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DI- N-BUTYLPHTHALATE AND THE RENAL FUNCTIONS OF ADULT MALE ALBINO WISTAR RATS.

Biochemistry

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ABSTRACT

This study investigated the effects of di-n-butylphthalate on the kidneys after oral administration, to adult male albino Wistar rats. Twenty rats, weighing between 146.10g and 301.20g were arranged into groups A,B,C,D, of five rats each, and were fed with graded concentrations, 0 mg/kg, 2,000 mg/kg, 4,000 mg/kg and 6,000 mg/kg body weight of di-n-butylphthalate respectively for thirty days. Serum urea and creatinine levels were used as test of renal function, In addition, the cell histology of the kidneys of the rats were also examined. There were significantly higher levels ($P < 0.05$), of creatinine and urea in groups B (71.80 ± 1.43µmol/L, (3.44 ± 0.10Mmol/L), C (85.40 ± 1.47µmol/L, 4.70 ± 0.18Mmol/L), and D (108.00 + 0.95µmol/L, 6.30 ± 0.13Mmol/L), when compared with the control group A. The histological examination of the kidney in groups B, C, and D indicated glomerular expansion, generalized inflammation of some glomeruli and vascular congestion, After thirty days of treatment, the control group showed a mean weight gain of 1.31%, whereas the treated groups B, C and D recorded a significant decrease in weight of 2.02%, 2.11% and 1.19% respectively. This study indicates that the chemical, di-n- butylphthalate is organotoxic, and may affect kidney functions rats at high concentrations.

KEYWORDS

Di-n-butylphthalate, kidneys, urea, creatinine.

INTRODUCTION

Background of study

Di-n- butylphthalate, (DBP) belongs to a widely used group of chemicals called phthalates. It is a phthalic acid ester with the molecular formualr $C_{16}H_{22}O_4$. It is an inert, colourless, oily liquid with mild aromatic smell, low vapour pressure and is soluble in most organic solvents like benzene, ether and alcohol (Duty, 2005).

DBP is used mainly as a specialty plasticizer for nitrocellulose, polyvinyl acetate and polyvinyl chloride (PVC), where it may account for 20% W/W or more of the plastic materials. Its production has increased since 1950's when PVC was introduced (Centre for Disease Control and Prevention, CDC, 2005). When added to plastics, it allows the long polyvinyl molecules to slide against one another, hence softening and increasing their flexibility, transparency and durability. Its end applications include medical devices like plastic tubing for nasal or oral feeding, blood bags and catheters; pharmaceuticals, like enteric coatings of drugs; cosmetics and body care products like perfumes, nail polish, fingernail elongators, body lotions, hair spray, powder, liquid soap, eye shadow, and moisturizer. Other products, like some building materials, aluminum foil used as food wraps, plastic tanks and containers, detergents, adhesives, printing ink, insect repellant, textile, paints, di-electric fluid in condensers, carpet backing, toys and rocket fuel contain phthalates (Brandt, 2005).

Statement of problem

Almost all the DBP present in the environment arose form anthropogenic sources rather than natural ones, and people are exposed to DBP daily through contact with everyday products and occupationally (National Occupational Exposure Survey, (1987). In Nigeria today, the use of products made from DBP and plastic materials for food packaging has been on the increase.

DBP and other phthalates are easily released into the environment because there is no covalent bond between the phthalates and plastics in which they are mixed. As the plastic ages and breaks down, the release of phthalates accelerates (Rudel and Perovich, 2008). DBP can easily leach out into the water or food contained in these packages or containers, like margarine, candy sweet, magi, fruit juice. It can also penetrate the skin and accumulate in the body through the use of some drugs, cosmetics and other body care products made from DBP. Through poor waste management and incineration of waste plastic materials, it can find its way into the air and water sources like streams. The persistent and ubiquitous distribution of this environmental contaminant has caused it to remain a potentially serious threat to human and animal health. The current research was therefore carried out to study some of the effects of DBP on male albino Wistar rats.

AIMS AND OBJECTIVES

This research is aimed at studying the toxicological effects of orally administered di-n-butylphthalate on the kidneys of adult male albino Wistar rats. The work therefore aims to:

1 Estimate the levels of serum urea and creatinine in the rats in order to assess the effects of DBP on the renal functions.
2 Examine the cell histology of their kidneys for morphological changes due to the toxic effects of DBP.
3 Compare the levels of these biochemical parameters and the histological changes in the treated animals with those of the control animals.

Significance of the study

The data generated from this study are assumed relevant to prediction of hazard to humans and will give some indications of the possible health implications of exposure to DBP. Further studies can be extended to humans. It will also help the policy makers to come up with a policy that will regulate its use in cosmetics, food, medical devices and everyday products to guarantee public safety.

Scope of study

This is a sub-acute study, and is limited to animal model - adult male albino Wistar rats of age 90 – 150 days old. This age was chosen based on the range of 70 -180 days stated as the peak age of male albino Wistar rats.

MATERIALS AND METHODS

SUBJECTS – RATS

Twenty adult male albino Wistar rats, weighing between 146.10g and 301.20g were used for this study. They were obtained from the Animal House of University of Nigeria Teaching Hospital (UNTH), Enugu. The rats were arranged into four groups (A, B, C, D) of five rats each, and were kept for three days to acclimatize before initiating the study. They were allowed free access to clean drinking water, and were fed on standard rat feed (top feed), throughout the period of the study.

The rats in group A received no treatment and served as control, while those in groups B, C and D were treated orally with graded concentrations of DBP 2,000mg/kg, 4,000mg/kg and 6,000mg/kg body weight respectively for thirty days. Doses were chosen based on the LD50 value of 8,000mg/kg body weight. Two rats from each of the groups were sacrificed at the end of the treatment period, and their kidneys were harvested.

Materials
The chemical, DBP was purchased from Analar grade Laboratory regents and chemical dealer at bridge Head market, Onitsha, Anambra State.

Specimen collection and preparation
The specimens were blood samples collected through the retro-bulbar plexus of the nasal canthus, at the end of thirty days, for the estimation of the chemical parameters. Serum samples were prepared from the whole blood as follows:

Three millitres of whole blood was collected into clean plain test tube. This was allowed to clot and retract, and then centrifuged at 3,000 rpm for five minutes. The supernatant (serum) was separated from the sediment (red cells) and transferred into another clean plain test tube, for the chemical analyses.

Two rats from each group were killed by euthanasia, with chloroform, their kidneys dissected out and fixed in 10% formol saline solution, for histological studies.

Serum Urea and Creatinine Estimation
Serum urea concentration was estimated by the modified diacetylmonoxime method of Wybenga et al. 1971; while serum creatinine, concentration was determined using alkaline picrate method of Fabiny and Ertingshaysen, (1971).

Histopathological examination
The testes tissues were processed in an automatic tissue processor and embedded in paraffin wax. Thin sections (about 4-5 microns thick) were cut using rotary microtome, and stained by heamatoxylin and eosin (H & E) method, and examined using a light microscope. (magnification X100)

Staining principle: Haematoxylin was used as a nuclear stain preceding staining of cytoplasm and connective tissues with eosin. Sections were stained with haematoxylin with a stain of sufficient power and for long enough to ensure some overstraining of nuclei, the superfluous and obscurative coloration of structures being removed by treatment with acid alcohol. Eosin stains connective tissues and cytoplasm in varying intensity and shades of primary colour giving a most useful differential stain with haematoxylin.

ANALYSIS OF RESULTS
Results were expressed as mean ± standard error of mean. Significant difference between means were determined by one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS).

RESULTS
The results showed that the rats in the control group A, which received no treatment with the chemical, DBP, recorded 1.31% weight gain but the rats in groups B,C and D which were treated with graded concentrations of DBP recorded 2.02%, 2.11% and 1.19% weight loss respectively as detailed in table 1.

There were significantly higher values, ($P < 0.05$) of urea and creatinine in groups C (4.70±0.18Mmol/L, 85.40±1.47µmol/L) and D (6.30+0.13Mmol/L, 108.00±0.95µmol/L), which were fed with higher doses (4,00mg/kg and 6,000mg/kg respectively) of the toxicant, but their levels in group B (3.44±0.10Mmol/L, 71.80±1.43µmol/L) were not as highly significant ($P<0.05$) when compared with the control group A (3.28±0.08Mmol/L, 60.00±1.14µmol/L) as can be seen in table 3.

In summary, there was a progressive increase in variation in the levels of the biochemical parameters, with increase in doses of DBP given to the rats. The renal parameters showed increases.

The kidney cell histology revealed vascular congestion, glomerular expansion and inflammation of some glomeruli when compared with the control.

Table 1: Weights Of The Rats At The On-set And End Of The Experiment (mean ± Standard Error Of Mean).

Mean Weight(g)	GROUP A	GROUP B	GROUP C	GROUP D
On– set of exp:	228.22±11.87	157.38±11.87	175.20±11.87	293.68±11.87
End of exp:	231.20±11.97	154.20±11.	171.50±11.97	290.18±11.97
Diff in wt:	2.98±0.6	3.30±0.06	3.70±0.06	3.50±0.06
Percentage diff. in wt	1.31%	2.02%	2.11%	1.19%

TABLE 2: The Levels Of Indices Of Renal Function (Mean ± Standard Error of Mean)

Parameter	GROUP A	GROUP B	GROUP C	GROUP D
Urea (Mmol/L)	3.28±0.08	3.44±0.10	4.70±0.18	6.30±0.13
Test of significance: F	(4,16) =115.145;	P < 0.05		
Creatinine (µmol/L)	60.00±1.14	71.80±1.43	85.40±1.47	108.00±0.95
Test of significance: F	(4,16) = 265.342	P < 0.05		

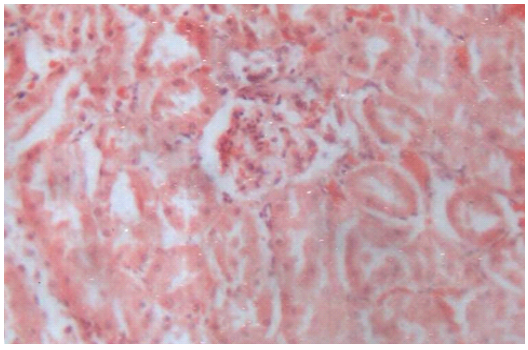
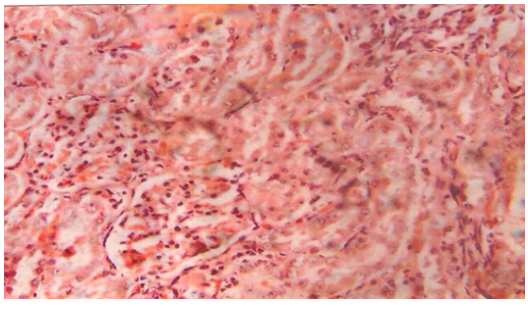


Figure 2: Photomicrograph of kidney cell histology

Control (No treatment with DBP)



Test (Treated with DBP)

DISCUSSIONS
The results of the sub-acute study on male albino Wistar rats, showed that di-n-butylphthalate (DBP) given orally to rats on daily doses of 2,000mg, 4,000mg and 6,000mg per kilogram body weight, produced some biochemical and pathological changes in the rats.

It was observed that there was significantly higher ($P < 0.05$) serum urea and creatinine levels in the treated rats when compared with the rats in the control group. This can be attributed to the impairment of the kidney, muscle wasting (weight loss), dehydration (diarrhea) caused by the toxicant. In addition, the reference interval for the ratio of serum urea nitrogen to serum creatinine for a normal subject on normal diet is 12 - 20:1 (Burtis *et al,* 2001). But from the results, all the rats in the treated groups had higher ratios (Group A 18:1, Group B 22:1, Group C 26:1 and Group D 27:1) with elevated creatinine, which is usually seen in post renal obstruction, pre-renal uremia superimposed on renal disease.

Dosage effect was also observed among the treated rats, suggesting a hepatobilliary saturation occurring at the higher doses, hence clearance of DBP and its metabolites from the plasma being higher at lower dose than at higher doses; implying more severe damage to the rats on higher doses.

It was also noted that after the first seven days of treatment, the treated groups began to feed less readily, showed dullness and had watery stool, in comparison with the control group. At the end of thirty days, the body weights of the control rats had significantly increased, unlike the treated rats that lost weight. All these are the symptoms of the

damage done by the toxicant on the internal organs like intestines, liver and the kidney.

The kidney morphology showed some mild changes in the glomerular structure of the treated rats in comparison with the control group as indicated by glomerular expansion, inflammation and vascular congestion. These alternations in the structures of the kidneys of the treated rats can be implicated in the corresponding alteration from normal, of the biochemical parameters assayed for, in the rats.

In conclusion, the toxicant, DBP altered the biochemical parameters in question in the treated animals by significant elevations in the parameters. Its toxicity on the kidneys is also evident from their cell histology. By its ubiquitous and persistent distribution in the environment, reasonable quantities can easily be ingested.

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