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Di-2-ethylhexylphthalate Induced Peroxidative Stress in Rat Liver

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The potential of phthalate ester plasticizers as environmental pollutants was known (Peakall 1975). Meyer et al (1972) emphasised the role of phthalate esters as environmental contaminants. The question of potential toxicity of phthalate plasticizers to humans was dealt with in detail (Lawrence 1978).

Di-2-ethyl hexyl phthalate (DEHP) is widely used as a plasticizer in the manufacture of plastics. Reddy et al (1976) showed that dietary DEHP in varying doses, brought about dose-linked biochemical changes in rodents. Osumi et al (1978) reported significantly high increases in the liver peroxisomal fatty acyl-CoA oxidation in rats exposed to 2% DEHP in diet for a period of 2 weeks. The peroxisomal β-oxidation appears to be independent of an electron transport chain resulting in production of H_2O_2 by direct transfer of electrons to O_2. Any increased production of H_2O_2 would lead to formation of highly reactive oxygen species, hydroxy (OH) free radicals (Chance et al 1979). Reddy et al (1976) showed that the hepatic catalase activity responsible for degradation of H_2O_2 was increased by dietary DEHP.

Moody et al (1976) concluded that the peroxisomal proliferation induced increase in hepatic peroxisomal catalase activity was comparatively smaller than the extent of H_2O_2 produced, suggesting peroxidative stress on the system.

Ames et al (1981) outlined the potential of uric acid as a biological antioxidant in mitigating cellular damage caused by oxygen free radicals.

The protective action of ascorbic acid against lipid peroxidation in the tissues was reported (Kawai Kobayashi & Yoshida 1986). The sparing effect of uric acid on ascorbic acid was elucidated (Davies et al 1986).

The present study evaluates the effects of DEHP feeding on factors linked with peroxidative stress in female rats.

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MATERIALS AND METHODS

Inbred female Wistar rats (135-150 g) were housed individually in cages with free access to food and water. The animals received either 20% casein diet (control diet) or 20% DEHP in the casein diet (DEHP diet) for a period of seven days. The diets were isocaloric (390 Kcals/100 g). Urine was collected over a period of 24 hours. At the end of seven days feeding the rats were killed and livers removed. Blood was collected through cardiac puncture. Uric acid was estimated immediately according to the method of Mahler et al (1955). Ascorbic acid was determined by the method of Roe and Kuether (1943). Malonaldialdehyde was estimated as per the described procedure (Kawai-Kobayashi & Yoshida 1986). Catalase activity was determined as per the method of Cohen et al (1970). The method of Mahler et al (1955) was employed to determine urate oxidase activity. All data were statistically analysed for levels of significance by Student's 't' test.

RESULTS AND DISCUSSION

The food consumption, body weight changes and fresh liver weights expressed as percent body weights are given in Table 1.

Table 1. Food consumption, body weight and fresh liver weights of rats fed DEHP

Rat Group	Initial Body weight g	Final Body weight g	Daily Food intake g	Fresh liver weight (% Body weight) g
control diet	141.6±1.4	164.5±0.9	14.5±0.4	5.1+0.1
DEHP diet	143.6±1.4	140.0±1.1	7.0±0.5	6.7±0.1*

Values are Mean ± SEM of 6 animals
* $P < 0.001$

The effect of DEHP feeding on the activities of liver catalase and urate oxidase is shown in Table 2. The enzyme activities are expressed as units per fresh liver weight.

Table 2. Effect of DEHP feeding on activities of liver enzymes in the rat

Rat Group	Catalase x 10^3 units (a)	Urate oxidase x 10^3 units (b)
control diet	24.5±2.0	1.3±0.2
DEHP diet	54.3±4.2*	2.4±0.1*

Values are Mean ± SEM of 6 animals
* $P < 0.005$
a) 1 unit = Δ A of 0.1/15 sec/g. fresh tissue
b) 1 unit = Δ A of 0.1/min/g. fresh tissue

The liver ascorbic and uric acids, malondialdehyde urinary ascorbic acid and serum uric acid levels of the two groups are indicated in Table 3. Liver parameters are expressed per wet weights.

Table 3. Effect of DEHP feeding on some biochemical parameters

Rat Group	Liver Ascorbic acid x 10^{-3}M	Liver Uric acid x 10^{-4}M	Liver Malondi-aldehyde x 10^{-9}M	Ascorbic acid in 24 h urine mg	Serum Uric acid mg/100 ml
control diet	7.0±0.5	16.2±3.0	137.3±15.2	0.3±0.0	0.7±0.0
DEHP diet	28.4±3.3*	22.9±2.1	211.5±16.4	0.4±0.1	0.7±0.0

Values are Mean ± SEM of 6 animals
* $P < 0.005$

The hepatic catalase and urate oxidase activities of control diet fed rats as given in Figure 1 show positive correlation ($r = 0.8$). This correlation does not exist in the DEHP diet fed rats. On the other hand the hepatic levels of biological antioxidants ascorbic acid and uric acid in DEHP diet fed rats establish a positive correlation ($r = 0.8$) as shown in Figure 2. This relationship does not exist in the control diet fed rats.

The present study indicates the onset of peroxidative stress on the system as shown by increased Thiobarbituric acid reactive species measured as malondialdehyde. DEHP induces increased hepatic biosynthesis of ascorbic acid to mitigate the peroxidative damage. Increased urinary ascorbic acid serves as an index of the stress imposed on the liver. Statistically significant correlation between hepatic ascorbic and uric acids in DEHP fed rats from controls suggests the existence of a sort of mechanism in which one is complementing the other. In spite of increased hepatic urate oxidase activity, there is no corresponding significant change in serum uric acid levels of the experimental rats. This phenomenon requires further study.

Lazarow et al (1982) did not find any significant increase in liver catalase activity of female rats exposed to Clofibrate, an established peroxisomal proliferating hypolipidemic drug. But, the present study shows a statistically significant increase in the hepatic catalase activity of rats exposed to dietary DEHP, a compound similar to Clofibrate in its biochemical manifestation. The balance maintained between the hepatic catalase and urate oxidase activities in the control diet fed rats appears to be upset in DEHP diet fed rats, possibly due to DEHP induced peroxidative stress.

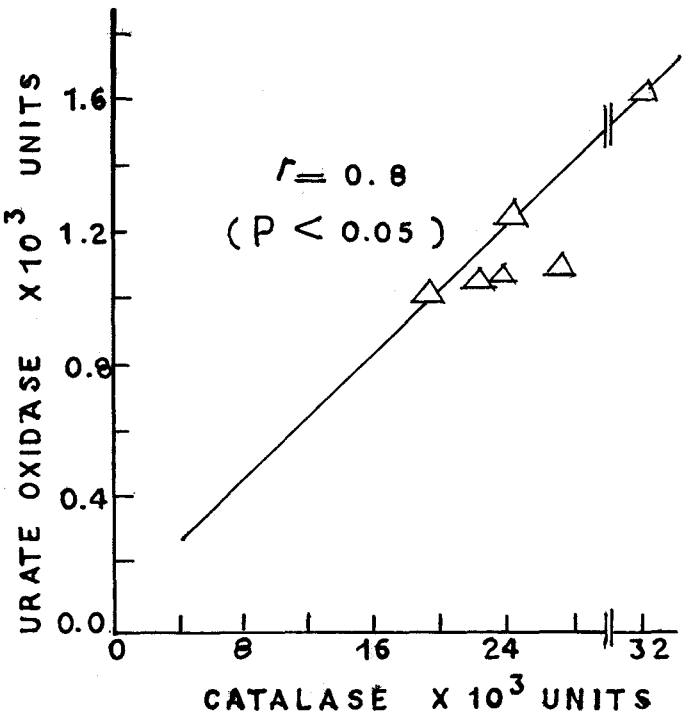

FIGURE.1.

HEPATIC CATALASE AND URATE OXIDASE ACTIVITIES_ RELATIONSHIP IN CASEIN DIET FED RATS .

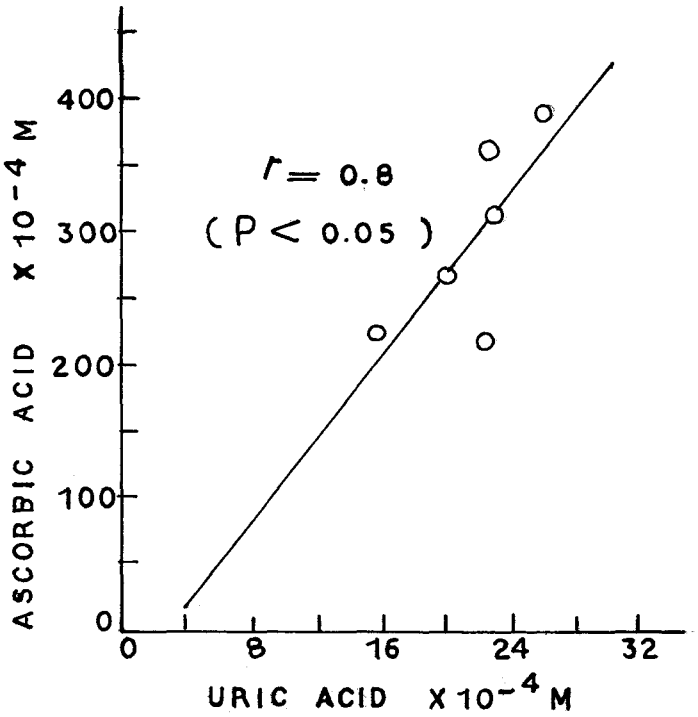

FIGURE.2.

CORRELATION CURVE OF HEPATIC URIC ACID VS ASCORBIC ACID IN DEHP_ DIET FED RATS .

Further work is warranted to elucidate this phenomenon.

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