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Some Toxicological Observations on Paraphenylene Diamine (Hair Dye) in Rats and Chickens

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Abstract: Paraphenylene diamine (PPD) is commonly used in the Sudan as hair dye and to intensify Henna colour. Several cases of poisoning with this compound have been reported in humans. Therefore, in the present work, we have examined the haematological, pathological and biochemical actions of PPD in rats and chickens. We also attempted to extract and detect PPD in tissues from treated rats and chickens. The toxic effects of PPD were qualitatively similar in the two species, but the onset of toxicity was faster and incidence of mortality higher in rats than in chickens. The clinical signs of toxicity included anorexia, decreased mobility, hind leg weakness and death. Histopathologically, PPD caused congestion, fatty change and necrosis in the liver, kidney, heart and muscles. This was accompanied by significant increases in plasma enzymes and metabolites indicative of tissue damage. Haematologically, there was also significant decreases in the packed cell volume, haemoglobin and erythrocytes counts. PPD could not be detected in the tissues of treated animals, probably suggesting that metabolite(s) of PPD and not the parent compound were responsible for the toxicity.

Key words: Paraphenylene diamine, rats, chicks, toxicity

Introduction

Paraphenylene diamine (PPD) is a synthetic compound that is widely used as a hair dye, in photochemical measurements and also in manufacturing of tire cords and photographic developer (MacPhee and Podger, 1975; Burnett and Corbett, 1977). Several cases of accidental, homicidal and suicidal poisoning with PPD have been reported in the Sudan, where it is mainly used to intensify the black colour produced by Henna (Lawonia inermis) and to reduce the time required for dying and decorating hands and feet with Henna (El-Ansary *et al*., 1983; Suliman *et al*., 1983; Yagi *et al.*, 1991; Abdelkarim *et al*., 1992). PPD has also been identified as one of the constituents of 'home made' analgesic remedies prepared by local "witch doctors' (Averbukh *et al*., 1989). In a recent companion paper, we have studied the basic *in vivo* and *in vitro* pharmacological actions of PPD in a variety of animal models. Although description of clinical cases of PPD toxicity in humans (resulting mainly from suicidal attempts) have been made (Suliman *et al*., 1983; Brown *et al*., 1987; Yagi *et al*., 1991) little seem to have been reported on the toxic effects of PPD in laboratory animals (Kiese and Rauscher, 1968; Mascres and Jasmin, 1974; Mathur *et al*., 1990). Therefore, in the present work we have examined the gross and histopathological, haematological and biochemical effects of acute and subchronic administration of PPD in rats and chicks.

Materials and Methods

Animals: Male Wistar rats, about 200 g in weight and 12 weeks of age and chickens (Brown Hisex strain) weighing about 400 aged 8 weeks were used. The rats were housed, six to a cage, at a temperature of 22-24°C and relative humidity of 50-60, with artificial light from 6 a.m. to 6:00 p.m. and were provided ad libitum with pelleted diet and water. Chickens were housed in one pen with artificial light from 6:00 p.m. to 6:00 a.m. and had free access to mashed feed and water. At the end of the experiment, rats were killed by stunning and decapitation and chickens by cervical dislocation and decapitation. Blood was collected in vials containing potassium citrate as an anticoagulant (for haemtology) and heparin sodium plus sodium fluoride (to obtain plasma, after centrifugation, for biochemical measurements). Tissues were rapidly excised from the animals and used immediately or frozen at -20°C pending analysis.

Experimental Design: Four experiments (designated experiment 1, 2, 3 and 4) were conducted:

Experiment 1: In the first (acute) experiment, groups of rats and chicks (n = 6 animals in each group) were given authentic (pure) or commercial PPD at doses of 35, 70 105, or 140 mg/kg by the oral (p.o.), intramuscular (i.m.), or subcutaneous (s.c.) routes. The animals were sacrificed 24h after the treatment.

Experiment 2: In the second (subchronic) experiment authentic PPD was injected weekly by the s.c route to rats at a dose of 17.5 mg/kg and to chicks at a dose of 45 mg/kg, for six consecutive weeks. The animals were killed 24 h after the last dose.

Experiment 3: In the third experiment, 18 rats were injected i.m. with authentic PPD (17.5 mg/kg /day) and six rats were then killed at intervals of 1, 3 or 5 days after the start of the treatment. Three (control) rats given distilled water (2ml/kg, i.m.) were killed with each group.

Experiment 4: Here, we attempted to compare different methods to extract PDD from liver, kidney and heart tissues of rats treated s.c. with authentic PPD (17.5 mg/kg/week for 6 weeks).

Measurements:

Haematological methods: The following parameters were measured by standard methods: haemoglobin (Hb) concentration, haematocrit (PCV), erythrocyte (RBC) count, leukocyte (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH).

Biochemical methods: Plasma was used to measure spectrophotometrically the following enzymes (using kits bought from Sigma Diagnostics, St. Louis, MO, USA) and metabolites: alkaline phosphatase (AP), Aspartate aminotransferase (AST or GOT), alanine aminotransferase (ALT or GPT), lactate dehydrogenase (LD), aldolase (ALS), creatinine phosphokinase (CPK). The following metabolites wee also measured using standard kits: cholesterol (Dia-Med International, Jordan), urea (Crescent Diagnostic, Saudi Arabia), glucose (Plasmatic Laboratory, UK). Total protein was measured as described by

Doumas (1975) and uric acid as reported by Jung and Parekh (1970). Creatinine was measured by the Jaffe's method (Husdan and Rapoport, 1968). The concentrations of calcium (Ca), potassium (K) and magnesium (Mg) were estimated by an atomic absorption spectrophotometer.

Pathological methods: The gross appearance of the organs of treated and control animals was inspected and recorded immediately after killing. Thereafter, small pieces of liver, heart, kidney and thigh muscle were fixed in 10% formaldehyde, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin. Specimens were examined by a histopathologist unaware of the treatments.

PPD extraction and detection: PPD was extracted by different methods from liver, heart and kidneys of rats and chicks treated with PPD using the following methods:

Direct extraction method: Specimens from the above tissues (2 g) was homogenized in chloroform from an alkaline (60% KOH) medium. PPD was then detected in the chloroform extract by thin layer chromatography (TLC) using a solvent system of acetone: hexane (30:90) and visualized by 1% potassium ichromate.

Protein-precipitation methods: (1) Tissues (2 g) were homogenized with chloroform and placed in a boiling water bath for 30 min. The solution was made alkaline (60% KOH) and extracted with chloroform. In the extract, PPD was detected by TLC using the above solvent system. (2) Tissues (5 g) were macerated with distilled water (10 ml) and concentrated HCl (13.5 ml) and then placed in boiling water bath for 5 min. After cooling in an ice-bath, 60% potassium hydroxide (12 ml) was added. The solution was then extracted with chloroform and detected by TLC as above. (3) Tissues (2 g) were homogenized in acetonitrile (20 ml). The homogenate was centrifuged and the supernatant was evaporated to dryness. The residue was dissolved in chloroform and PPD was detected by TLC as above. (4) The tissues were homogenized in trichloroacetic acid (30%) and alkalinized with 60% KOH .The homogenate was then centrifuged and the supernatant was extracted with chloroform and PPD was detected by TLC as above.

Drugs and Chemicals: Chemicals used were Analytical Reagent grade, except where mentioned.

Statistical Analysis: Values reported are means ± SEM (number of observations). Differences between group means were assessed by a one-way analysis of variance, followed by Dunett's test. P less than 0.05 has been considered significant.

Results

Experiment 1: (acute experiment): Authentic and commercial PPD at s.c., i.m., or oral doses of 35 and 70 mg/kg in both chickens and rats produced weakness, anorexia and reduced mobility during the 24 h prior to killing. No other overt clinical signs were seen. At the higher doses of 105 and 140 mg/kg, the animals showed hind limbs weakness, tremors, shivering and respiratory distress. Urine produced was darker than in the controls. This was followed by oedema in the neck, pharynx and the head region. Animals had severe convulsions and respiratory distress 2-4 h after the administration of PPD, followed by death. The onset of these effects was faster and mortality incidence higher in rats than in chickens. The gross changes in the tissues of treated animals were, in general, dose-dependent and included: generalised congestion in heart, liver, kidney and intestine (Fig. 1 and 2). The heart was flappy and was enlarged in animals receiving the highest dose (Fig. 1). The biochemical changes induced by PPD treatment in chickens and rats are summarised in Table 1 and 2. There was a dose-dependent increase in the activity of plasma enzymes and in glucose, cholesterol and K, Mg and Ca levels. The haematological changes produced by acute PPD treatment included a dose-dependent decrease in PCV and HB values and a concomitant decrease in MCHC Histpoathologically, lesions varied according the dose administered. In the kidney there was tubular necrosis, lymphocytic infiltration, blood vessel congestion and haemorrhage and shrinkage in the glomeruli (Fig. 3). In the liver, there was severe centrolobular necrosis, congestion in the central veins, fatty changes and portal lymphocytic infiltrations (Fig. 4). In the heart generalised congestion was seen. Skeletal muscles showed congestion of blood vessels, lymphocytic infiltration and necrosis. Some of the muscle bundles were apart from each other and esinophilic homogenous material was seen between the muscle bundles.

Experiment 2: (subchronic experiment): In this experiment, rats and chickens receiving a weekly dose of 17.5 and 45 mg/kg, respectively, for six weeks were anorectic and slightly weak. The PCV and Hb levels in these animals were significantly decreased. (Table 3). Post mortem changes seen in both species included flappy heart, congested liver and intestine and pale kidneys. There was dose-related increase in plasma enzymes, decreased total proteins, hyperglyceamia, hypercalcaemia and hyperkalaeamia. Histopathologically, there was hepatic centrolobular necrosis, bile duct hyperplasia, fatty change and congestion of central veins. The portal tract was infiltrated with lymphocytes. In the kidney there was severe necrosis congestion and haemorrhage. There was hyalinisation of renal tubules and the glomeruli were detached from the basement membrane. There was necrosis in cardiac muscle fibers, with lymphoctic infiltration and scattered haemorrhage.

Experiment 3: The clinical, haematological, pathological and biochemical effects of PPD (35 or 70 mg/kg) in chickens that were killed 24 h after dosing were similar to those reported in experiment 1. On the whole, the severity of the above effects in animals sacrificed 72 and 120 h post treatment were significantly less than in those killed 24 h post treatment, except in case of GPT and ALP activities which were similar to or more than in animals killed 24 h post treatment (Table 1 and 2). In rats receiving PPD at a dose of 17.5 mg/kg and killed 24, 72 or 120 h post treatment the severity of the above effects, generally, decreased with time. However, some biochemical variables either remained similar to those in rats killed 24 h after treatment (e.g. ALP and cholesterol) or even higher (e.g. Mg and Ca concentrations).

Experiment 4: Under our experimental conditions we were unable to detect any traces of PPD in tissues of treated animals.

Discussion

The present results indicated that the commercial PPD available in our local markets and used in this work has the same toxic potential as the authentic (pure) compound. In other (unreported) experiments and using various chemical and x-ray fluorescent methods, we have shown that the commercial and authentic compounds share the same chemical and physical characteristics. Although the signs of PPD toxicity in the present work were qualitatively similar in rats and chickens, we have found that rats are more susceptible to PPD toxicity than chickens, as the onset of toxicity was faster and the mortality incidence was higher in the former species than in the latter. In general, it is assumed that

humans are more vulnerable to toxicity than experimental animals by a factor of 10. The administration of PPD by s.c, i.m. and p. o. routes resulted in quantitatively dissimilar effects.

Table 1: Plasma constituents of chicks that received different oral doses of commercial PPD

Parameter	Group1 (Control)	Group2 (105 mg/kg)	Group 3 (140 mg/kg)
GOT(U/L)	38.35 ± 0.07a	57.15 ± 0.02b	60.96 ± 0.01c
GPT (U/L)	1.91 ± 0.02a	2.86 ± 0.01b	3.84 ± 0.01c
Aldolase (U/L)	15.46 ± 0.02a	25.84 ± 1.00b	28.12 ± 0.01c
LDH (U/L)	3.36 ± 0.01a	16.86 ± 0.01b	23.63 ± 0.01c
CPK (U/L)	4.56 ± 0.01a	12.46 ± 0.01b	15.32 ± 0.01c
ALP (U/L)	5.05 ± 0.04a	5.84 ± 0.01b	6.67 ± 0.01c
T. Proteins (g/dl)	3.87 ± 0.08a	3.14 ± 0.22b	2.97 ± 0.19b
Glucose (mg/dl)	247.04 ± 7.12a	208.23 ± 4.30b	184.71 ± 7.91c
Uric acid (mg/dl)	24.77 ± 1.01a	19.25 ± 0.70a	26.02 ± 6.42a
Cholesterol (mg/dl)	129.09 ± 1.24a	140.78 ± 7.41a	154.53 ± 11.18b
K (mg/dl)	5.91 ± 0.01a	6.51 ± 0.01b	6.26 ± 1.01c
Mg (mg/dl)	3.26 ± 0.01a	3.17 ± 0.01b	3.61 ± 0.01c
Ca (mg/dl)	8.64 ± 0.02a	15.12 ± 0.01b	16.92 ± 0.01c

Table 2: Plasma constituents of rats that received oral doses of authentic PPD

Parameter	Group1 (Control)	Group2 (35 mg/kg)	Group 3 (70 mg/kg)
GOT(U/L)	37.00 ± 1.61a	148.33 ± 4.01b	171.00 ± 33.32c
GPT (U/L)	19.17 ± 0.48a	87.33 ± 6.92b	109.17 ± 4.67c
ALP (U/L)	77.00 ± 0.70a	79.75 ± 1.32b	83.60 ± 1.10c
Creatinine (mg/dl)	0.27 ± 0.02a	1.00 ± 0.18b	1.60 ± 0.17c
T. Proteins (g/dl)	7.36 ± 0.17a	7.65 ± 0.20a	5.68 ± 0.41b
Glucose (mg/dl)	102.56 ± 2.77a	115.74 ± 12.14b	81.89 ± 7.94c
Urea (mg/dl)	29.67 ± 0.61a	35.78 ± 3.34b	41.72 ± 2.91c
Cholesterol (mg/dl)	39.17 ± 2.17a	49.38 ± 3.23b	51.83 ± 3.82b
K (mg/dl)	5.17 ± 0.30a	7.30 ± 0.21b	9.15 ± 0.22c
Mg (mg/dl)	2.56 ± 0.02a	2.41 ± 0.03b	2.27 ± 0.05c
Ca (mg/dl)	9.68 ± 0.07a	9.95 ± 0.10a	11.12 ± 0.22b

Table 3: Haematological changes in blood of rats following oral administeration of PPD

Parameter	Group1 (Control)	Group2 (105 mg/kg)	Group 3 (140 mg/kg)
Hb (g/dl)	13.13 ± 0.11a	13.77 ± 0.32a	11.30 ± 0.25b
PCV (%)	40.33 ± 0.42a	53.83 ± 1.62b	32.67 ± 2.26c
RBC (x $10^6 mm^3$)	5.48 ± 0.09a	4.06 ± 0.26b	4.11 ± 0.06b
TWBC (mm^3)	5030.83 ± 140.38a	4716.68 ± 119.03b	5383.33 ± 235.82a
MCH (µg)	24.83 ± 0.97a	34.36 ± 1.55b	27.52 ± 0.77a
MCV (μ^3)	73.66 ± 1.20a	135.23 ± 9.02b	79.56 ± 5.77a
MCHC (%)	32.59 ± 0.56a	25.68 ± 0.94b	35.48 ± 2.60a

Fig. 1: Enlargement of the heart in chicks that received 140 mg/kg Bwt of PPD administered subcutaneously

For example, death was caused in chickens by a s.c dose of 105 and 150 mg/kg, whereas in rats a s.c. dose of 35 mg/kg was lethal. Treatment of both species by the oral route did not produce the same effect. This may suggest slower absorption of PPD by the oral route, probably due to the chemical nature of the compound, or to its degradation in the gut, or to other unknown factors. It should be mentioned, however, that oral ingestion of PPD is the commonest route of intake in humans. Previously reported data are different from ours and have indicated that the LD50 of PPD when given s.c. in rats is 170 mg/kg and when given p.o. is 80 mg/Kg (Spector, 1956; Burnett and Corbett, 1977). The reason for the discrepancy is uncertain, but may be related to the strain of animals used, experimental conditions, or to other unknown factors. The histopathological changes seen in PPD-treated rats and chickens were basically cell necrosis and fatty change in liver, kidney, heart and muscles. Interestingly, it has been shown that PPD in mice causes damage in skeletal muscles and not liver or kidneys (Averbukh *et al*., 1989). This may be genuine species difference and confirms the need to study the toxicity of compounds in several species and not to depend on a single animal species and to extrapolate the data therefrom to humans. The PPD-induced histopathological damage is not certain but could have resulted from lipid peroxidation and free radical formation, as these two

Fig. 2: Congestion of the intestine in hicks that received 140 mg/kg Bwt of PPD administered subcutaneously

Fig. 3: Degeneration of the cells of renal tubules in chicks that received 70 mg/kg bwt of PPD administered subcutaneously

processes have previously been shown to be associated with exposure to PPD (or metabolites thereof) in G. pig liver (Mathur *et al*., 1990) and human keratocytes (Picardo *et al*., 1992). As far as we are aware, the cardiac damage seen in this work has not been reported before .The protective mechanisms against free radicals in the heart are probably lower than that in other organs, which renders the heart tissue more susceptible to the effects of chemicals. PPD-induced tissue damage resulted in haematological and biochemical changes. In chickens the treatment caused significant increases in the plasma enzymes AST, GPT, LDH, ALP, ALS and CPPK and in the metabolites creatinine, urea, glucose, uric acid, potassium and calcium. There were decreases in total proteins, cholesterol and magnesium. Broadly similar effects were produced in the rat, although some differences were noted. For example, in rats, PPD caused hyperglycaemia and not hypoglycaemia as in the case in chickens .The reasons for these differences is not known. PPD was not detected in the tissues of treated rats or chickens. This suggests that the toxic effects produced were possibly produced by metabolite(s) of PPD and not by the parent compound itself. WE have found that PPD was ineffective in eliciting any action when added directly to isolated pharmacological preparations. It is also possible that strong binding of PPD with tissue protein makes its detection difficult.

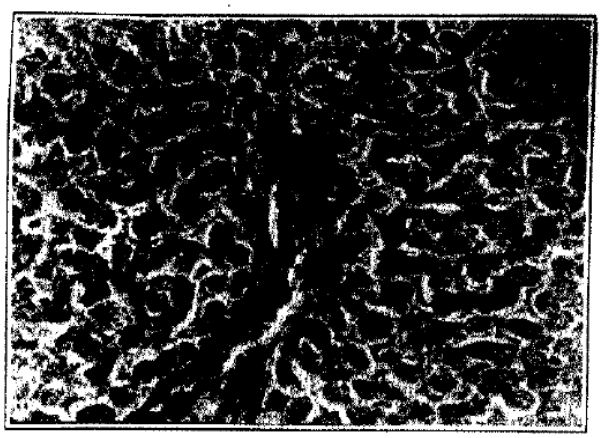

Fig. 4: Degeneration of hepatic cells in chicks that received 60 mg/kg Bwt of PPD administered subcutaneously

In conclusion, the present work has shown that treatment of chickens and rats with PPD damages the liver, kidney, heart and muscles. This resulted in haematological and biochemical changes consistent with the tissue damage.

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References

Abdelkarim, E.E., H.M. Ali, D.W.G. Harron and K.M. Ali, 1992. Suicide attempt with paraphenylene diamine (PPDA) dye in Sudan. Health Serv. J. Eastern Mediterr. Region WHO, 6: 44-48.

Averbukh, Z., D. Modai, Y. Leonov, J. Weissgarten and G. Lewinsohn *et al*., 1989. Rhabdomyolysis and acute renal failure induced by paraphenylenediamine. Hum. Exp. Toxicol., 8: 345-348.

Brown, J.H., M.G. McGeown, B. Conway and C.M. Hill, 1987. Chronic renal failure associated with topical application of paraphenylenediamine. Br. Med. J., 294: 155-155.

Burnett, C.M. and J.F. Corbett, 1977. The Chemistry and Toxicology of Hair Dyes. In: Cutaneous Toxicity, Drill, V.D. and O. Lazar (Eds.). Academic Press, New York, USA., ISBN-13: 9780122220500, pp: 203-221.

Doumas, B.T., 1975. Standards for total serum protein assays-A collaborative study. Clin. Chem., 21: 1159-1166.

El-Ansary, E.H., M.E.K. Ahmed and H.W. Clague, 1983. Systemic toxicity of para-phenylenediamine. Lancet, 321: 1341-1341.

Husdan, H. and A. Rapoport, 1968. Estimation of creatinine by the jaffe reaction: A comparison of three methods. Clin. Chem., 14: 222-238.

Jung, D.H. and A.C. Parekh, 1970. An improved reagent system for the measurement of serum uric acid. Clin. Chem., 16: 247-250.

Kiese, M. and E. Rauscher, 1968. The absorption of *p*-toluenediamine through human skin in hair dyeing. Toxicol. Applied Pharmacol., 13: 325-331.

MacPhee, D.G. and D.M. Podger, 1975. Hair dye. Med. J. Aust., 2: 32-33.

Mascres, C. and G. Jasmin, 1974. [Pathogenic study of the muscular lesions caused by *p*-phenylenediamine]. L'union Medicale du Canada, 103: 672-677, (In French).

Mathur, A.K., B.N. Gupta, S. Narang, S. Singh and N. Mathur *et al*., 1990. Biochemical and histopathological changes following dermal exposure to paraphenylene diamine in guinea pigs. J. Applied Toxicol., 10: 383-386.

Picardo, M.C., C. Zompetta, C. Marchese, A. De Luca, R.J. Faggioni, R. Schmidt and B. Santucci, 1992. Paraphenylenediamine, a contact allergen, induces oxidative stress and ICAM-1 expression in human keratinocytes. Br. J. Dermatol., 126: 450-455.

Spector, W.S., 1956. Handbook of Toxicology, Volume 1: Acute Toxicities of Solids, Liquids and Gases to Laboratory Animals. Saunders Publishing Co., Philadelphia, USA., pp: 232.

Suliman, S.M., M. Homeida and O.I. Aboud, 1983. Paraphenylenediamine induced acute tubular necrosis following hair dye ingestion. Hum. Toxicol., 2: 633-635.

Yagi, H., A.M. El Hind and S.I. Khalil, 1991. Acute poisoning from hair dye. East Afr. Med. J., 68: 404-411.