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Toxicity Effects of Hair Dye Application on Liver Function in Experimental Animals

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Abstract

Objective: This study was conducted to assess the hair dye toxicity by using hair dye among experimental rats in order to verify the biochemical and haematological abnormalities and liver dysfunction.

Methods: Albino Wistar Rats were obtained from the Faculty of Pharmacy, University of Khartoum– Sudan. The rats were divided into two batches on the basis of using the commercial hair dye as oral or subcutaneous administration respectively; each batch has four groups (control and three test groups) each comprising six rats. Batch-1 (group-2, 3, and 4 orally administered with 10, 20, and 30mg/kg body weight of the commercial hair dye, respectively); and Batch-2 (group-2, 3, and 4 subcutaneously administered with 10, 20, and 30 mg/kg body weight of the commercial hair dye, respectively).

Results: The clinical features were shown in all rats batches, administered orally or subcutaneously with the commercial hair dye. These clinical features rates from slight weakness in group 2 to head, neck, and pharyngeal oedema in group-3 up to severe weakness in hinds and fore limbs with election of hair, tremors, shivering of the whole body and respiratory distress, severe convulsions, and respiratory difficulty prior to death in group-4. The Biochemical parameters showed significant ($P<0.05$) increase in the activities of the liver enzymes concomitant with the increase of the commercial hair dye dosage in the two batches, and decrease in the total plasma protein levels, albumin, and cholesterol with the increase of commercial hair dye dosage in the two batches. Hematological parameters showed a significant (p value <0.05) decrease in complete blood count (associated with significant decreases in neutrophils and significant increases of lymphocytes) concomitant with the increasing of commercial hair dye concentration.

Conclusion: The study highlighted the major toxicity of commercial hair dye and its association with liver dysfunction.

Keywords: Hair dye; Paraphenylenediamine; Toxicity; Liver atrophy; Parameters; Experimental animals; Sudan

Introduction

Henna is very popular culture in Sudan; it is part of the traditions which used to adorn women's body during marriage ceremonies and other social celebrations since the Bronze Age. Henna is commercially cultivated in Sudan and other countries. Despite the wide spread use of natural henna, reports of allergic contact dermatitis to natural henna are very rare in the literature. It can therefore be assumed that natural henna is safe [1]. The first artificial dye was synthesized in the laboratory in 1856, and permanent hair colorants have been in commercial use for over 100 years [2].

Para-Phenylenediamine (PPD) is an organic compound; its chemical formula is C6H8N2 [3]. This derivative of aniline is a white solid, but samples can darken due to air oxidation. It is also an ingredient used in Sudan and other countries in combination with henna "lawasonia Alba" for tattooing to give black color in a short time in traditional and during local and social festival. It was found to be toxic and there are some reports from these countries showing its toxicity on different systems of the body. The consumers use this product because its price is 20-30 times less expensive than pharmaceutical hair dye preparations [4].

Many accidental cases of toxicity and mortality have been reported in Sudan, Egypt and other countries in cases of suicidal and homicidal due to oral ingestion or subcutaneous mistaken used of hair dyes containing Para-phenylenediamine [5]. There are many studies showed effects on respiratory, renal, and muscular system, but no study determines the effects on all these systems together, and no study describes the correlation of PPD toxicity to body's biochemical alterations in liver [6].

There was a continuous inflow of suicidal and homicidal cases in Sudanese hospitals and the causes of poisoning with PPD are much conflicting in the determination of clinical order of PPD Patients [7].

As PPD is the main ingredient on hair dyes, and whose toxicity is directly related to human health. So this paper studied the toxicity of hair dye in vivo, to determine the biochemical and haematological abnormalities associated with major toxicity of commercial hair dye and liver dysfunction among experimental animals.

Methodology

This study was conducted at national research center-University of Khartoum. The commercial hair dye was collected from local markets (Libya Market–Omdurman).

Albino Wistar male rats at age of 11 weeks, weighting 140-160 g were obtained from the Faculty of Pharmacy, University of Khartoum–Sudan. The animals were housed in cages provided with rice husk as bedding materials and kept under ambient temperature of 23 ± 2°C. The animals were kept in the laboratory condition for 1 week to adapt the climate condition and for the commencement of treatment protocol. The rats were divided into two batches on the basis of using the commercial hair dye as oral or subcutaneous administration respectively; each batch has four groups (control and three test groups) each comprising six rats. Batch-1 (group-2, 3, and 4 orally administered with 10, 20, and 30 mg/kg body weight of commercial hair dye, respectively); and Batch-2 (group-2, 3, and 4 subcutaneously administered with 10, 20, and 30 mg/kg body weight of commercial hair dye, respectively). The animals were killed after 3-6 days after the administration. The lethal dose of commercial hair dye for rats was determined as 80 mg/kg body weight [8] and the lethal subcutaneous dose was determined as 37 mg/kg body weight [9]. Hence, we tested the toxicity of various sub lethal doses through different routes considering the LD50 of PPD is 37 mg/kg.

Two milliliter of blood samples were collected from eye blood vessels of each rat in ethylenediamine tetra acetic acid (EDTA) container for hematological tests and other 2 ml of blood samples were collected in heparinized containers for biochemical tests. Plasma was separated by centrifugation at 3000 rpm for 5 min.

Total proteins, glucose, cholesterol, albumin, and the enzyme activities of GOT, GPT, and ALP were measured spectrophotometrically by using commercial kits. Determination of hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs) count, and total white blood cell (TWBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH), PLT count, (Lymphocytes–Basophil, Neutrophil) were analyzed by a semi-automated hematological analyzer (Sysmex Corporation; Mundelein, Illinois, Sysmex America, Inc.).

Statistical analyses were performed using statistical package for social sciences (SPSS) version 11.5 and excel 2007 statistical program. Continuous and categorical variables were analyzed using student's t-test and Chi-square test respectively. P value was considered significant if it was less than 0.05.

Results

Clinical features were shown in all rats administered orally or subcutaneously with the commercial hair dye, however, the clinical features rate from slight weakness in group 2 to head, neck, and pharyngeal oedema in group-3 up to severe weakness in hinds and fore limbs with election of hair, tremors, shivering of the whole body and respiratory distress, and there were severe convulsions and respiratory difficulty prior to death which occurred at about four hours post oral ingestion of the commercial hair dye in group-4. As seen in Table 1 and Table 2, the biochemical parameters showed significant (P<0.05) increase in activities of the liver enzymes glutamate oxalotranserase (GOT), glutamate pyruvate transferase (GPT), and alkaline phosphatase (ALP), and there is a decrease in the total plasma protein levels, albumin, and cholesterol when compared with the control groups.

Groups / Parameters	Group 1 (Control)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (30 mg/kg)
GOT (U/L)	41.3 ± 2.1	1219.5 ± 12.1***	1581.8 ± 30.9***	1690.0 ± 23.7***
GPT (U/L)	40.1 ± 1.7	127.8 ± 1.2***	242.8 ± 7.2***	295.0 ± 28.8***
ALP (U/L)	115.3 ± 3.2	113.7 ± 2.8	129.0 ± 1.4*	136.0 ± 2.2**
T. proteins (g/dl)	7.5 ± 0.7	7.0 ± 0.6	6.7 ± 0.3	6.3 ± 0.5*
Glucose (mg/dl)	105.3 ± 11.0	137.8 ± 1.7**	127.8 ± 0.8*	113.5 ± 3.6
Cholesterol (mg/dl)	88.5 ± 15.8	60.2 ± 5.9***	67.5 ± 4.4**	79.0 ± 3.7*
Albumin (g/dl)	4.2 ± 0.7	4.8 ± 0.3	3.7 ± 0.3*	3.2 ± 0.2*
* = P<0.05; ** = P<0.01; *** = P<0.001				

Table 1: Showing the mean differences of Biochemical parameters between the study groups when received different oral ingestion doses (10-20-30 mg/kg b.w.) using the commercial hair dye.

Groups / Parameters	Group 1 (Control)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (30 mg/kg)
GOT (U/L)	41.3 ± 2.1	1311.7 ± 3.1***	1663.7 ± 2.3***	1790.5 ± 1.0***
GPT (U/L)	40.1 ± 1.7	138.0 ± 0.9***	242.3 ± 2.2***	302.0 ± 2.1***
ALP (U/L)	115.3 ± 3.2	112.2 ± 1.9	129.0 ± 1.4*	136.7 ± 2.1**
T. proteins (g/dl)	7.5 ± 0.7	6.8 ± 0.4	6.5 ± 0.3	6.0 ± 0.2*
Glucose (mg/dl)	105.3 ± 11.0	138.8 ± 2.1**	127.3 ± 2.2*	115.3 ± 2.2
Cholesterol (mg/dl)	88.5 ± 15.8	59.5 ± 1.9***	65.8 ± 1.5**	77.2 ± 3.0*
Albumin (g/dl)	4.2 ± 0.7	4.8 ± 0.3	3.7 ± 0.1*	3.9 ± 0.3
* = P<0.05; ** = P<0.01; *** = P<0.001				

Table 2: Showing the mean differences of Biochemical parameters between the study groups when received different subcutaneous doses (10-20-30 mg/kg b.w.) using the commercial hair dye.

These differences associated with the increase of the commercial hair dye dosage in the two batches. Blood glucose showed significant

increase among different doses of oral or subcutaneous commercial hair dye compared with the control groups. Despite the different route of commercial hair dye administration, the results showed slight increase in the mean results of GOT, GPT and total protein when the commercial hair dye administered subcutaneously.

Compared with the control groups, hematological parameters showed significant (p value <0.05) decrease in Hb, RBCs, PCV, TWBCs count (associated with significant decreases in neutrophils and significant increases of lymphocytes), MCH, and MCV relevant to the increasing of the commercial hair dye concentration. Despite the significant decreases (P<0.05) in the percentage of neutrophils count, the platelets and lymphocytes showed significant (P<0.05) increase associated with increasing concentrations of the commercial hair dye in the different routes (Table 3 and Table 4).

Groups / Parameter	Group 1 (Control)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (30 mg/kg)
Hb (g/dl)	12.85 ± 0.67	10.38 ± 0.73*	9.59 ± 0.68*	8.67 ± 0.82**
PCV (%)	44.67 ± 0.52	37.57 ± 0.88*	27.00 ± 2.83***	25.33 ± 2.73***
RBCs$^{\times 10^3}$ /CMM	5466.00 ± 859.45	5116.67 ± 231.66*	4083.33 ± 365.60**	3483.33 ± 172.24***
TWBCs /CMM	7600.00 ± 2182.65	5133.33 ± 2182.66***	4133.33 ± 659.29***	2900.00 ± 209.76***
MCH (pg)	29.83 ± 2.32	20.0 ± 2.19*	18.33 ± 1.97**	16.67 ± 2.07***
MCV (fl)	88.67 ± 5.32	65.00 ± 4.15***	59.67 ± 7.61***	52.17 ± 4.83***
MCHC (g/dl)	34.00 ± 1.41	37.50 ± 2.74	42.83 ± 3.71*	45.50 ± 2.51**
PLT /CMM	240833.3 ± 82366.1	203333.3 ± 25819.9**	373333.3 ± 25819.9***	558333.3 ± 34302.6***
LYM%	27.83 ± 6.18	54.33 ± 3.61***	67.50 ± 5.13***	87.33 ± 3.27***
BASO%	0.52 ± 0.37	0.57 ± 0.34	0.72 ± 0.21***	0.87 ± 0.61***
NEUT%	54.50 ± 9.29	24.67 ± 2.16***	10.35 ± 1.65***	6.67 ± 1.63***
*=P<0.05; **=P<0.01; ***=P<0.001				

Table 3: Showing the mean differences of Hematological parameters between the study groups when received different oral doses (10-20-30 mg/kg b.w.) using the commercial hair dye.

Groups / Parameters	Group 1 (Control)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)	Group 4 (30 mg/kg)
Hb (g/dl)	12.85 ± 0.67	10.23 ± 0.64*	9.32 ± 0.5*	8.67 ± 0.82**
PCV (%)	44.67 ± 0.52	29.87 ± 2.25***	27.83 ± 2.14***	25.50 ± 1.87***
RBCs$^{\times 10^3}$/CMM	5466.00 ± 859.45	5400.00 ± 740.27	3883.33 ± 147.20**	3700.00 ± 442.72***
TWBCs /CMM	7600.00 ± 2182.65	4983.33 ± 231.66***	3366.67 ± 463.32***	2816.67 ± 318.85***
MCH (pg)	29.83 ± 2.32	20.98 ± 2.04*	19.17 ± 1.94*	15.33 ± 1.37***
MCV (fl)	88.67 ± 5.32	65.17 ± 1.94***	65.67 ± 3.31***	57.17 ± 3.31***
MCHC (g/dl)	34.00 ± 1.41	34.17 ± 2.14	36.83 ± 2.93	46.50 ± 2.43**
PLT /CMM	240833.3 ± 82366.1	277666.7 ± 29024.5**	358333.3 ± 29268.9***	555000.0 ± 32710.9***
LYM%	27.83 ± 6.18	55.50 ± 2.43***	76.83 ± 2.86***	88.33 ± 4.27***
BASO%	0.52 ± 0.37	0.38 ± 0.17***	0.53 ± 0.27	0.73 ± 0.22***
NEUT%	54.50 ± 9.29	20.67 ± 2.88***	10.17 ± 1.17***	6.83 ± 1.47***
*= P<0.05; **= P<0.01; ***= P<0.001				

Table 4: Showing the mean differences of Hematological parameters between the study groups whenreceived different subcutaneous doses (10-20-30 mg/kg b.w.) using the commercial hair dye.

Discussion

This study was carried out to evaluate the hair dye toxicity by using commercial hair dye in a way to estimate the hazards of this dye on rats, since it is known that toxic effects in humans are usually in the same range as those of experimental animals. PPD is the main constituent in hair dye and is an organic derivative of paranitroaniline, when ingested in a dose-dependent manner, results in severe hypersensitivity (itching, angioedema, asphyxia) and rhabdomyolysis (paresis of extremities, cola-colored urine, oliguria, markedly elevated creatinine phosphokinase and lactate dehydrogenase, hyperkalemia, hypophosphatemia and hypocalcaemia) [10,11]. Other features such as anemia, leukocytosis, hemoglobinemia, hemoglobinurea, and liver necrosis have been reported [12]. In animal model, PPD induces rhabdomyolysis leakage of calcium ions from the smooth endoplasmic reticulum, followed by continuous contraction and irreversible structural changes in the muscles [13].

In this study, we used commercial hair dye given to Albino Wistar rats in order to provide information about the effect of commercial hair dye on liver as hepatocellular necrosis accompanying hair dye poisoning in human [12].

The commercial hair dye was introduced in this study through oral and subcutaneous routes, although the variation in systemic effect between the two routes was not great. As shown in the results, subcutaneous injection results in a rather faster absorption of commercial hair dye than oral ingestion but the difference are not great.

At higher doses of the commercial hair dye, there was broad deviation from the normal values in biochemical and hematological parameters compared to lower doses of hair dye administered via the same route in all batches, because the concentration of a toxic agent influence its rate of absorption.

Our results showed significant increase in liver enzymes (GOT, GPT, ALP) activities in a dose-dependent manner in the two batches, and there is a decrease in the total plasma protein levels, albumin, and cholesterol associated with the increase of the commercial hair dye dosage in the two batches. Our finding is in agreement with others [14-18] when their administration of PPD to rats revealed a significant increase in GOT, GPT, and ALP, and a significant decrease in total proteins and glucose. Blood glucose showed significant increase among different doses of oral and subcutaneous administration of commercial hair dye compared with the control groups. Our result showed inconsistency with other study reported that the PPD leads to renal failure resulting in appreciable amount of urine glucose which causes low blood glucose level in rats [17]. Changes in the aforementioned biochemical parameters in our study indicate possible hepatic toxicity, pointed out by the substantial leakage of enzymes contained in the cells of hepatic tissues to the blood. It has been reported that low cholesterol level is usually associated with hepatocellular damage [18-19]. The decrease in the level of cholesterol in our study may be associated with hepatic lipidosis and obstructive liver diseases [20].

The hematological investigations showed significant decrease in Hb and PCV values which may be attributed to the escape of plasma from circulation to the surrounding tissues, in addition to significant decreases in RBCs, MCH and MCV values. These hematological changes indicate that, anemia may occur as a result of exposure to commercial hair dye. The possible cause for anemia is the hemolytic effect of PPD on RBCs; anemia was noticed in rats that received sub lethal doses of PPD, however, in chronic toxicity experiments, all batches showed hematological changes indicating anemia. The effect of commercial hair dye on RBCs may extend to bone marrow leading to inadequate production of red blood cells and other elements. The decrease in MCH and MCV has been associated with macrocytic anemia, while the decrease in MCHC values indicates anemia and iron deficiency.

In this study, TWBC count was found to be decreased in rats that received different doses of commercial hair dye and have been associated with significant decrease in neutrophil cells and significant increase in lymphocyte cells. This may be due to the action of PPD in the immune system, which triggers neutrophils apoptosis and massive production of immunocompetent cells [21].

The changes in biochemical and hematological parameters were reported more significantly among rats exposed to higher doses of commercial hair dye.

Our study highlighted the experimental correlation between commercial hair dye administration and liver dysfunction, and reflects the importance of public awareness regarding the potential lethality of commercial hair dye and the governmental legislations and restriction of sale of commercial hair dye.

Authors' Contribution

The main investigator of this work is Dr. Ehab Salih and all other authors contributed equally in this work.

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