Diethyl Phthalate Causes Oxidative Stress: An in Vitro Study

Heena Prajapati[1], Ramtej Jayram Verma[1]*

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ABSTRACT

Background: Phthalates are a group of multifunctional chemicals. Diethyl phthalate (DEP) is one of the most frequently used phthalates in solvents and fixatives for numerous industrial products .

Method: The present experiment was designed to assess oxidative stress, if any, caused by diethyl phthalate. For this the homogenates of liver and kidney were treated with different concentrations (10-40 µg/mL) of DEP. 10% liver and kidney homogenates were prepared in phosphate buffered saline and used for estimation of lipid peroxidation.In final step lipid peroxidation and total protein content were analyzed .

Results: The result revealed significant and dose - dependent increase in lipid peroxidation, whereas protein content reduced significantly. Maximum increase in LPO and decrease in protein content was observed at 40 µg/mL of DEP concentration .

Conclusion: From this study, it can be concluded that different concentrations of DEP leads to dose- dependent significant increase in lipid peroxidation and decrease protein content.So at the different concentration of DEP cause oxidative stress.

Keywords: Diethyl Phthalate, Lipid Peroxidation, Oxidative Stress, Protein Content.

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INTRODUCTION

Phthalate esters are a group of synthetic chemicals, which are used to plasticize polymers, such as polyvinyl chloride (PVC), polyvinyl acetates, cellulosic, and polyurethanes. These polymers are widely used in food wraps, plastic tubing, furniture, toys, shower curtains, cosmetics, etc.[1,2,3]. The release of phthalates esters into the environment during manufacturing, use, and disposal has been extensively reviewed [4,5]. These esters have been identified in all environmental compartments (water, air, sediment, and biota) in gulfs [6] and rivers [7] around the world. Phthalates are usually taken up into the food chain and are present in fish meat and dairy products [8].

Manufacturers use low-molecular weight phthalates DEP and DBP in various personal care products, like colognes, deodorants, and fragranced products [9].

Among potential sources of DEP contamination and accumulation in human beings, one is cosmetic products and the other is dietary meat of fish particularly those obtained from unknown contaminated sources [10,11]. Phthalates have also been detected in pooled breast milk samples of American women [12].

DEP is used in pharmaceutical coating as a fixative in cosmetics, manufacturing varnishes and ropes, denaturation of alcohol, and perfume binders [13]. Diethyl phthalate (DEP) has been found to have diverse acute and chronic toxic effects on several species at different trophic levels, as well as endocrine disrupting properties [14, 15, 16]. Central nervous system effects and damage to the spleen and kidneys occurred in laboratory animals that were given single injections of diethyl phthalate and the liver was affected in subjects exposed through dialysis equipment [17]. Diethyl phthalate produced mitochondrial swelling, smooth endoplasmic reticulum focal dilation and vesiculation, and increased interstitial macrophage activity associated with the surface of Leyding cells of rats [18]. At culture concentration of 0.05, 0.167, and 0.5 µg/litre, DEP produced a concentration-related increase in the number of relative sister chromatid exchanges per

1. Department of Zoology, School of Sciences, Gujarat University, Ahmedabad, India.
*Corresponding Author: E-mail: ramtejverma2000@yahoo.com

chromosomes in the presence of S9 fraction from rat liver homogenates [19].

Oxidative stress, on the other hand, is a condition in which the generation of reactive oxygen species (ROS), a ubiquitous by-product of aerobic metabolism, overwhelms the cellular antioxidant defence mechanism. Lipid peroxidation has been taken as an indicator of cellular oxidative damage [20]. Lipid peroxidation-derived free radicals can attack the backbone of proteins and side chains of specific amino acid residues [21].

The main aim of the present study was to evaluate the effect of DEP on lipid peroxidation and protein content in liver as well as kidney homogenates in vitro.

MATERIALS AND METHODS

Chemicals

Analytical grade diethyl phthalate was procured from Sisco Research Laboratories, Mumbai, India. All other chemicals used in the present study were of analytical grade.

Liver and kidney samples

Inbred healthy adult female Swiss strain albino mice weighing 30-35 g were obtained from Zydus Research Centre, Ahmedabad, India. The animals were kept in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India, under controlled conditions (temperature 25 ± 2OC, 12 h light/dark cycle and relative humidity 50-55%). They were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and water ad libitum. All procedures described were reviewed and approved by the University Animals Ethical Committee. The animals were handled according to the guidelines published by Indian National Science Academy, New Delhi, India (1991).

The mice were sacrificed by cervical dislocation and their liver and kidneys were isolated, blotted free of blood and used for biochemical studies.

Lipid peroxidation

In this study, 10% liver and kidney homogenates were prepared in phosphate buffered saline and used for estimation of lipid peroxidation. The following sets of tubes were prepared:

1. Control tubes: These tubes contained 0.2 mL of tissue homogenates and 0.8 mL of phosphate buffered saline .
2. DMSO control tubes: These tubes contained 0.2 mL of tissue homogenates and 0.4 of mL DMSO and they were made up to 1 mL by phosphate buffered saline.
3. DEP-treated tubes: These tubes contained 0.2 mL of tissue homogenates with different concentrations of DEP (0.1-0.4 mL) and their final volume was made up to 1mL with phosphate buffered saline.

All tubes were subjected to incubation for 30 min at 37˚C. The total lipid peroxidation in control, DMSO control, and toxin-treated samples was measured by quantification of thiobarbituric acid reactive substance (TBARS) determined by the method applied by Ohkawa et al. [22].

Protein

Liver and kidney tissues were homogenized in chilled water and used for estimation of protein.

The following sets of tubes were prepared.

1. Control tubes: These tubes contained 0.2 mL of tissue homogenates and 0.8 mL of distilled water.
2. DMSO control tubes: These tubes contained 0.2 mL of tissue homogenates and 0.4 mL of DMSO and they were made up to 1mL by distilled water.
3. DEP treated tubes: These tubes contained 0.2 mL tissue homogenates with different concentration of DEP (0.1-0.4 mL) and their final volume was made up to 1mL with distilled water.

The total protein content in control, DMSO control, and toxin-treated samples was estimated by the method of Lowry et al. [23] using bovine serum albumin as the standard.

Statistical analysis

All data are expressed as the means ± SEM. Statistical analysis and linear regression were performed using Graphpad Instat Software, version 5.03. The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's

test. The level of significance was considered P<0.05 .

RESULTS

Results shown in Table 1 revealed significant (P<0.05) dose-dependent increases in lipid peroxidation in liver (r2 = 0.965, Figure 1) and kidney (r2 = 0.971, Figure 2) homogenates. At 40 µg/mL DEP concentrations, the maximum lipid peroxidation was observed in both liver and kidney homogenates.

Results shown in Table 2 revealed significant (P<0.05) dose-dependent decreases in the protein content of liver (r2 = 0.977, Figure 3) and kidney (r2 = 0.953, Figure 4) homogenates. The maximum decrease in protein content was observed at 40 µg/mL DEP concentrations.

The coefficient of regression r2 was obtained by linear regression. All result exhibited coefficient of regression r2>9 (P<0.05). Linear regression shown in Figures 5 and 6 indicates a strong correlation between lipid peroxidation and protein content in liver (r2 = 0.974) as well as kidney homogenates (r2 = 0.894).

Table 1. Effect of diethyl phthalate on lipid peroxidation in mice liver and kidney homogenates.

Concentration of diethyl phthalate (µg/ml)	Lipid peroxidation (n moles MDA formation /100 mg tissue wt./60 min)	
	Liver	kidney
0	153.10 ± 2.84	96.65 ± 14.59
10	232.00 ± 11.49 [a]	150.90 ± 10.61 [a]
20	267.00 ± 21.33 [a]	172.20 ± 11.96 [a]
30	300.10 ± 21.82 [a]	188.60 ± 12.18 [a]
40	368.80 ± 25.72 [a]	224.30 ± 14.69 [a]

Values are given as mean ± S.E.M.; n=10; Significant.
at [a] P<0.05 in control vs. treated homogenates.

Table 2. Effect of diethyl phthalate on protein content in mice liver and kidneys homogenates.

Concentration of diethyl phthalate (µg/ml)	protein (mg protein / 100 mg tissue wt)	
	Liver	kidney
0	12.61 ± 0.71	9.05 ± 0.34
10	8.93 ± 0.65 [a]	7.38 ± 0.20 [a]
20	7.79 ± 0.689 [a]	6.97 ± 0.38 [a]
30	7.27 ± 0.63 [a]	6.02 ± 0.29 [a]
40	6.50 ± 0.57 [a]	5.76 ± 0.19 [a]

Values are given as mean ± S.E.M.; n=10; Significant.
at [a] P<0.05 in control vs. treated homogenates.

Legends to figures

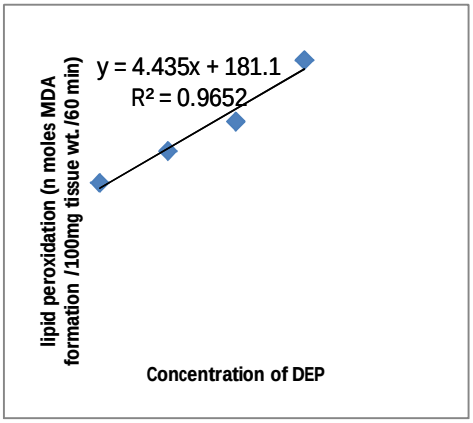

Figure 1. Linear regression curve between different concentrations of diethyl phthalate and lipid peroxidation in liver homogenate.

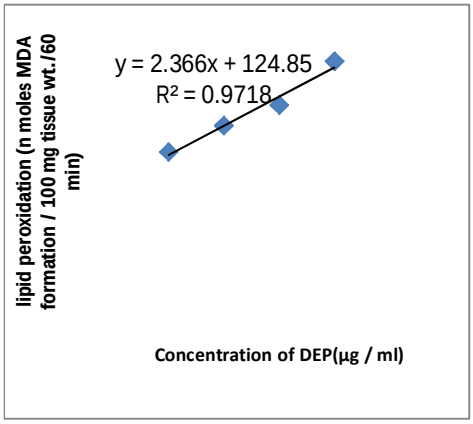

Figure 2. Linear regression curve between different concentrations of diethyl phthalate and lipid peroxidation in kidney homogenate

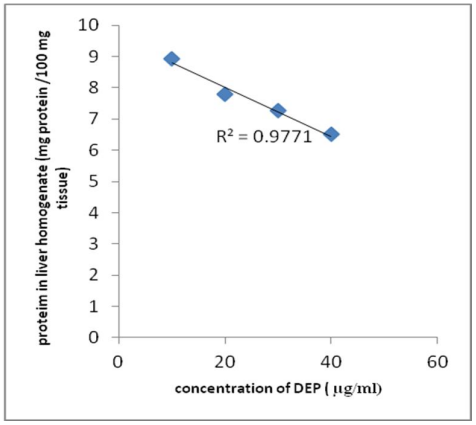

Figure 3. Linear regression curve between different concentrations of diethyl phthalate and protein in liver homogenate.

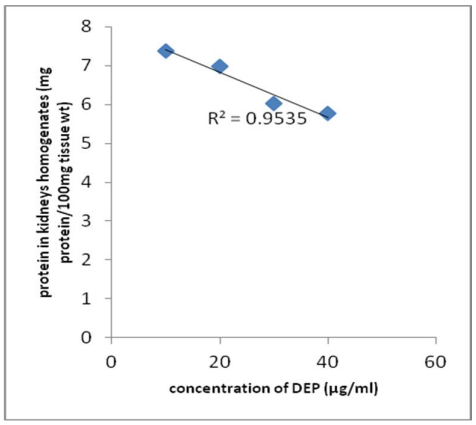

Figure 4. Linear regression curve between different concentrations of diethyl phthalate and protein content in kidney homogenate.

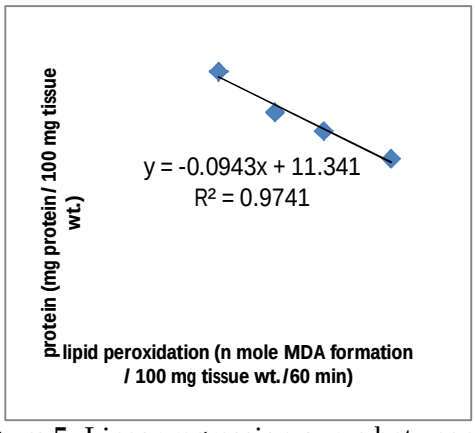

Figure 5. Linear regression curve between the level of lipid peroxidation and protein content in the liver.

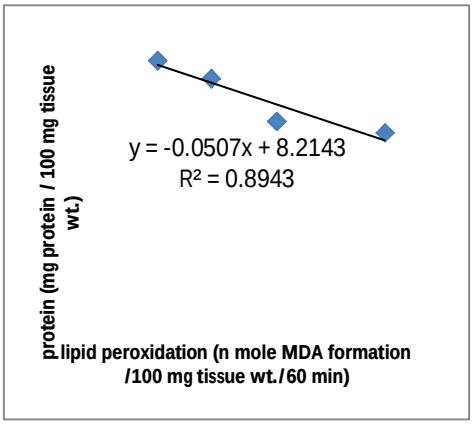

Figure 6. Linear regression curve between the level of lipid peroxidation and protein content in the kidney.

DISCUSSION

In the present study, it was found that DEP induces oxidative stress by increasing thiobarbituric acid reactive substances (TBARS) in mice liver and kidney homogenates .

Lipid peroxidation is a major harmful consequence of reactive oxygen species (ROS) formation [24, 25]. Increased lipid peroxidation could lead to severe cell organelle damage leading to impairment in the various metabolic functions of the cell [26]. Elevation of oxidative stress in liver indicates the high level of ROS production due to liver damage by varying exposure to DEP. The higher level of TBARS in Wistar rats and olive flounder, Paralichthys olivaceus, a marine culture fish, was also observed by Pereira et al. [27] and Kang et al. [28], respectively.

Determination of malondialdehyde (MDA) by TBARS is used as an index of the extent of lipid peroxidation. In the present study, increased MDA formation was observed in liver and kidney homogenates. This is due to the oxidative damage of protein by elevated oxygen free radicals. In the present study, decrease in protein content correlated with oxidative stress (Table 2.(

Chemically, oxidative stress is associated with increased production of oxidizing species or significant decrease in the effectiveness of antioxidants defence [29]. Severe oxidative stress can cause cell death.

Even moderate oxidation can trigger apoptosis while intense stress may cause necrosis. According to Sun et al. [30], DEP affects the apoptotic system in PC12 cells. Diethyl phthalate may enhance oxidative stress like that induced by reactive oxygen species in PC12 cells.

CONCLUSION

Considering the results of this study, it can be concluded that different concentrations of DEP lead to a significant dose-dependent increases in lipid peroxidation as well as a decrease in protein content.

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