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Toxic Impact of Phthalate (Di-n-butyl phthalate) on Haematological Profile of Mice

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Abstract : The haematological toxicity of phthalate was studied in mice in laboratory experiments. The sub-lethal concentration of 1.666 ml of phthalate/Kg body wt. of mice was administrated subcutaneously in the experiments. The mice were kept at room temperature and kept on normal diet. The hematological profile was analyzed at an interval of 3 days and continued up to 18 days. A significant decrease in haemoglobin % and total erythrocyte count was observed. The maximum reduction in haemoglobin % (6.5%) was noticed on 3rd day but the reduction in % was slightly improved on 18th day. In total erythrocytes count, the highest % (8.92%) was on 18th day. An increasing pattern was also noticed in the total counts on these intervals. The differential counts % of neutrophils (up to 40.9%) and lymphocytes (up to 388%) were maximum on 9th day. The present study suggests that phthalate was a potent hematological toxin.

Key words : Phthalate, Hematological profile, Toxicity.

Introduction

The name phthalate, a potent pollutant from plastic industry, has been derived from phthalic acid and it is dialkyl or alkyl aryl esters of 1, 2-benzenedicarboxylic acid. Phthalates allow the long polyvinyl molecules to slide against one another when added to plastics. The phthalates show low water solubility, high oil solubility and low volatility. The polar carboxyl group contributes little to the physical properties of the phthalates, except when R and R' are very small (such as ethyl or methyl groups). They are colorless, odorless liquids produced by reacting phthalic anhydride with an appropriate alcohol (usually 6-13 carbon).

Di-n-butyl phthalate is used mainly as a specialty plasticizer for nitro-cellulose, polyvinyl acetate and polyvinyl chloride, a lubricant for aerosol valves, an antifoaming agent, a kin emollient and a plasticizer in nail polish, fingernail elongators and hair spray.

Studies on rodents involving large amount of phthalates have shown damage to the liver, kidney, lungs and in the developing testes (Hallmark *et al.*, 2007). The phthalate di-n-butyl-phthalate (DBP) or its metabolite monobutyl phthalate (MBP) has been reported to suppress steroidogenesis by fetal type Leydig cells in primates as in rodents (Hallmark *et al.*, 2007). Anti androgenic effect of DBP has been reported in rats (Ashby and Lefevre, 2000b). They also reported (Ashby and Lefevre, 2000 a) delayed preputial separation in rats. Gangolli (1982) reported decrease in testis weight in 2000 mg/kg/day treated male mouse. There is paucity of information pertaining to hematological impact of phthalate on mammalian species. The present communication deals with the toxic impact of phthalate on certain haematological parameters in laboratory experiment on mice.

Materials and Methods

The hematological toxicity of phthalate was studied in mice in laboratory experiments. The sub lethal concentration of 1.666 ml of phthalate per Kg body wt. of mice was administrated subcutaneously in experimental specimen and simultaneously control set was maintained. The mice were kept at room temperature with proper ventilation and on normal diet during the period of experiments. After treatment, 6 samples were examined at an interval of every 3 days. RBC and WBC were counted with standardized Neubauer haemocytometer and haemoglobin was determined by acid haematin method (Schalam *et al.*, 1975).

Results and Discussion

The haemoglobin (Hb) % decreased by 6.5, 6.1, 4.95, 4.79, 4.51, and 4.25% on 3rd, 6th, 9th, 12th, 15th and 18th day respectively in comparison to the control values after the administration of sublethal dose of phthalate

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(Table-1). The result was statistically significant ($p < 0.001$). Similarly, in treated mice with respect to their corresponding control values, the TEC decreased significantly ($p < 0.001$) by 6.55, 6.74, 7.67, 8.66, 8.87 and 8.92% on 3rd, 6th, 9th, 12th, 15th and 18th day of experiment (Table-2).

The TC values as shown in Table-3 exhibit increase % pattern in comparison to control group. The treated group showed a % increase *i.e.*, 26.8, 25.0, 24.3, 24.6, 23.1, and 22.8 on 3rd, 6th, 9th, 12th, 15th, and 18th day. The readings were statistically significance at $p < 0.001$.

Table 1: Percentage change in haemoglobin (Hb) content in phthalate treated mice

Date	Control	Treated	% Decrease	Significance
24.5.08	12.2g	11.4g	6.5	$p < 0.001$
27.5.08	12.3g	11.5g	6.1	
30.5.08	12.1g	11.5g	4.95	
03.6.08	11.0g	10.9g	4.79	
06.6.08	12.2g	11.6g	4.51	
09.6.08	12.4g	11.5g	4.25	

Table 2: Percentage change in total erythrocyte count (TEC) in phthalate treated mice

Date	Control (million/mm^3)	Treated (million/mm^3)	% Decrease	Significance
24.5.08	4.27	3.99	6.55	$p < 0.001$
27.5.08	4.3	4.01	6.74	
30.5.08	4.3	3.97	7.67	
03.6.08	4.27	3.9	8.66	
06.6.08	4.28	3.9	8.87	
09.6.08	4.26	3.88	8.92	

Table 3: Percentage change in total count (TC) in phthalate treated mice

Date	Control (no/mm^3)	Treated (no/mm^3)	% Increase	Significance
24.5.08	4100	5200	26.8	$p < 0.001$
27.5.08	4200	5250	25	
30.5.08	4100	5100	24.3	
03.6.08	4050	5150	24.6	
06.6.08	4100	5050	23.1	
09.6.08	4150	5100	22.8	

Table 4: Percentage change in differential count (DC; Lymphocyte) in phthalate treated mice

Date	Control (no/mm^3)	Treated (no/mm^3)	% Decrease	Significance
24.5.08	90	60	33.3	$p < 0.001$
27.5.08	85	55	35.2	
30.5.08	88	52	40.9	
03.6.08	95	65	31.5	
06.6.08	90	63	30	
09.6.08	92	65	29.3	

Table 5: Percentage change in differential count (DC; Neutrophil) in phthalate treated mice

Date	Control (10^2/mm^3)	Treated (10^2/mm^3)	% Increase	Significance
24.5.08	10	40	300	$p < 0.001$
27.5.08	8	36	350	
30.5.08	9	44	388	
03.6.08	12	42	320	
06.6.08	11	44	300	
09.6.08	12	38	216	

A marked significant increase ($p<0.001$) in neutrophils (40.9%) and a significant decrease (388) in lymphocytes ($p<0.001$) have been noticed due to the effect of the sublethal dose (Table - 4 and 5). The results show that the toxicant used is similar to other haematological toxins to produce anemia after exposure. Shih *et al.* (2003) reported a significant decrease in haemoglobin content and red blood count by exposure of 2-methoxyethanol (2-ME). The haematological changes in many cases have been reported to recover to normal. In the present study the value of Hb% showed a gradual recovery from 6.5% to 4.25% on 18 days but the rising trend in the level of TEC has been noticed. Regarding total counts, the trend was similar to that of Hb%. Lymphocytes decreased up to 9th day and there after recovery occurs. A similar pattern of fall and rise was noted for neutrophils. Jeevarathanam *et al.* (1991), reported after subcutaneous administration of half of LD_{50} of methyl isocyanide in female rabbits found a significant increase in haemoglobin concentration, hematocrit and leukocyte count in blood. The present study suggestes that methyl isocyanide is not a haematological toxin and does not affect the erythropoietic tissue . The present study denotes that of the phthalate esters have been reported to adversely affect the process of erythopoiesis even in low doses and interferes with protein turn over on erythropoietic tissue (Ganning *et al.*, 1987). Hence, it can be concluded that phthalate, apart from being potent toxin of liver, kidney, lung and other developing testes (Ashby and Lefevre, 2000 a and b; Hallmark *et al.*, 2007) is also a potent toxin haematologically.

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