ORIGINAL ARTICLE

Renal function profile of Di (2-Ethylhexyl) phthalate exposed adult Wistar Rats

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Abstract

Background: The study was designed to investigate renal function profile upon exposure to graded oral doses of Di (2-ethylhexyl) phthalate (DEHP) in the adult Wistar rats considering that DEHP leaches easily from plastic to foods, drinking water and the environment

Methods: Twenty (20) Wistar rats were administered DEHP in the doses of 0.02mg/kg, 20mg/kg and 200mg/kg body weight in blocks A, B and C respectively through the intrapharyngeal route. Rats in block D, serving as control, were administered distill water. Both treatments and distilled water were administered at a convenient dose of 0.1ml over a 30 day duration. Serum obtained from each rat was analyzed for electrolytes (Na^+, K^+ and Cl^-), creatinine and urea

Results: Oral exposure of the rats to DEHP at the graded doses used in this study increased only the mean serum urea from 32.6 ± 2.63 mg/dl in control to 42.01 ± 3.05 mg/dl, 41.75 ± 1.93 mg/dl and 40.2 ± 2.82 mg/dl in rats treated with 0.02mg, 20mg and 200mg DEHP/body weight respectively. Other parameters of serum Na^+, K^+, Cl^- and creatinine remain unchanged.

Conclusion: Exposure to oral DEHP induced an elevation of the blood urea nitrogen irrespective of dose without affecting serum creatinine and the electrolytes

Keywords: Di (2-ethylhexyl) phthalate, DEHP, Renal function, Plasticizer, Azotaemia

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Introduction

Exposure of humans to di (2-ethylhexyl) phthalate (DEHP) occurs throughout life, from intraembryonic life (via maternal exposure) to adult life. The most probable route of exposure being via food processed and stored in plastics (at the dose of about 0.25mg/day) and drinking water (at the dose of 0.04 to 30 parts per million) with the maximum daily exposure for the general population estimated at about 2mg/day[1]. High DEHP exposures are known to occur in blood exchange transfusions, extracorporeal membrane oxygenation (ECMO), in cardiovascular surgery[2] as well as in peritoneal dialysis procedure[3]. DEHP (C_6H_4 ($C_8H_{17}C00)_2$) is an organic, colorless and almost odorless liquid with a molecular weight of 390.57g/mol[1].

It is the most widely used plasticizer of poly vinyl chloride (PVC), commonly known as plastic. The amount of plasticizer in plastic is determined by the intended target use; the softer plastic containing higher amount of DEHP. Thus, plastics may contain as little as 1% to as much as 40% of the plasticizer[4]. In the plasticized form, plastic could become useful in clothing and upholstery, flexible hoses and tubing, flooring to roofing membranes and electrical cable insulation. Others applications may include inflatable products such as waterbeds, toys and inflatable structures. Medically, plasticized plastics have wide range of uses in face masks, intravenous and blood bags and giving sets, hand gloves etc[5]. With increasing demand for plastic and its use, exposure of man to DEHP is bound to persist, if not increase.

As a plasticizer, DEHP easily leaches out of plastics into its immediate environment (such as the soil, water or the air) or its content such as food, water, serum and blood following contact since it does not bind to the plastics. It is highly hydrophobic and hence extract faster into non-polar solvents such as oil and fats than the polar solvents like water. Leaching of DEHP from plastic medical devices and deposits in tissue has been well documented[6.7]; this leaching property is both time and use dependent.

Aside its use as plasticizer, DEHP could itself used directly as , dielectric fluid in capacitors and as solvent in lipsticks. Approximately three billion kilograms are produced annually and is metabolized by hydrolysis to mono-(2-ethylhexyl) phthalate (MEHP) and subsequently to phthalate salt releasing alcohol which

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can be oxidized to an aldelyde[8]. The breakdown products of DEHP may be measured in urine or blood to determine exposure to it. However, this test only provides a measure of recent exposure to the chemical.

DEHP exposure has been to shown to exhibit diverse organic and systemic effects in both man and experimental animals. These include i) distortion of the Sertoli cell, leading to reduced fertility and changes in sperm production in males[9,10,11], ovarian dysfunction and decreased hormone production in females[12,13], ii) implication in American men with abdominal obesity or insulin resistance who have been observed to have relatively high levels of DEHP and dibutyl phthalate (DBP) metabolites in their urine[14,15], iii) increased irregular cardiac rythms in invitro DEHP-exposed rat and chick cardiac cell culture, in conjunction with reduced gap junctional connexins proteins in cardiomyosites[4;] iv) increased incidence of liver tumors in DEHP-exposed rats and mice[16,17] as well as centrilobular hepatic necrosis in rats with carbon tetrachloride (CCL4) co-administration[18]; v) respiratory distress and changes in kidney function[6,18]. In more specific terms, chronic DEHP exposure has been shown to induce pathologies such as multiple cysts in the kidneys of mice both with intact and knocked-out PPARs (peroxisome proliferation activator receptors), significant weight loss and lesions in the liver in PPAR intact mice and testicular lesions in PPAR knocked-out mice[19]. This study evaluates renal function in DEHP exposed rats.

Materials and Methods

Twenty (20) Wistar rats were procured for the study. They were weighed using ae Adam® (UK) AQT compact digital weighing scale, housed in wooden cages and maintained in a controlled environment with a 12hr light/dark cycle, temperature of 30 ± 2°C and relative humidity of 80 ± 18%. They were all equally exposed to free growers chow, water ad libitium, and cared for according to international animal care regulations[20]. They were then randomized into 4 experimental blocks of A, B, C and D with 5 rats each.

Based on the observed dose of 19mg/kg body weight as the optimal safe exposure dose for DEHP (as fixed USA Environmental Protection Agency (EPA)[21], doses of 0.02mg, 20mg and 200mg per kg body weight of DEHP (procured from Sigma-Aldrich® (distributors) as dioctyl phthalate (DOP) manufactured by Eastman chemical company, all of USA) were administered on the rats in blocks A, B and C respectively through intrapharyngeal route. Rats in block D, taken as the control, had no treatment, but were instead administered distill water. The treatment dosages and the distilled water were administered at a convenient dose of 0.1ml over 30 days. At the end of the treatment days, 2 mls of fasting tail blood was extracted from each rat into a clean non-EDTA bottle from where serum was extracted for analysis.

Serum obtained from each rat was analyzed for electrolytes (Na^+, K^+ and Cl^-), creatinine and urea using semi automated chemistry analyzer (Humalyzer junior®. Results were expressed as mean ± standard deviation (SD) mean. Data obtained were analyzed using the USA Center for disease control (CDC) EPI Info statistical software version 7.1.1.14, 2013. Mean pairs were tested for significance using ANOVA. Significant level of probability was fixed at 0.05 or less.

Results

The result data obtained are expressed in Tables 1. Oral exposure of the rats to DEHP at the graded doses used in this study increased only the mean serum urea from 32.6 ± 2.63 mg/dl in control to 42.01 ± 3.05 mg/dl, 41.75 ± 1.93 mg/dl and 40.2 ± 2.82 mg/dl in rats treated with 0.02mg, 20mg and 200mg DEHP/body weight respectively. Other parameters of serum Na^+, K^+, Cl^- and creatinine remain unchanged.

Table 1: Renal functions of DEHP exposed adult Wistar rats

Test	Mean ± SD					Standard
Parameters	A (0.02mg DEHP)	B (20mg DEHP)	C (200mg DEHP)	D (Control)	P	References
Na^+(mmol/L)	140.78 ± 3.64	139.65 ± 1.11	135.95 ± 7.63	141.95 ± 3.29	0.224	135–155
K^+(mmol/L)	5.0 ± 0.24	5.0 ± 0.63	5.05 ± 0.27	4.75 ± 0.69	0.659	3.5-5.6
Cl^-(mmol/L)	74.8 ± 5.06	75.0 ± 5.32	78.5 ± 7.99	75.0 ± 8.58	0.801	90–108
Urea (mg/dl)	42.01 ± 3.05	41.75 ± 1.93	40.2 ± 2.82	32.6 ± 2.63	<0.001	15–50
Cr (mg/dl)	0.84 ± 0.17	0.9 ± 0.07	0.65 ± 0.04	0.7 ± 0.03	0.119	0.7-1.4

Discussion

The kidney is known to carry out several functions that are critical to health of the human body and cells. Such functions include water and electrolyte balance, blood volume control, regulation of acid/base status, the production/secretion of hormones such as vitamin D, erythropoietin as well as moderating the rennin/angiotensin/aldosterone functions and the excretion of soluble wastes like urea and creatinine as foreign materials like drugs. Thus, renal function assessment is clinically important as it is helpful in a) identifying renal impairments, b) monitoring disease progress or even c) in assessing baseline measurements prior to starting treatment with certain drugs. Clinically, the plasma concentrations of creatinine and urea, as well as electrolytes are commonly used to determine renal function. These measures are used to determine whether a patient is suffering from kidney disease or not.

Significant decrease in kidney function as demonstrated by creatinine clearance has been

documented by authors in DEHP-exposed rats[18]. This, they attributed to the ability of DEHP to induce polycystic kidney disease in the experimental animals. While no morphological study of the kidneys was undertaken in the present study to collaborate the findings of these authors, there was however, evidence of some degree of renal function derangement induced by the exposure to DEHP. This observed derangement was characterized by elevation of serum urea concentration irrespective of dose.

Elevation of urea in the blood (azotaemia) is normally an indication of inadequate excretory, regulatory and endocrine functions of the kidneys[22]. In fact, ureamia/ureamic syndrome has been used to describe terminal renal failure in clinical practice[23]. In such state of renal dysfunction however, it is expected that electrolyte imbalance will be one of the apparent laboratory manifestations contrary to the findings of this study where the serum electrolytes remain largely unchanged. The selective azoteamia observed could be arising from extra-renal/pre-renal sources as observed with high protein diet or excessive body protein catabolism and not due to renal pathology. This argument is strengthened by previous findings that DEHP induced weight loss in rats exposed to it[23,24]. These authors observed that there was elevated leptin concentration in the blood of these rats, accounting for a likely disordered fat metabolism.

We conclude that the oral exposure to DEHP induced an elevation in blood urea nitrogen with little or no effect on the serum creatinine and electrolytes. There is the need to study the effect of DEHP on human kidney function.

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