Research Article

Effect of single dose of Di isobutyl Phthalate on hepatic development in Wister rats treated prenatally

Shetty PN[1], Ray B[2], D'Souza AS[3], Sushma RK[3], Nayak D[4], Rao ACK[5],Chakraborti S[6]

[1]Department of Anatomy, Manipal College of Medical Sciences, Pokhara, NEPAL.
[2]Department of Anatomy, ESICMC - PGIMSR, Rajajinagar, Banglore, Karnataka, INDIA.
[3]Department of Anatomy, Kasturba Medical College, Manipal, Karnataka, INDIA.
[4]Department of pharmaceutical chemistry ,Manipal college of Pharmaceutical sciences ,Manipal ,Karnataka, INDIA.
[5]Departmentof Pathology International Centre, Manipal, Karnataka, INDIA.
[6]Department of Pathology, Kasturba Medical College, Manglore, Karnataka, INDIA.
Email: dr.prasadnshetty@gmail.com

Abstract

Phthalate esters are plasticizers widely used in the manufacture of plastics. Di iso butyl phthalate (DIBP) is used as a plasticizers, ranging from the plasticization of PVC to the production of paints, printing inks and adhesives. Therefore, DIBP exposure in people in adhesive industries and pharmaceutical industries are higher in comparison to general population where it is low. Major route of excretion of DIBP is through urine, with some excretion in the faeces, presumably due to biliary excretion. Aim of this study was to determine the effect of single dose of DIBP on developing liver of Wistar rat. One hundred and eight adult pregnant Wistar rats were divided into control and experimental groups. Rats in experimental group were given DIBP on10, 12and 14day of gestation at 0.375, 0.75 and 1.25ml/kg body weight doseintraperitoneally in a single dose.Sections of liver collected on day 21of gestation were stained with haematoxyline and eosin and examined histologically.The remarkable histo-pathological changes were not observed, when exposed to above dose of DIBP.But, there was a significant reduction in the weight of foetalrat liver, when mother rat exposed to DIBP on 10thday of gestation compared to 12th and 14th days. As pregnant women are constantly exposed, effect of DIBP on the liver of a developing fetus would denote the consequence in future generation.

Keywords: Di isobutyl Phthalate, Wister rats.

Address for Correspondence
Dr. Prasad N. Shetty, Lecturer, Department of Anatomy, Manipal College of Medical Sciences,Pokhara, NEPAL.
Email: dr.prasadnshetty@gmail.com
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INTRODUCTION

Phthalates are organic chemicals and the most commonly used plasticizers in the world. In general, they are colorless, high-boiling liquids, soluble in organic solvents but immiscible in water. And they are degraded very slowly in the ambient environment.[1]They have been part of the revolutions to provide low cost, high performance materials that make our everyday lives much easier. Phthalates are not used alone as they always incorporate into an end product such as something that is made of Poly vinyl chloride (PVC). They include everything from lifesaving medical devices to footwear, electrical cables, packaging materials, stationery toys and roofing; In addition specific phthalates are used in other non PVC applications such as paints, rubber products and some adhesives. When phthalates are added into the vinyl manufacturing process they act as a softener and a lubricant.[2, 3, 4]At present, there are six medicinal products on the market that contain Phthalatesi.e, Litare, Modifenac, Mesasal, Vivotif, Omeprezole, and Theophylline.[5] A large number of tubing's, blood bags, infusion containers, surgical gloves, catheters, surgical

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drapes, and other medical items are of PVC, containing rather large concentrations of phthalate esters.[2,3,4] Phthalates do not persist in the environment as they biodegrade readily they do not accumulate in animals or humans, they breakdown quickly and are excreted.[6, 7] Because phthalates are so widely used, they have undergone extensive testing for possible health and environment effects and are among the most widely researched of all chemical substances. Di-isobutyl phthalates(DIBP)are a high-production-volume class of chemicals that are used in many consumer products like toys, baby products, lotions, cosmetics, fragrances, medical devices, pharmaceuticals, and automobile parts. They are present everywhere in our environment and most people including pregnant women and their fetuses are exposed to phthalates.[8, 9] Several studies have shown that although DIBP exposure in humans are generally low, almost near the limit of detection, asmall percentage of peoples working in adhesive and pharmaceutical industries are exposed to higher levels of DIBP.[10]

Toxicities

According to national toxicology program of U.S.A. Phthalates is listed as a substances 'seasonally anticipated to be a human carcinogen.[11] According to the national institute of environmental health sciences of U.S.High levels of exposure to phthalates through the use of medical tubing and other plastics devices for feeding, medicating, and assisting the breathing of newborn infants, may affect the development of the male reproductive system.[12] According to a data produced by the Australian Government, Department Of Health And Ageing, NICNAS, the toxic level of DIBP by Intraperitoneal route (LD5O) is 4.5 gm/Kg body weight of rat or greater than 1600mgm/kg body weight of rat.[13]

Former reports on DIBP

DIBP has a low order of acute toxicity by the oral, intraperitional and dermal route. DIBP is reported to cause minimal skin irritation in guinea pigs. No eye irritation or skin sensitization was reported in animals.[14]Intraperitional (ip) injection of DIBP to pregnant Sprague Dawley rats, doses of 0.375, 0.75 and 1.25 ml/kg bw (approximately 390, 780 and 1300 mg/kg bw) on gestational day 5, 10 and15. Showed decreased average weight of foetuses at all dose levels and increased numbers of resorptions. And also observed skeletal abnormalities ("partially elongated and fused ribs") at the highest dose (1300 mg/kg bw) even dead fetuses were found.[15] A 4-month repeated DIBP dose toxicity study reported low body and testes weight. Increased liver weights in rats with a 5% diet.[16]Administration of 600 mg/kg bw of DIBP to pregnant Wistarrats fromgestational day 7 to 19 or 20/21 day, resulted in significant reduction in anogenitaldistance (AGD) in male pups and increased AGD in female pups at gestational day 20/21 together with reduction in bodyweights of male and female foetuses and reductions in testicular testosterone production. Testicular pathological changes were also noted.[17]Feeding a diet containing 2% (approximately 2000 mg/kg bw/d) of DIBP for a week resulted in significantly decreased absolute and relative weight and Zinc concentrations in the liver.[18]The study reported that chronic toxicity of DIBP increased liver weight and as well as liver cancer.[14] A short term study was conducted on dogs, One male and one female dog were fed with DIBP via diet at a daily rate of 0.1 ml/kg and 2.0 ml/kg feed respectively for 2 months. Weight loss was noted in the female dog. There was an increase in relative liver weight in the female dog compared to controls. No histological changes in liver were observed. In the male dog, histological examination of testes revealed few abnormal sperm[16] .In an oral study with mice increased incidence of hepato - cellular carcinoma were seen at dose level of 3000-6000 mg /kg in the diet.[7]

MATERIALS AND METHODS

108Female Wistar rats of an average weight of 200 g and an average age of 120 days were used in this study and they were housed individually in plastic cages in noise-free, air conditioned animal house with temperature maintained at 75°F and on a light dark cycle of 12:12 hours. Humidity was maintained with a minimum of 50%. Rats were fed on diet pellets (Hindustan Lever, Bombay, India) and tap water ad libitum and treated with utmost human care. All experiments were carried out with prior approval from the institutional animal ethical committee. The female rats in their pro-estrous were caged overnight with males of the same stock (Female: Male = 3:1). Presence of sperms in the vaginal smear on the following morning confirmed start of gestation and the day was numbered as the day 'zero' of pregnancy.

Experimental groups

108 adult pregnant Wistar rats were used in the present work.They were divided into two main groups. Group I (54 rats) was treated with equal amount of distilled water and served as control. Group II (54 rats) were given intraperitional injection of DIBP on 10, 12 and 14 day of gestation. The animals of treatment in each group (n=6) were administered DIBPintraperitoneally in a single dose of 0.375 (Dose 1), 0.75 (Dose 2) or 1.25 (Dose 3) ml/kg (approximately 390, 780 or 1300 mg/kg) body weight with the help of a sterile tuberculin syringe. Along with this experimental group, a control group was maintained and administered equal amount of distilled water alone.

The Pregnant rats were anesthetized by chloroform on day 21st and the fetuses were removed by Caesarian operation. The Liver was surgically exposed, washed with 0.9% normal saline, fixed in formalin, embedded in paraffin and sectioned at 8µm thickness. Sections were stained with haematoxyline and eosin and examined under microscope. The photomicrographs for histological studies were taken with the help of LetizOrthoplan Photomicroscope.

RESULTS

1. Morphological findings

Maternal administration of DIBP in rats has shown Significant (P<0.05) reduction in weight of the foetal rat liver on the 10th day when compared to 12th and 14th days of gestational period and also with control group.

2. Histo-pathological findings

There are normal hepatocytes, healthy environment of central vein and other blood vessels, extra medullary haematopoiesisaround the sinusoids, which is denoted by immature nucleated RBCs and Megakaryocytes containing platelets in the experimental groups. These findings are within the normal limits when compare with the control group.

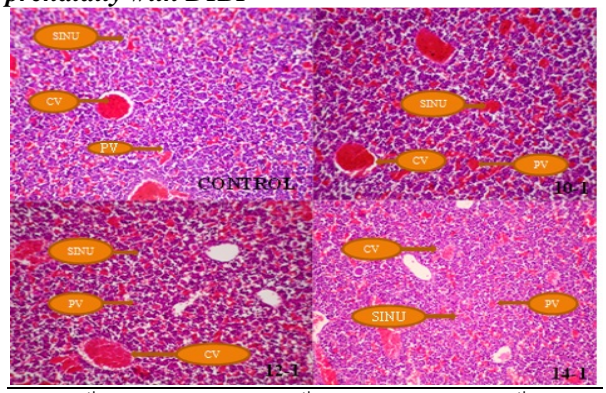
Table 1: Showing the relative weights of the liver of rat foetuses treated prenatally with diisobutylphthalats

Days	Doses	Mean ±S.D	P-Value
10	Dose 1 Control	0.50 ± 0.0137	
	Dose 1 Experimental	0.19 ±0.0081	0.000
	Dose 2 Control	0.50±0.0403	
	Dose 2 Experimental	0.18±0.0189	0.000
	Dose 3 Control	0.55±0.0206	
	Dose 3 Experimental	0.17±0.0163	0.000
12	Dose 1 Control	0.50±0.0089	
	Dose 1 Experimental	0.45±0.0089	0.001
	Dose 2 Control	0.50±0.0034	
	Dose 2 Experimental	0.47±0.0752	0.471
	Dose 3 Control	0.58±0.0147	
	Dose 3 Experimental	0.42±0.0432	0.001
14	Dose 1 Control	0.50±0.0453	
	Dose 1 Experimental	0.47±0.0863	0.296
	Dose 2 Control	0.50±0.0339	
	Dose 2 Experimental	0.40±0.0136	0.001
	Dose 3 Control	0.61±0.0116	
	Dose 3 Experimental	0.46±0.0783	0.006

Level of significance at P<0.05

Histo-pathological findings

Tissues of control pups compared with pups treated prenatally with DIBP



10-1: 10th day dose 1, 12-1: 12th day dose 1, 14-1: 14th day dose 1
SINU- sinusoids, CV-central vein, PV- Portal vein

DISCUSSION

In the present study maternal administration of the considered dosages of DIBP during the organogenetic period (10-14 days) in the rat do not cause any histo-pathological effects in the fetal liver, although a marked reduction in the fetal liver weight was observed on exposure to 10th day when compared with 12th and 14th days of gestational period and also with control group. Fetotoxic and embryopathic effects are greater during the early organogenitic period (10th day) than during the later stages (12th and 14th day). A single injection of DMEP induced a pronounced fetotoxicity during the organogenetic period regardless of the day of injection. The fetotoxic and the embryopathic effects were greater during the early stages(days 10 or 11 of gestation) of organogenesis thanduring the later stages (days 12, 13 or 14 of gestation).[19]DIBP appears to be readily absorbed via the oral and dermal routes. Based on a dermal absorption study, DIBP undergoes primary metabolism into the hydrolytic monoester, monoisobutyl phthalate (MIBP), before excretion.[20] Urine is the major route of excretion with minor biliary excretion being observed. There was little accumulation in the rat tissues. Therefore, effect of DIBP on developing fetal liver was insignificant.[21]This is consistent with present study. Diethyl phthalate (DEP) was the cause of several cases of hepatitis in a hemodialysis unit . The plasticizer was present in PVC tubing used in the dialysis apparatus. It is possible that these agents may have played a role in causing hepatitis. However, experimental evidences does not indicate that diethyl phthalate alone will cause hepatic damage in rats.[22] In a study, feeding high levels of DEHP to rats and mice throughout their lifetimes results in an increased incidence of liver cancer. Acute and chronic administration of DEHP caused variable effects on liver

after intra peritoneal administration caused enlargement of liver weight and histo-pathological examination showed phthalate induced enlarged liver with vacuolization, congestion and dilation of veins followed by cloudy swelling, excessive fatty degeneration, dilation of the smooth and rough endoplasmic reticulum, mitochondrial swelling and Increase in the micro body in rat liver have been reported on intra peritoneal administration of DEHP for 21 days.[23] In spite of their low order of toxicity, phthalates have been shown to exert hepatotoxic, cytotoxic, teratogenic and mutagenic effects. Liver and testis appear to be the main target organs in phthalates toxicity. Phthalates have been shown to elicit contrasting effects on the testis and the liver, causing testicular degeneration and promoting abnormal hepatocyte proliferation and carcinogenesis.[24]Experimental results indicate that chronic exposure to Phthalic esters may be responsible for a number of adverse health effects. Phthalates change the structure and function of the liver in a profound manner by inducing peroxisomes, mitochondria and enzymes which participate in fatty acid transport and β-oxidation. Prolonged administration of phthalate esters, in doses comparable to those occurring in human exposures, seems to have an accumulative effect on the liver. Liver biopsies taken from dialysis patients show peroxisome proliferation which again warn the possibility that human health may be influenced by plasticizers.[25] DEHP at high concentration can cause functional hepatic damage as reflected by morphological changes and alterations in the activity of energy linked enzymes and metabolism of lipids and carbohydrates.[26]In rats, at certain levels of exposure, phthalates can cause liver cancer.[27] Maternal administration of DIBP has shown hepatomegaly and carcinoma of the liver in the mother rat and decreased liver weight in foetal rat .[28]

CONCLUSION

In the present study, exposure to the DIBP in early days of pregnancy (organogenetic period), has shown significant reduction in the weight of foetal rat liver butdoes not affect developing foetal liver significantly at histological level. Prolonged and sustained exposure of mother rat to DIBP may it affect the normal histology of foetal liver.Manufacturers should be knowledgeable enough about Phthalate toxicity and it should include in prescribed amount.

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