

Environments and Health Hazards of Phthalate (Di-*n*-Butyl Phthalate) Present in Plastics

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Abstract: Plastics are extremely diverse in terms of chemical composition, properties and possible applications and are widely distributed in the society and the environment. Several of the chemicals used to produce plastics are hazardous for human health and the environment. Phthalate (Di-*n* butyl phthalate) is a major chemical constituent of plastic. Phthalates are also frequently used in soft plastics, fishing lures, nail polish, adhesive, caulk, paint pigments and sex toys. The present studies aims to examine the change in serum glucose, serum protein, serum creatinine, serum cholesterol and serum triglyceride. The sub lethal concentration of 1.66 mL of Phthalate per Kg body weight of mice while simultaneously control set was also observed. The mice were kept on room temperature and normal diet during the experimental period. At the end of the terminal day of treatment, mice were decapitated the blood was collected by cardiac puncture and serum was separated by centrifugation method at 2000 rpm for 15 min. After overnight the serum samples were stored at 20°C for serum analysis. Serum protein, serum cholesterol and serum triglyceride are decreased in treated animal. The toxicity of Phthalate on the above said parameters will be discussed.

Key words: Plastics • Di-*n* Butyl Phthalate • Serum • Toxicity

INTRODUCTION

The term phthalates, or phthalate esters, refers to a large group of compounds that share basic chemical similarities. However, the individual members of the group have unique physical and chemical properties and studies to data suggest that they also affect biological organisms differently. There is large number of sources of phthalates, most of which reflects their use as plasticizer for polyvinyl chloride (PVC). These compounds are particularly useful in the production of soft PVC products such as plastic tubing, gloves and toys. In addition phthalates are also used in building material, clothing's, personal care products and other consumer products [1]. Plastics used in food industries processing and packaging unit, are the major source of phthalates in foods, milk and drinking water. Foods seem to be the main source of human exposure to DBP [2, 3], although air and dust appear to be significant contributor in infants and children. Phthalates have been measured in residential

indoor environments in both house dust and indoor air [4]. However, the relative contribution from the various sources and routes of exposure to phthalates is unknown [5]. Studies on rodents involving large amount of phthalate have shown damage to the liver, kidney, lung and developing testis [6]. Anti androgenic effect of DBP has been reported in rat [7]. Gangolli [8] reported decreases in testis weight in 2000 mg/ kg/Day treated male mouse.

Di-*n*- butyl phthalate is generally used in dyes, latex adhesives, plasticizer in cellulose plastics, medicines coatings and cosmetics products [9]. It is also found in food due to leaching from DBP containing products that come into contact with food during processing or storage. However use of DBP in plastic toys appears rare because the result of careful analysis of toys indicate that it is either not present or present at low level [10]. Release of DBP into the ecosystem occurs during the production phase and via leaching and volatilization of plastics product during their usage or after disposal. There is

paucity of information pertaining to serological impact of Phthalates (DBP) on mammalian species. The present paper deals the toxicity values that are generally used to characterize the potential adverse outcomes from exposure to DBP with reference to serological parameters.

MATERIALS AND METHODS

Male Albino rats were kept at room temperature with proper ventilation and normal diet during the period of experiment. The experimental animals have divided into two groups, control and treated group in which 10 male albino rats each in both groups. They treated orally with sub lethal concentration of 1.66 ml [Di-*n-Butyl* phthalate, Merck Company] per kg body weight, after treatment 10 samples were examined at interval of every three days while simultaneously control set was observed. Serological test have been done by following methods.

Serological Parameters: Routine serological tests were conducted periodically to evaluate changes in serum creatinine, serum glucose and serum cholesterol and serum triglycerides [11-16].

Serum Glucose Estimation: The serum glucose estimation of experimental animals were done by running the experimental serum against a glucose reagent and a glucose standard by using a colorimeter [11]. The amount of glucose was estimated by the development of color.

Thus,

$$\text{The average serum glucose level of the test animal} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Serum Protein Estimation: The serum protein estimation of test animal was done by running the test serum against standard protein solution and a Biuret reagent using calorimeter [12-13]. The amount of protein was estimated by intensity of blue- violet color produced by the reagent.

Thus,

$$\text{The average serum protein level of the test animal} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Serum Creatinine, Cholesterol and Triglyceride Estimation: Serum creatinine, cholesterol and triglyceride all were done by running the test serum against the standard solution or reagent provided and then by measuring the intensity of color produced. The entire samples were measured in g/dl [11, 14-16].

RESULTS AND DISCUSSION

Initially the experimental animals which under control had the serum glucose level was 190 mg/dl. The serum glucose level increased by 5.2, 3.7, 6.25, 6.87, 4.73 and 4.16% on 3rd, 6th, 9th, 12th, 15th and 18th day respectively in comparison to the control values after the administration of sub lethal dose of DBP (Table 1). Similarly, in treated rats with respect to their corresponding control values, the serum creatinine value increased significantly ($p < 0.001$) by 21.4, 20, 18.5, 22.9, 19.19 and 12.24% on 3rd, 6th, 9th, 12th, 15th and 18th day of experiment (Table 2).

On first day the experimental animals which were under control had the serum protein level 7 g/dl. Then those which were kept under treated group were continuously exposed to 1.6 ml DBP/ kg body weight. After every three days interval the treated animals shows 6±1.73, 7±2.02, 6±1.01, 6±1.12, 6±1.20 and 5±1.32 g/dl respectively. On the basis of observation we can infer that the serum protein level in treated group decreases with respect to the control one (Table 3).

The serum cholesterol values as shown in Table 4 exhibit percentage decrease pattern in comparison to control group. The treated group showed the % decrease 33.3% on 3rd day and highest % decrease was 35.6% on 12th day of the observation. Also the % decreases were observed in serum triglycerides level in treated rats 25% decrease in triglycerides level was observed in respect to control one (Table 5). Bibra [17] and Barbar *et al.* [18] reported that serum triglycerides and cholesterol level decreased in all exposed males and cholesterol level in all exposed females. The serum level of DBP was significantly higher than those in control serum samples [19]. In the study by NTP [20] there was decrease in triglycerides, cholesterol and total protein level starting at 10000ppm in male rats and 20000ppm in female rats.

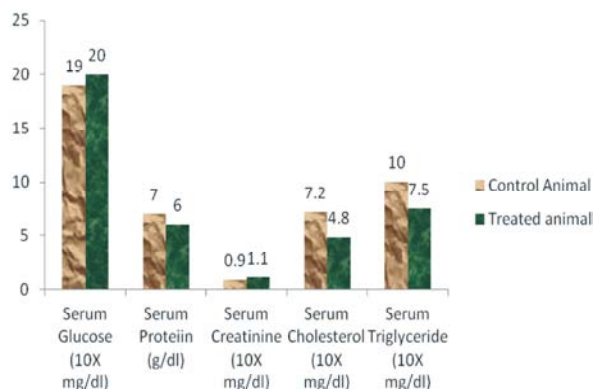


Fig. 1: Average biochemical parameters of control and Phthalate treated mice

Table 1: Biochemical parameters of Serum Glucose (mg/dl)

Days	Control animal (M ± SD)	Treated animal (M ± SD)	% Increase
3	190±4.58	200±1.73	5.2
6	188±9.29	195±2.51	3.7
9	192±8.50	204±3.05	6.25
12	189±8.88	202±5.56	6.87
15	190±3.51	199±2.51	4.73
18	192±8.50	200±1.76	4.16

Table 2: Biochemical parameters of Serum Creatinine (mg/dl)

Days	Control animal (M±SD)	Treated animal (M ± SD)	% Increase
3	0.98±0.01	1.19±0.21	21.4
6	1.00±0.06	1.20±0.15	20.0
9	0.97±0.02	1.15±0.22	18.5
12	0.96±0.02	1.18±0.08	22.9
15	0.99±0.10	1.18±0.08	19.1
18	0.98±0.01	1.10±0.11	12.2

Table 3: Biochemical parameters of Serum Protein (g/dl)

Days	Control animal (M±SD)	Treated animal (M ± SD)	% Decrease
3	7±1.00	6±1.73	14.2
6	8±1.00	7±2.02	12.5
9	7±1.52	6±1.01	14.2
12	8±1.12	6±1.12	25.0
15	7±1.50	6±1.20	14.2
18	7±1.52	5±1.32	28.5

Table 4: Biochemical parameters of Serum Cholesterol (mg/dl)

Days	Control animal (M±SD)	Treated animal (M±SD)	% Decrease
3	72±1.52	48±2.51	33.3
6	70±2.51	46±2.08	33.3
9	71±1.01	49±1.52	30.9
12	73±1.52	47±2.51	35.6
15	71±2.01	48±2.52	32.39
18	73±1.61	49±1.62	33.3

Table 5: Biochemical parameters of Serum Triglyceride (mg/dl)

Days	Control animal (M±SD)	Treated animal (M±SD)	% Decrease
3	100±3.51	75±3.51	25
6	95±3.020	72±3.51	24.2
9	105±1.52	78±2.01	25.7
12	98±2.080	74±3.01	24.4
15	99±2.510	73±3.02	26.2
18	103±3.51	78±2.07	24.2

A marked significant increase ($p<0.001$) in serum glucose (6.87%) and significant decrease in serum triglyceride and cholesterol were 35.6% and 26.2% respectively (Fig. 1), which is in agreement with above finding. Nikonorow *et al.* [21] reported that hypocholesterataemia in both sexes at 20000 and 40000 mg/kg hypotriglyceridemia in all exposed male and female at ≥ 10000 mg/kg elevation in alkaline phosphatase activity and bile concentration in both sexes was considered indicative of cholestasis, which have been

published [20]. In an inhalation study, effect at lowest level were observed by Zacharewski [22], the high levels of urea nitrogen and low level of cholesterol and triglycerides in the serum of rats exposed to the high concentration were indicating to hypolipidemic activity of DBP.

Studies performed on laboratory animal have shown that there is direct evidence that certain Phthalates plasticizers have carcinogenic effect *in vivo* [6]. Because their readily miscible in organic solvent like plasma and

saliva. Human have a chance of ingesting and absorbing them during common medical procedure. Once they are absorbed they are accumulated in fat storage tissues that cause teratogenic effect in human. An acute case of exposure of healthy male worker accidentally ingesting 10g DBP reported delayed nausea, vomiting and dizziness followed by headache, pain and irritation of eyes, lacrimation and photophobia and conjunctivitis [3, 23].

DBP has so many uses therefore widespread in the environment. DBP concentration is more in indoor air than outdoor air [17] thus people exposed to DBP by indoor environment and also with water and food. DBP released into the air, water and soil are also of concern near garbage dump and landfills. This is because large amount of products that have DBP in them are thrown away at this sites and the DBP can slowly leach out of these product and contaminate air water and soil. The highest exposure of DBP is most likely to come from some dairy, poultry product, fish and seafood as these foods comprise a major part of human diet. DBP is also an indirect food additive that is present in food containers and may be transferred to foods. Every person and creature on the earth whose lives are impacted by the use of plastics.

The present study have explored that phthalate esters adversely affect the process of cholestasis and susceptibility to diabetes. Hence it can be concluded that phthalate, apart from being potent toxin of liver, kidney, lung and developing testis [6, 7, 24] is also a potent toxin serologically.

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