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The para isomer of dinitrobenzene disrupts redox homeostasis in liver and kidney of male wistar rats

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ABSTRACT

Background: Para-Dinitrobenzene (*p*-DNB) is one of the isomers of dinitrobenzene which have been detected as environmental toxicants. Skin irritation and organ toxicities are likely for industrial workers exposed to *p*-DNB. This study evaluated the effect of sub-chronic exposure of rats to *p*-DNB on cellular redox balance, hepatic and renal integrity.

Methods: Forty eight male Wistar rats weighing 160–180 g were administered 50, 75, 1000 and 2000 mg/kg b.wt (body weight) of *p*-DNB or an equivalent volume of vehicle (control) orally and topically for 14 days. After the period of treatment, the activities of kidney and liver catalase (CAT), alkaline phosphatase (ALP) and superoxide dismutase (SOD) as well as extent of renal and hepatic lipid peroxidation (LPO) were determined. Serum ALP activity and plasma urea concentration were also evaluated.

Results: Compared with control animals, *p*-DNB -administered rats showed decrease in the body and relative kidney and liver weights as well as increased renal and hepatic hydrogen peroxide and lipid peroxidation levels accompanied by decreased superoxide dismutase and catalase activities. However, *p*-DNB caused a significant increase in plasma urea concentration and serum, liver and kidney ALP activities relative to control. In addition, *p*-DNB caused periportal infiltration, severe macro vesicular steatosis and hepatic necrosis in the liver.

Conclusions: Our findings show that sub-chronic oral and sub-dermal administration of *p*-DNB may produce hepato-nephrotoxicity through oxidative stress.

1. Background

One of the global health problems is liver disorders and diseases which are due to exposure to various toxic chemicals. The major site of xenobiotic metabolism is the liver and it can be damaged by toxic chemicals, drugs and environmental agents which can lead to deleterious effect on the liver cells and functions. Furthermore, in preclinical toxicity studies, renal toxicity is one of the major concerns. Renal toxicity can be a result of hemodynamic changes, direct injury to cells and tissue, inflammatory tissue injury, and/or obstruction of renal excretion. The kidney has important roles in plasma filtration and maintenance of metabolic homeostasis. Toxic effects on the kidney as a result of environmental toxicants can impair these kidney roles and induce changes in kidney function and structure [1].

Dinitrobenzene (DNB) has been detected has environmental contaminants of ground water and soil near sites and at military munitions test grounds. Also, it has been characterized as occupational toxicants/pollutants, by its toxicity [2–4]. DNB production yields a mixture of the three isomers namely *ortho-isomer* (*o*-DNB), *meta*-isomer (*m*-DNB) and *para*-isomer (*p*-DNB) [5,6]. Both DNB mixture and the individual isomers of DNB are available commercially. (*p*-DNB) or 1,4-DNB forms colourless to yellow monoclinic needles and is used in the synthesis of dyes, explosive and in plastics industry. DNB induces serious peroxidation in membrane structures.[5]. Many investigators have described testicular toxicity associated with an exposure of rats to 1,3-dinitrobenzene or trinitrobenzene (TNB) [7–10]. It has been reported that in rats DNB cause encephalopathy, hematological alterations and toxicity to brain, testes, epididymis and spleen [11–14,54].

List of abbreviations: p-DNB, para-dinitrobenzene; CAT, Catalase; ALP, alanine phosphatase; SOD, Superoxide dismutase; LPO, lipid peroxidation; o-DNB, ortho-dinitrobenzene, m-DNB, meta-dinitrobenzene; TNB, trinitrobenzene; GSH, glutathione; GST, glutathione –s –transferase, GPX, glutathione reductase, NIH, national institute of health; PHS, public health service; OECD, Organisation for economic co-operation and Development; TBA, thiobarbituric acid; H & E, hamatoxilin eosin; SPSS, Statistical Pucteage for Social Sciences; MDA, malodialdehyde

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Free radical is a reactive atom or group of atoms that has one or more unpaired electrons. They are produced in the body by natural biological processes or introduced from an exogenous source such as drugs and environmental toxicants [15]. Excessive production of free radicals which are not neutralised can oxidize macromolecules, such as DNA, proteins, carbohydrates, and lipids and ultimately damage the cell [16]. Toxins, toxicants and free radicals are distributed to the liver and kidney, thus exposing them to a state of induced toxicities. The liver and kidney tissues have evolved an array of antioxidant defense systems to protect themselves against harmful effect of metabolites and free radicals [15].

Oxidative stress occur when there is a relative imbalance between pro-oxidant and antioxidant molecules in the body thereby leading to an accumulation of reactive oxygen species [17,18]. Reactive oxygen species are chemically active molecules that contain oxygen and are formed in normal physiology as by-products [19]. Oxidative stress can be linked to the pathophysiology of liver and kidney tissues such as inflammation, hypertrophy, apoptosis and fibrosis and it is characterized by a disruption of redox signaling and control. processes [20–23] Substances that inhibit oxidation or reactions by oxygen, peroxides, or free radicals or their actions are called antioxidants [24]. They include both enzymatic and nonenzymatic antioxidants. Some examples are reduced glutathione (GSH), ascorbic acid, vitamin E, glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT) [25–27].

One of the mechanisms of *p*-DNB is their ability to generate free radicals and trigger oxidative stress in vivo [28]. In Nigeria, the increasing use of dynamite, plastics, dyes, petrochemicals products and improperly managed waste products from manufacturing plants may contribute to *p*-DNB toxicity and possibly introduce high concentrations of this potential hepatorenal toxicant into the environment. Consequently, the present study was designed to investigate the effect of sub-chronic oral and dermal administration of *p*-DNB on target tissues (kidney and liver) and to evaluate the redox balance of *p*-DNB target tissues in male rats. For this purpose, we conducted oxidative stress biomarkers assays on rat kidney and liver treated with *p*-DNB to evaluate hepatotoxicity and nephrotoxicity through oxidative stress. In addition, we evaluate the kidney enzyme biomarkers for toxicity to evaluate *p*-DNB -type kidney toxicity.

2. Methods

2.1. Chemicals

p-DNB was purchased from Sigma Aldrich. All other chemicals used in the experiment were of analytical grade.

2.2. Animals and experimental design

Forty eight male Albino Wistar rats, weighing 150–170 g, were purchased from the Department of Biochemistry, Bingham University, Nigeria. They were housed in plastic cages placed in a well-ventilated rat house and allowed ad libitum access to rat chow (Vital Feeds Ltd, Nigeria) and water and subjected to natural 12-h light: dark cycle. All the animals received humane care according to the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science, published by the National Institute of Health (NIH). Also, the ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments (Public Health Service (PHS), 1996). After 1 week acclimation period, rats were randomly assigned to three groups of eight rats per group following oral administration. Group I rats received corn oil alone at 2 ml/kg bw. Group II were orally treated with 50 mg/kg bwt *p*-DNB dissolved in corn oil for 14 days While group III rats orally treated with 75 mg/kg bwt *p*-DNB dissolved in corn oil for 14 days of the experiment. Also, for sub-dermal administration, rats were randomly divided into three groups of eight rats per group. Before treatments, the dorsal fur of the rats where shaved at 2 cm by 2 cm length, then *p*-DNB dissolved in corn oil where applied topically to the skin of the animals. Group I rats were treated topically with corn oil alone at 2 ml/kg. Group II rats were treated with 1000 mg/kg *p*-DNB while Group III rats were treated with 2000 mg/kg for 14 days. The doses of *p*-DNB were chosen based on the Acute Oral Toxicity Up-and-Down-Procedure OECD 2008 described by Dixon and Mood [29–32].

2.3. Serum collection and tissue preparation

At the end of experimental period approximately 24 h after the last *p*-DNB treatment, blood samples were collected by retro orbital bleeding. Blood samples were left to clot, centrifuged at 3000 rpm for 15 min, serum was collected and stored for further biochemical analysis. Afterwards, rats were sacrificed by cervical dislocation, liver and kidney were rapidly excised from each animal, connecting tissue and fats deposits were trimmed from the liver and kidney, and they were washed free of any blood and clots with ice cold 1.15% KCl solution. They were then blotted over a piece of filter paper.

2.4. Biochemical analyses

The liver and kidney were homogenised in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride and the homogenate was then centrifuged at 10 000*g* for 15 min at 4 °C. The supernatant was collected for the estimation of catalase (CAT) and Superoxide dismutase (SOD) activities. Serum was used for estimation of liver and renal injury according to manufacturer protocol.

2.4.1. Plasma biomarkers of Renal Toxicity assay

Plasma urea was determined with Randox diagnostic kits. Method for Plasma urea assays was based on the Fenton reaction [33] with the diazine chromogen formed absorbing strongly at 540 nm.

2.4.2. Renal and hepatic level of Lipid Peroxidation Assay

The extent of lipid peroxidation (LPO) in the kidney and liver was estimated by the method of Varshney and Kale [34]. The method involved the reaction between malondialdehyde (MDA) a product of lipid peroxidation) and thiobarbituric acid (TBA) to yield a stable pink chromophore with maximum absorption at 532 nm.

2.4.3. Renal and hepatic Antioxidant Enzymes Assay

Oxidative stress markers were assessed such as SOD and CAT. Renal and hepatic superoxide dismutase (SOD) activity was determined by measuring the inhibition of autooxidation of epinephrine at pH 10.2 and 30 °C by the method of Misra and Fridovich, (1972), [35]. Renal and hepatic catalase activity was determined by the method described by Sinha [36]. The method was based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2). The chromic acetate produced is measured spectrophotometrically at 570 nm.

2.4.4. Determination of kidney, liver and plasma alkaline phosphatase

Plasma alkaline phosphatase (ALP) activities were determined using Randox diagnostic kits. ALP activity was determined in accordance with the principles of Tietz et al. [37]. The p-nitrophenol formed by the hydrolysis of p-nitrophenyl phosphate confers yellowish colour on the reaction mixture and its intensity can be monitored at 405 nm to give a measure of enzyme activity.

2.5. Histopathological examination

Liver sections were taken immediately from the liver, fixed in 10% buffered formalin, cleared in xylene, and embedded in paraffin. Sections (4–5 mm thick) were prepared and then stained with hema-