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Impact of Exposure To Di- N-Butylphthalate on The Liver of Adult Male Albino Wistar Rats

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ABSTRACT

This study investigated the effects of di-n-butylphthalate on the liver, 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 levels of bilirubin, alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase AST, served as indices of liver function. In addition, the cell histology of the liver of the rats was also examined. The results of all the liver parameters were significantly high ($P<0.05$), in groups B, C. and D. when compared to the level in the control group A. Conjugated bilirubin also recorded a significantly low level ($P<0.05$) in the treated groups B (2.14±0.04µmol/L), C (2.18±0.05µmol/L), and D (2.22±0.02µmol/L). The histological examination of the liver cell revealed occasional portal inflammation mild fibrosis and moderate amount of nuclear pyknosis. 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 organ functions for example liver functions, at high concentrations.

Key words*:* Di-n-butylphthalate, liver, bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase

INTRODUCTION

Di-n- butylphthalate, (DBP) belongs to a widely used group of chemicals called phthalates. It is a phthalic acid ester with the molecular formula $C_{16}H_{22}O_4$. It is an inert, colorless, oily liquid with mild aromatic smell, low vapor 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 (Centre for Disease Control and Prevention, CDC, 2005). 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).

Almost all the DBP present in the environment arose from 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.

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

i. Estimate the levels of serum bilirubin, ALP, ALT, and AST in the rats to assess the effects of DBP on the liver function.
ii. Examine the cell histology of their liver, for morphological changes due to the toxic effects of DBP.
iii. Compare the levels of these biochemical parameters and the histological changes in the treated animals with those of the control animals.

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.

MATERIALS AND METHODS

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 reproductive age of male albino Wistar rats.

Study Animals:

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 liver, kidney and testes 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 liver, dissected out and fixed in 10% formol saline solution, for histological studies.

Analytical Methods.

Histopathological examination:

The liver tissue was 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)

Biochemical analysis:

Methods of analysis

Total and conjugated bilirubin analysis was carried out using the method of Jendrassik and Gruff (1938) whereas Aklaline phosphatase was assayed with the method of King (1954). The transaminases were analyzed by the method of Reitman and Frankel (1957).

Statistical Analysis

Results were expressed as mean ± rmined standard error of mean. Significant differences between means were determined by one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 20 software.

RESULT

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.

Table 1: Weights of the rats (Mean ± SEM) at the on-set and end of the experiment.

MEAN WEIGHT(g)	GROUP A	GROUP B	GROUP C	GROUP D
Onset 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±11.97	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%

All the liver parameters of the treated rats were significantly high (P<0.05), except conjugated bilirubin which was significantly low (P<0.05) when compared with the untreated control group A, as shown in table 2.

Table 2: The Levels (Mean ± SEM) of Indices of Liver Function

PARAMETER	GROUP A	GROUP B	GROUP C	GROUP D
Total Bilirubin (μmol/L)	4.26 ± 0.06	5.68 ± 0.12	6.0 ± 0.13	6.30 ± 0.15
Test of significance:	F (4,16) =58.229; P < 0.05			
Conju. Bilirubin (μmol/L)	3.40 ± 0.06	2.14 ± 0.04	2.18 ± 0.05	2.22 ± 0.02
Test of significance:	F (4,16) =183.937; P < 0.05			
ALP (U/L)	32.40 ±2.20	50.60 ± 1.29	76.80 ± 0.92	100.00 ± 1.78
Test of significance:	F (4,16) =337.454; P < 0.05			
ALT (U/L)	15.00 ± 1.10	19.00 ± 0.55	26.60 ± 1.08	27.20 ± 1.40
Test of significance:	F (4,16) = 30.780; P < 0.05			
AST (U/L)	23.00 ± 0.84	31.60 ± 1.70	44.40 ± 1.40	64.20 ± 1.50
Test of significance:	F (4,16) =165.292 P< 0.05			

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.

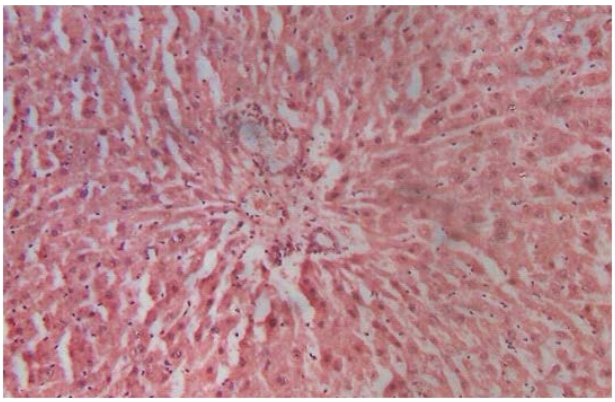

Fig. 1a: Photomicrograph of liver cell histology of Control (No treatment with DBP)

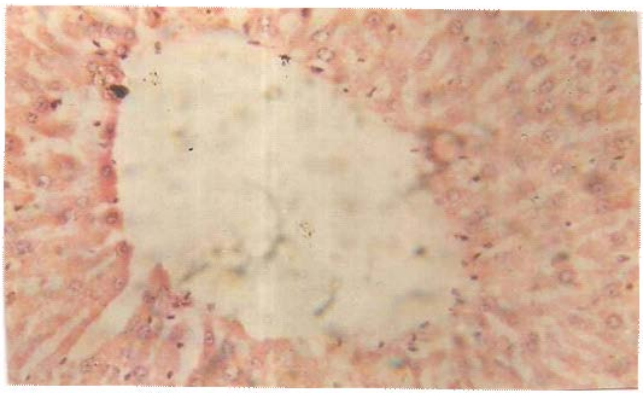

Fig. 1b: Photomicrograph of liver cell histology of Test (treated with DBP)

The liver cell histology showed portal inflammation, mild fibrosis and nuclear pyknosis.

DISCUSSION

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. The results revealed a significantly higher total bilirubin in the treated rats and a significantly lower ($P < 0.05$) conjugated bilirubin level when compared with the control group. Foster et al. (1982) investigated the metabolism of DBP following oral and intravenous doses in rats and hamsters, and reported that monobutylphthalate (MBP) undergo glucuronidation in the liver forming monobutylphthalate glucuronide (MBP-GLUC), which is the major metabolite in urine. The decrease in the level of conjugated bilirubin may be attributed to the depletion or diversion of the available glucuronic acid to conjugation of MBP, hence elimination of the toxicant from the system.

The levels of serum ALP, ALT, and AST from the study were significantly higher ($P<0.05$) in the treated rats in comparison with the control rats. Elevated activities of these enzymes are a common sign of toxic injury to the liver. It has also been shown by Litterest et al. (1972) that polychlorinated biphenyls, compounds structurally similar to DBP, can induce hepatic microsomal enzymes in rats in an attempt to eliminate the poison quickly. Similar process of enzyme induction and hepatocellular damage may also apply in this study and implicated as the cause of the increased liver enzyme activities.

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 pathological changes in the liver morphology of the treated groups manifested as portal inflammation, mild fibrosis and nuclear pyknosis. These alternations in the structures of the liver 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 liver function parameters in the treated animals. This toxicity on the liver is also evident from the cell histology of this organ. By its ubiquitous and persistent distribution in the environment, reasonable quantities can easily be ingested posing a serious threat to public health.

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