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Experimental Correlation between Different Routes of Commercial Hair Dye Administration and Renal Toxicity

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Abstract: ***Objective:Toexamine the effect of hair dye toxicity on the renal function among experimental rats by using different Administration routes. Methods:A randomized controlled trail was conducted. Albino Wistar Rats were obtained from the Faculty of Pharmacy, University of Khartoum–Sudan. The rats were divided into two batches based on oral or subcutaneous administration; each batch had four groups (control and three test groups) each comprising six rats. Batch-1 and batch-2 (group-2, 3, and 4 with 10, 20, and 30mg/kg body weight (BW)where orally and subcutaneously administered respectively. The study was conducted in the period from July 2013 to February 2014. Results:The clinicalfeatureswere shown in all rats batches ranged from slight weakness in group 2 to pharyngeal oedema in group-3 up to severe weakness in hinds and fore limbs with severe convulsions, and respiratory difficulty prior to death in group-4. The Biochemical parameters showed significant increase in serum creatinineand urea concomitant with the increase of the commercial hair dye dosage in the two batches(P<0.05 and P< 0.005 respectively), and significant decrease in serum albumin (P<0.05) and uric acid(P<0.005). Potassium was significantly high among treated rats (P<0.05).Oral administration showed more renal toxicity compared with the subcutaneous route.***

Keywords: Hair dye, Paraphenylenediamine, Renal toxicity,Biochemical parameters, Experimental animals, Sudan

1. Introduction

Para-Phenylenediamine (PPD) is an organic compound; its chemical formula is $C_6H_8N_2$ [(1)]. 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. The consumers use this product because its price is20-30 times less expensive than pharmaceutical hair dye preparations[(2)].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 PPD [(3)]. There are many clinical studies showed the cause of acute renal failure due to hair dye use [(4-8)]. It has also been reported that this chemical induce renal histopathological changes in rats when PPD applied topically [(9,10)] or when applied orally and Subcutaneously[(11)]. Since, PPD is the main ingredient on hair dyes, and whose toxicity is directly related to human health. Keeping this view, we tested the toxicity of hair dye by using two different routes of administration to determine the biochemical abnormalities associated with renaltoxicity among experimental rats.

2. Materials and Methods

A randomizedcontrolled trail was conducted to examine the effect of hair dye toxicity on the renal function among experimental rats by using different administration route. It was conducted at National ResearchCenter-University of Khartoum. The commercial hair dye was collected from local markets (Libya and Omdurman).

Albino Wistar male rats at age of 11 weeks, weighting 140-160g 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 randomly into two batches on the basis of using the commercial hair dyewhich is

administered as oral Ingestion or subcutaneousInjection 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 BW of commercial hair dye, respectively); and Batch-2 (group-2, 3, and 4 subcutaneously administered with 10, 20, and 30mg/kgBW of commercial hair dye, respectively).The animals were killed 3 - 6 days after the administration. The study was conducted in the period from July 2013 to February 2014. The randomization of the batches and groups were conducted in order to match the groups before enrollment in the study.

The lethal dose of commercial hair dye for rats was determined as 80mg/kg BW[(12)] and the lethal subcutaneous dose was determined as 37mg/kg BW[(13)]. Hence, we tested the toxicity of various sub-lethal doses through different routes considering the LD_{50} of PPD is 37mg/kg. All experimental protocol was approved by the Ethical Approval Committee for Animal Experimental of National Research Institute, Ministry of Science and Technology. Ethical clearance was obtained from the Faculty of Laboratory Sciences at Omdurman Islamic University-Sudan prior to enrollment in the study.

2 ml of blood samples were collected from eye blood vessels of each ratin heparinized containers for biochemical tests. Serum was isolated and kept at -20 C^{o} till analysis. SerumCreatinine, Urea, Albumin, andUric Acid concentrations were evaluated by using kinetic colorimetric or enzymatic methods.Serum Na^{+} and K^{+}were measured by using flame emission spectrophotometer (FES) method, while $Ca^{+2}$and Mg^{+2}were determined by usingthe atomic absorption spectrophotometer.

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.

3. Findings

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 ofthe commercial hair dye in group-4.

The Biochemical parameters in renal toxicity exhibited dose-related changes in the different route of administration correspondingly.

As compared with the control group,**Table 1** showed significant increase in the levels of serum creatinine and urea, this riseconcomitants with the increase of the commercial hair dyewhen administered subcutaneously($P<0.05$ and $P<0.005$ respectively), also there is a significant decrease in serum albumin ($P<0.05$) and uric acid levels ($P<0.005$). The same results were reported in batch-2correspondingly**(Table 2)**, however the oral route of administration showed more effect on the aforementioned parameters compared to the subcutaneous dosage.The serum K^{+} was significantly high among treated rats compared with the control ($P<0.05$).Serum Ca^{++} showed no alteration, similarly, magnesium and sodium were also increased with increasing dose of both routes but they didn't report significant differences**(Table 1 and 2)**.

Table 1: Biochemical parameters between the groups when received different subcutaneous doses (10- 20- 30 mg/kg BW).

Groups / *Parameters*	*Group 1* Control	*Group 2* 10 mg /kg	*Group 3* 20 mg /kg	*Group 4* 30 mg /kg
Creatinine (mg/dl)	0.93±0.16	1.88±0.22*	4.00±0.20*	5.18±0.42*
Urea (mg/dl)	25.00±7.51	32.67±1.75*	33.83±1.72*	55.17±1.94**
Albumin (mg/dl)	4.20±0.70	4.75±0.30	3.70±0.14*	3.88±0.32*
Uric Acid (mg/dl)	5.47±1.10	3.10±0.23*	2.45±0.20**	2.00±0.32**
K^{+}(mmol/L)	4.02±0.43	5.92±0.19	7.63±0.24*	9.62±0.24**
Mg^{++} (mmol/L)	2.16±0.19	1.82±0.12	1.75±0.15	1.62±0.25
Ca^{++}(mmol/L)	9.47±0.61	10.40±0.64	9.82±0.21	9.05±0.31
Na^{+} (mmol/L)	134.33±5.89	135.17±3.19	133.83±1.94	131.17±2.32

*** = P<0.05, ** = P<0.005**

Table 2: Biochemical parameters between the groups when received different oral doses (10- 20- 30 mg/kg BW)

Groups / *Parameters*	*Group 1* Control	*Group 2* 10 mg /kg	*Group 3* 20 mg /kg	*Group 4* 30 mg /kg
Creatinine (mg/dl)	0.93±0.16	2.10±1.91*	3.97±0.23*	5.37±0.27*
Urea (mg/dl)	25.00±7.51	31.67±1.75*	34.17±2.79*	56.83±2.71**
Albumin (mg/dl)	4.20±0.70	4.78±0.32	3.67±0.26*	3.22±0.16*
Uric Acid (mg/dl)	5.47±1.10	2.98±0.59*	2.48±0.28**	2.13±0.10**
K^{+} (mmol/L)	4.02±0.43	5.73±0.72	7.82±0.25*	9.79±0.71**
Mg^{++} (mmol/L)	2.16±0.19	1.99±0.33	1.60±0.28	1.35±0.27
Ca^{++} (mmol/L)	9.47±0.61	10.00±0.69	10.02±0.34	9.13±0.39
Na^{+} (mmol/L)	134.33±5.89	134.67±3.45	131.17±0.75	130.00±1.55

*** = P<0.05, ** = P<0.00**

4. Discussion

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 creatininephosphokinase and lactate dehydrogenase, hyperkalemia, hypophosphatemia and hypocalcaemia) [11,14].Human exposure to PPD mainly occurs through skin contact during a hair dyeing or tattooing process and due to accidental or deliberate oral ingestion. For this reason we applied the hair dye by using the different routes (orally and subcutaneously) in order to verify the toxicity effect of different exposure on renal efficacy.

The lethal dose of PPD in human is not known, however, in experimental animals, the lethal dose of commercial hair dye for rats was determined as 80mg/kg BW[12] and the lethal subcutaneous dose was determined as 37mg/kgBW[13]. Hence, we tested the toxicity of various sub lethal doses through different routes considering the LD_{50} of PPD is 37mg/kg in order to determine the cytotoxic effect of PPD on renal function by using two different routs (orally and subcutaneously).

The findings of the present study demonstrated the direct toxic action of the chemical (particularly PPD) or its by-products on the commercial hair dye when administered orally or subcutaneously as was evident from the sharp rise in the serum creatinine and urea levels in the treated groups as compared with the control. Available data also indicated the significant rising of urinary nitrogen urea and creatinine [9].In this study, serum albumin and uric acid were significantly declined among treated rats compared with the control, and this may be likely due to the defect in tubular re-absorption. Adecrease in serum albumin after exposure to PPD without significant difference has been previously reported [10].Altered membrane permeability and cations (e.g. Ca^{++}, K^{+}, and Mg^{++}) transport has been reported in nephrotoxicity of amino glycosides [15]. Our study reported markedly high levels of potassium ion, since PPD was found to cause an increase in the serum level of K^{+}[16]. Hyperkalemia is a common finding in renal failure due to the release of intracellular potassium ion stored in association with cell injury and death. Hyperkalemia resulting from renal failure is probably to occur if renal failure is acute, and extensive cellular necrosis may cause increase in plasma level of K since most of the K is intracellular located [17].

As reported before and inconsistent with our study, PPD toxicity was found to increase Ca levels[18], suggesting that hypocalcaemia is a secondary rather than a primary manifestation of renal insufficiency [19].

Generally, the changes in biochemical parameters were reported more among rats exposed to higher oral doses of commercial hair dye compared with subcutaneous dose.

Our study highlighted the experimental correlation between commercial hair dye administration and renaltoxicity, 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.

5. Authors' Contributions

EIE is the Chief Investigator with overall responsibility and the Principal Investigator for the study. WAA worked as statistician with responsibility for statistical aspects of the design, conduct and analysis of the study. EIE is the programme managers with responsibility for drafting and updating the study. KOA and MAG have the responsibility for data collection. All authors therefore involved in the development of the study. KEK assisted in creation of the first draft of the manuscript with assistance from EIE. All authors reviewed and commented on the draft and approved the final version of the manuscript.

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7. Ethical Clearance

The authors obtained permission from the ethical committee of Omdurman Islamic University, Sudan.

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9. Conflict of interest

The authors declared that this study has no conflict of interest. They also declared that they did not receive financial support for this work.

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