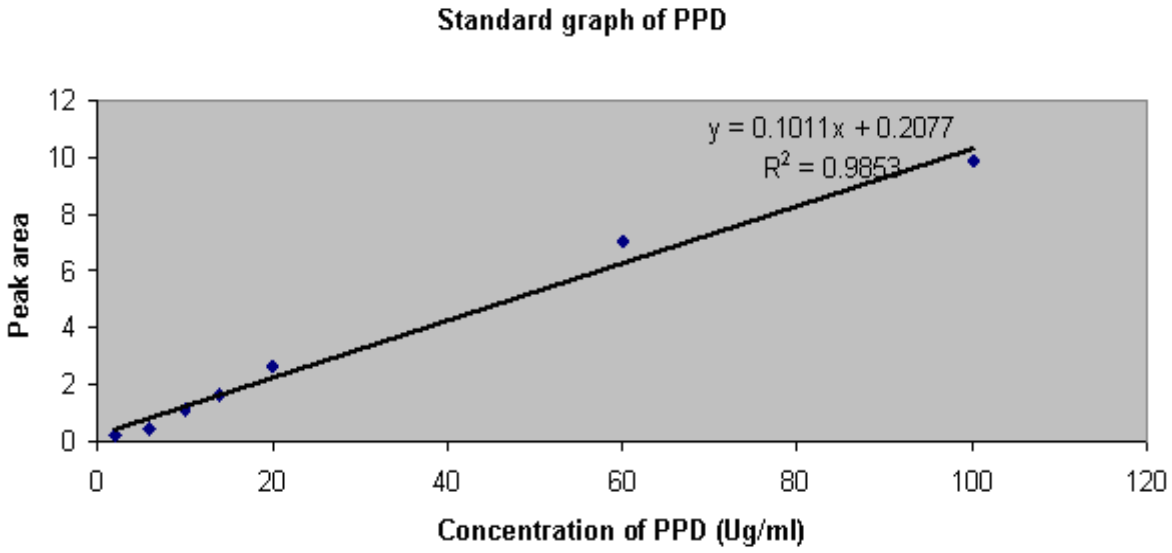

Fig 1: Standard graph of PPD

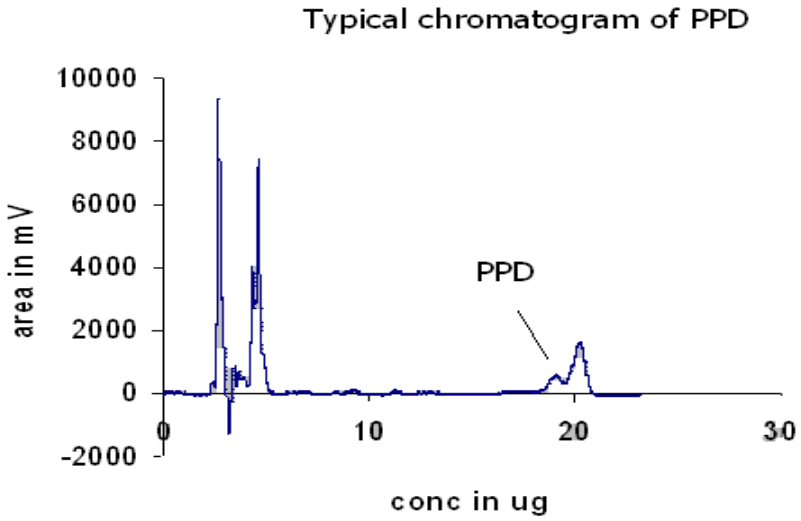

Fig 2: Chromatogram of PPD

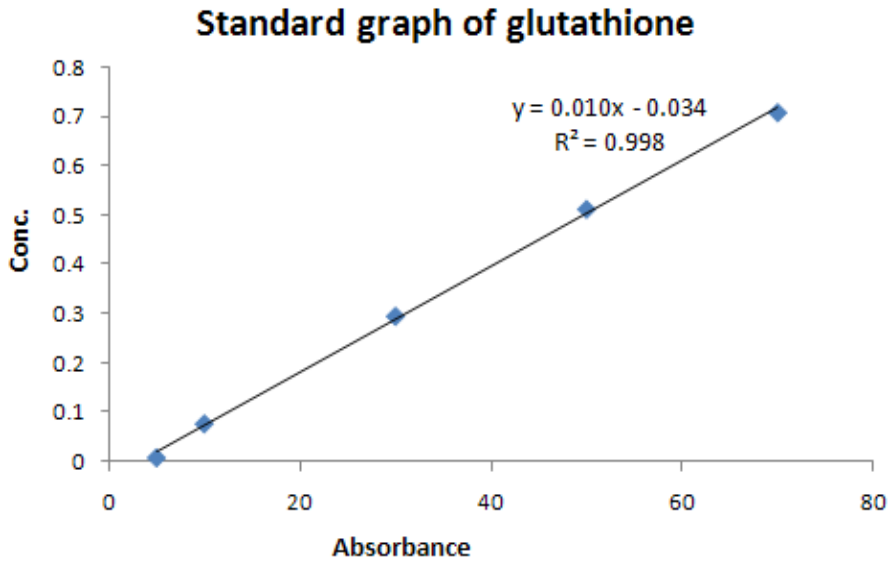

Fig 3: Standard graph of Glutathione

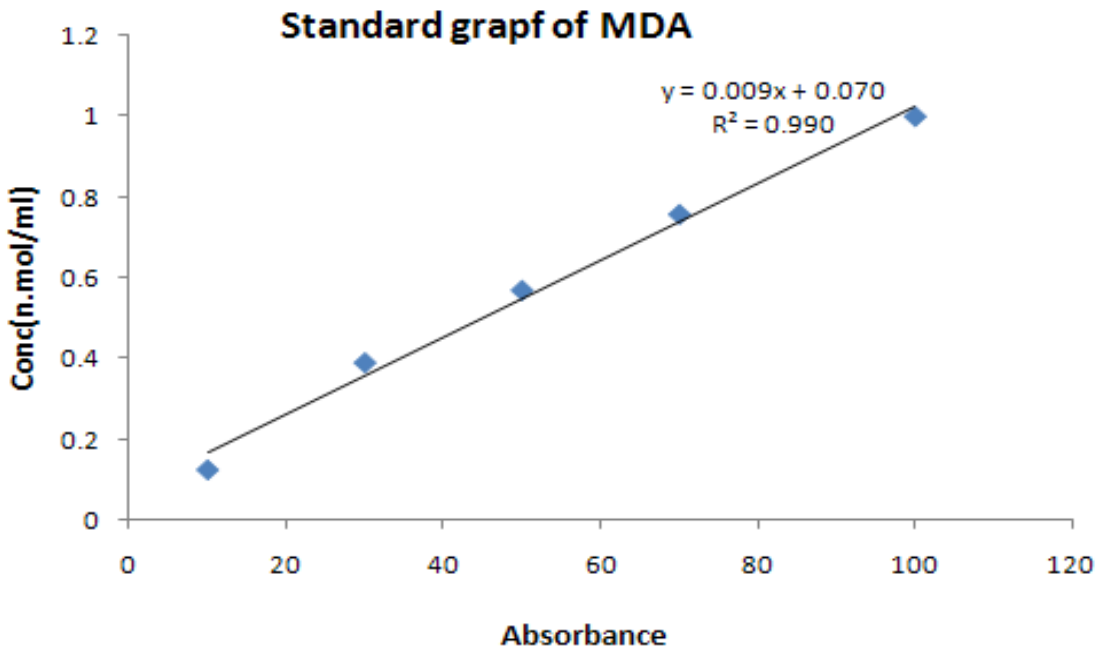

Fig 4: Standard graph of MDA

suggesting severe ongoing muscle damage, which could not be countered by symptomatic therapy.

4. CONCLUSIONS:

Ingestion of PPD can be fatal and the physician should asses the patient for early detection and treatment. This study suggests that apart from symptomatic treatment there is a necessity for the development of specific antidote for poisoning and a supportive antioxidant therapy to decrease the stress and the muscle damage. Further studies are required to elucidate the interactions between alcohol and hair dyes.

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