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RESEARCH ARTICLE

CHRONIC EFFECTS OF DI (2-ETHYLHEXYL) PTHALATE ON BIOCHEMICAL INDICES OF LIVER IN FEMALE SWISS ALBIINO MICE MUS MUSCULUS

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ABSTRACT

In present study Di (2-ethyylhexyl) phthalate orally administered at the dose of 10 mg/kg/b.wt/day selected on the basis of maximum tolerable dose for a period of six weeks showed very significant changes in liver weight($P<0.01$) and body weight($P<0.01$) in all DEHP treated groups except two weeks treated mice group . Increase in serum level of SGPT and ALP in DEHP treated group were not significant ($P>0.05$) in two weeks treated mice while significant($P<0.05$) in four weeks and very significant ($P<0.01$) in six weeks treated mice .Decrease in serum level of albumin and total protein in DEHP treated mice groups were statistically not significant ($P>0.05$) in two weeks while found very significant ($P<0.01$) in four weeks and statistically extremely significant($P<0.001$) in six weeks . The present study concludes hepatotoxic effects of the selected dose of DEHP (10mg/kg b.wt./day) on female swis albino mice observed as the significant biochemical changes in the liver function test parameters. Since variable concentrations of Di (2-ethylhexyl) phthalate are present in plasticizers and plastic containing consumer products it is necessary to investigate the toxic potential of DEHP.

Key words: Di (2-ethylhexyl) phthalate ,Female Mice , Plasticizer, Hepatotoxin, Chronic

INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) and other PAEs used as plasticizers to impart flexibility to plastics, particularly polyvinylchloride (PVC) polymers have a wide variety of biomedical and other uses. These are known to leach out from finished PVC products into blood, physiological fluids, commercial solvents and food materials (Tomita *et al.*, 1979). Ubiquitous presence of DEHP in environment has led to numerous studies on their toxicology. In spite of their low order of toxicity, DEHP have been shown to exert hepatotoxic, and cytotoxic effects. Liver is one of the main target organs in phthalate toxicity. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987). Jain *et. al.*, (2009) have reported that the oral administration of DEHP (1000mg/kg b. wt./day) for the period of six weeks in rat caused significant increase in serum marker enzymes. Similar study has been done by Kluwe *et. al.*, (1982) on B6C3FI mice at a dietary dose of 390mg/kg b. wt. /day and 780 mg /kg b. wt./day and they reported an increase in incidence of hepatocellular abnormalities. There is no reported chronic toxicity data available in detail, at a particular dose of 10mg/kg b. wt./day on liver biochemical parameters in swiss albino mice. Such information will be of great significance in assessing the toxic potential of DEHP at the selected dose. In liver different cells have different enzymes inside them, depending on the function of the cell. When cells die or are damaged, the enzymes leak out causing an increase or decrease in their level in the blood. Serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase are some liver-function test enzymes, the elevation of which, in serum, reflects some hepatic disorders. Albumin and total protein levels are known to be useful in assessing the functional integrity of the liver. Therefore, in order to evaluate the liver damage, estimation of these enzymes in serum is essential. In present study effect of di(2-thylhexyl) phthalate has been evaluated on female Swiss albino mice at a chronic dose of 10 mg/ kg body weight/day for a period of six weeks.

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MATERIALS AND METHODS

Chemicals

Di(2-ethylhexyl)phthalate(DEHP) was procured from Accustandered USA(Marketed by Rankem) and corn oil used as vehicle from Sigma Pvt. Ltd. All chemicals used were of analytical grade

Animals

Female swiss albino mice *Mus musculus* weighing 28g-30g (6-8 weeks, reared and bred in the animal house of Mahavir Cancer Sansthan, Patna))were housed in individual apartment cage in an air condition room (24 ±1 0^CTemp. and 55± 10% humidity)with a 12-hr alternating light- dark cycle. The animals were provided with slandered pellet diet (Amrut Laboratory Animal Feed ,Mysore Feed Limited, Banglore, India) and water *ad libitum* and allowed to acclimatize for a week before treatment. All experiments

were carried out as per CPCSEA guideline (approval no.-1129/bc/07/CPCSEA).

Experimental design

The animals were subsequently divided into seven groups (I-VII) with six mice in each group having similar mean body weight. The maximum tolerable dose of DEHP was found to be greater than 100 mg /kg/b wt in our laboratory and on the basis of this observation the 1/ 10^{th} value of the dose of 10 mg/kg/b.wt was selected for oral administration to the experimental groups. In our study 10 mg DEHP was dissolved in 10 ml of corn oil(vehicle) to obtained the experimental dose of 10mg/kg/bodyweight/day and was administered orally to the experimental mice groups daily, according to the body weight .The mortality and morbidity were recorded during experimental period.

Statistical analysis

Mean and standard error of mean (SEM) were calculated from the six replicates of each group. Statistical calculations of the data were performed using one way (ANOVA).P<0.05 was considered statistically significant.

Automated Biochemistry Analyzer(Model No-SELECTRA-"E",VITALAB BY MERCK) in the Dept. of Biochemistry of Mahavir Cancer Sansthan and Research Centre ,Patna, India.

RESULTS

The present study was carried out on female Swiss albino mice to evaluate the toxic potential of Di(2-ethylhexyl)phthalate at a dose of 10mg/kg/b. wt. on biochemical parameters of liver. In the present study 10% morbidity was recorded after oral administration of 10mg/kg/b. wt./day DEHP. About 40-52% mortality was recorded among the DEHP treated mice during the experimental period of six weeks . Only few deaths were recorded during the experimental period of six weeks in the control groups, which indicated that corn oil at the dose of 10 ml/kg body weight can be used as a vehicle for toxicological studies. In the experimental period, a progressive decrease in the body weight was observed after two weeks of oral administration of DEHP at a dose of 10mg/kg/b. wt/day . The changes in body weight were statistically not significant

Table 1. Body weight and organ weight of normal, control and DEHP treated female mice

S.L.	Parameters (in grams)	Normal female mice	Control (Corn oil treated mice)			DEHP treated female mice		
			Gr-II 2weeks	Gr-III 4weeks	Gr-IV 6weeks	Gr-V 2weeks	Gr-VI 4weeks	Gr-VII 6weeks
1.	Body weight	30.3 ±0.246	30.08 ±0.217	30.9 ± 0. 15	31.5 ± 0.26	30.01 ±0.123	30.11 ±0.221	28.95** ±0.438
2.	Liver weight	1.65 ± 0.20	1.7 ±0.017	1.73 ±0.020	1.76 ± 0.012	1.73 ±0.026	1.83** ±0.023	1.91** ±0.015

Values expressed as mean ±SEM.,P<0.05 , P<0.01* and p<0.001**
Were considered statistically significant

Table 2. Liver function test parameters of normal, control and DEHP treated female mice

S.L	Liver function test parameters	Normal female mice (Gr-I)	Corn oil treated mice(female)/control			DEHP treated female mice		
			Gr-II 2weeks	Gr-III 4weeks	Gr-IV 6weeks	Gr-V 2weeks	Gr-VI 4weeks	Gr-VII 6weeks
1.	SGPT(IU/L)	30.6 ±1.12	30.13 ±0.944	30.81 ±0.968	31.4 ±0.981	33.3 ±0.854	34.5* ±0.577	36.7** ±0.918
2.	ALP(IU/L)	232 ±30.63	228 ±27.25	274 ±6.874	290 ±6.378	303 ±10.140	307* ±8.236	315 ** ±8.129
3.	Total Protein (gm/dl)	5.9 ±0.241	6.08 ±0.168	6.26 ±0.176	6.34 ±0.212	6.3 ±0.131	5.26* ±0.080	5.1** ±0.113
4.	Albumin (gm/dl)	3.05 ±0.109	3.01 ±0.137	3.01 ±0.156	3.28 ±0.124	3.08 ±0.087	2.5* ±0.157	2.41** ±0.127

Values expressed as mean ±SEM.,P<0.05 , P<0.01* and p<0.001**
Were considered statistically significant

Biochemical study

Serum was obtained from the blood by centrifugation (3000 rpm for 15 minutes), for the estimation of various liver biochemical parameters. The serum biochemical liver function test parameters like ALP estimated by Alkaline phosphatase kit (Kind P.R.M et al 1954), SGPT by SGPT Kit (Reitman S et al 1957), total protein by Biuret method and albumin by BCG method(Douma BT et al 1971), using fully

(P>0.05) in two and four weeks(Gr.-V and Gr.-VI) while it was statistically very significant(P<0.01) in six weeks(Gr.-VI) DEHP treated female mice. The relative weight of liver increased in treated groups and the changes in liver weight were not quite significant in two weeks(Gr.-V) treated mice whereas it was statistically extremely significant (P<0.001) in four (Gr.-VI) and six weeks(Gr.-VII) treated mice (Table 1). Increase in serum level of SGPT in DEHP treated mice was observed from second week up to the sixth week of the

experimental period. The increase in level of SGPT was statistically not quite significant (P>0.05) in two weeks(Gr.-V) treated mice ,while it was statistically significant(P<0.05) in four weeks(Gr.-VI) and was statistically very significant (P<0.01)in six weeks(Gr.-VII) treated mice in comparison to control group. In our study the serum ALP level also showed a similar increase in DEHP treated groups of mice which was statistically not quite significant(P>0.05) in two weeks(Gr.-V) treated group, statistically significant(P<0.05) in four weeks(Gr.-VI) and statistically very significant(P<0.01) in six weeks(Gr.-VII) treated groups(Table-2). The present study revealed that the serum level of albumin decreased progressively from second week to sixth week and the decreased value was statistically significant(P<0.05) in two weeks(Gr.-V) and four weeks(Gr.-VI) treated mice while very significant (P<0.01) in six weeks(Gr.-VII) treated group. The total protein level also decreases in DEHP treated mice. The decreased value of total protein serum level in two week (Gr.-V) treated mice was found not quite statistically significant (P>0.05 while it was very significant (P< 0.01) in four week (Gr.-VI) and six week (Gr.-VII) treated mice (Table-2)

DISCUSSION

DEHP is a well known hepatotoxin in animals and belongs to a class of chemical called the peroxisome proliferators (PPs) (Moody and reddy 1978) as it produces liver hypertrophy (Rusyn *et al*., 2006). DEHP has potentially adverse effect on the liver and endocrine system (Ticner *et al*., 2001 and Sharp R M (2001). It has also been reported by Yuki ITo *et al*., (2007) and G C Jain *et al.,* (2009) that liver weight increases in rat after subchronic administration of DEHP (1000mg/kg b. wt). In present investigation an increase in liver weight has been recorded after sub-chronic and chronic administration of 10 mg/kg/b. wt./day of DEHP in female Swiss albino mice. Several workers have studied the hepatotoxic potential of phthalates in a variety of animal species. DEHP tends to be rapidly cleared from the body with the majority being eliminated within 24 to 72 hours after absorption as reported by Kluwe (1982) but chronic low dose feeding studies in rhesus monkeys have showed longer retention of this chemical, notably in liver (Jacobson *et. al*., 1977) . Oral administration of DEHP caused variable effects on liver depending upon the species of animal according to Carpenter, *et.al*, (1953). Liver function test are helpful screening tools to detect hepatic dysfunction (Thapa *et. al.,* 2007).SGPT and ALP are considered as the bio-markers for liver function (Mazumder *et al.*,1999).

Serum Glutamic Pyruvate Transminase (SGPT) is primarily localized in cytosol of hepatocytes (Sherlock *et. al.,* 1997 and Rosen *et. al*., 2000) and increased and decreased serum level of it is the specific indicators of hepatocellular necrosis. The present study showed the progressive increase in serum SGPT level (Table -2) in DEHP treated groups from second week onwards. The result indicates that the increased SGPT level may be due to hepatocellular necrosis which causes an increase in the permeability of the cell membrane resulting in the release of transaminase in blood circulation and probably the leakage of enzyme across damaged plasma membrane and or the increased synthesis of enzyme in the liver. ALP (Alkaline Phosphatase) is found histochemically in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes (Rosalki *et.al.,* 1999) and splits various phosphate esters at an alkaline pH and mediates membrane transport (Smith *et al*., 1983) and its inactivation leads to membrane damage of hepatic cells (Flora *et al.,* 1994). Highest level of alkaline phosphate occur in cholestatic disorders and that elevation occurs as a result of both intra hepatic and extra hepatic obstruction to bile flow as reported by Friedman *et. al.*,(2003). These enzymes are associated with transmembrane transport mechanism, ion transport, maintenance of ionic strength and cell growth in the organ (Moog, 1946). The significant increase in the activities of acid and alkaline phosphatases may be attributed to the destruction of all membranes and lysosomes which in turn might cause tissue damage (Ramalingam *et al*., 1999).

The present investigation showed an increase in serum ALP level in DEHP treated mice groups (Table -2).) indicating chronic DEHP toxicity at the selected dose. It has also been reported by G. C. Jain (2009) that the liver function test parameter like SGPT and ALP increased very significantly after the administration of DEHP(1000mg/kg b.wt./day) in rat. Therefore, it can be inferred that DEHP toxicity may cause intrahepatic and extrahepatic obstruction to bile flow. Albumin is a plasma protein, synthesized only by the liver (Rosalki *et al*., 1999) and its serum level is affected by liver diseases, nutritional status and also by hormonal imbalances. Albumin maintains the fluid volume within the vascular space and its low value is a sign of poor health. The present study revealed that serum albumin level and total protein level progressively decrease from second week onward in DEHP treated mice at the selected dose (Table -2). Jain *et al*., (2009) have also observed a decrease in serum total protein level in rat treated with DEHP (1000mg/kg/b.wt./day). The total protein represents the sum of albumin and globulin. The plasma protein is a function of the nutritional status which is one of the factors affecting the state of health of the animal (Igwebuike *et.al*., 2008).

The above mentioned results may be due to hepaic tissue damage which leads to increase in the permeability of cell membrane resulted the excessive release of these enzyme in blood circulation (Sanker Samipillai *et al*., 2010).As the liver is the center for detoxification of many foreign compound entering the body. DEHP (10mg/kg b.wt./day) which was administered to female swiss albino mice probably did not get detoxified in the system and ultimately caused the observable toxic effects on liver . Therefore, the results of the present investigation concluded hepatotoxic effects of DEHP at a dose of 10mg/kg b. wt./day on the female mice and since variable concentrations of Di(2-ethylhexyl)phthalate are present in plasticizers and plastic containing consumer products, so it is suggested that DEHP containing plastic consumer products should be used with caution.

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