The effects of di(2-ethylhexyl)phthalate and selenium on hepatic oxidant/antioxidant status in rat

P Erkekoglu¹, B Giray¹, N. D. Zeybek², M Kızılgün³, W Rachidi⁴, I Hininger-Favier⁵, AM Roussel⁵, AFavier⁴, E Asan², F Hincal¹

¹ Hacettepe University, Faculty of Pharmacy, Department of Toxicology, Ankara, Turkey
² Hacettepe University, Faculty of Medicine, Histology and Embryology Department, Ankara, Turkey
³ Turkish Health Ministry Ankara Child Health and Diseases Hematology Oncology Education and Research Hospital, Ankara, Turkey
⁴ CEA Grenoble, INAC/SCIB/LAN, 17 Rue des Martyrs, 38054 Grenoble Cedex 9, France
⁵ LBFA, Universite Joseph Fourier, Grenoble, F-38041, France

Coressponding author: easan@hacettepe.edu.tr

Keywords: Di(ethylhexyl)phthalate (DEHP), selenium, liver, lipid peroxidation, oxidative stress

The hepatotoxic effect of di(2-ethylhexyl)phthalate (DEHP) is well known. However, the underlying mechanisms is poorly defined [1]. The present study was performed to evaluate the effect of DEHP alone or with/without selenium supplementation on liver of rats.

All rats were divided randomly into six groups with 6 rats each; 1- Control group, 2- Selenium deficient group (SeD) 3-Selenium supplemented group (SeS), 4- DEHP group, 5-Selenium supplemented DEHP group (DEHP/SeS), 6- Selenium deficient DEHP group (DEHP/SeD). For SeD group rats were fed with ≤0.05 Se mg/kg diet for 5 weeks. Supplementation group was on 1mg Se/kg diet and DEHP treated groups received 1g/kg DEHP by gavage during the last 10 days of feeding period. Plasma biochemical parameters along with activities of antioxidant selenoenzymes [glutathione peroxidase 1 (GPx1), glutathione peroxidase 4 (GPx4), thioredoxin reductase (TrxR)], catalase (CAT), total superoxide dismutase (total SOD), and glutathione S-transferase (GST); concentrations of total glutathione (tGSH), thiols and thiobarbituric acid reactive substance (TBARS) levels were measured. The catalase immunoreactivity was detected by indirect immunperoxidase method. Apoptosis was detected by TUNEL. The tissues were processed for both light and electron microscopic observations. All the data was evaluated statistically.

Absolute liver weight increased only in DEHP/SeD group. However, relative liver weights increased in all DEHP-exposed groups. Plasma ALT activity increased in all DEHP-exposed groups while plasma AST activity changed markedly in DEHP and DEHP/SeD groups in comparison to control group. Focal necrosis was observed in DEHP/SeD group (Figure 1). The glycogen granules was increased in Selenium supplemented group and decreased in SeD, DEHP, Se deficient DEHP group as indicated by the incidence of PAS stained hepatocytes. This data was also confirmed by electron microscopic examination. There was a decrease in the immunoreactivity of catalase in the hepatocytes of SeD group and an increase in DEHP, DEHP/SeD and DEHP/SeS group compared to control group. There was no difference in the number of apoptotic cells in all groups. GPx1 activity decreased significantly in SeD, DEHP and DEHP/SeD groups and GPx4 activity decreased in both DEHP and DEHP/SeD groups compared to control. TrxR activity increased significantly in SeS, DEHP and DEHP/SeS groups. Total SOD, Mn-SOD and Cu-Zn-SOD activity increased in SeD, DEHP, DEHP/SeS and DEHP/SeD groups. This increase was statistically significant when compared to control group. CAT activity showed a marked decrease in SeD group while significant increases were observed in DEHP and DEHP/SeS groups (Figure 2). GST activities showed marked decrease in all DEHP-exposed groups compared to control group. Total GSH levels decreased significantly in SeD, DEHP and DEHP/SeD groups. TBARS levels increased significantly in: SeD, DEHP and DEHP/SeD.

These results indicate that one of the underlying mechanism for hepatic toxicity of DEHP is oxidative stress. Selenium supplementation along with DEHP treatment was able to ameliorate DEHP-induced hepatotoxicity; while Selenium deficiency with DEHP application caused drastic changes in the hepatic histology and oxidative stress parameters [2].
Reference

[2] This study was supported by Hacettepe University Research Fund (project no: 0701301001). The authors express their thanks to Prof. Dr. Figen Kaymaz for electron microscopic assistance.

**Figure 1.** Light micrographs of livers in experimental groups. Cytoplasmic eosinophilia in DEHP, focal necrosis and infiltration in DEHP/SeD groups. Control (A), Se supplementation (B), Se deficient (C), DEHP (D), DEHP/SeS (E), DEHP/SeD (F). (Hematoxylin-eosin A-F X400)

**Figure 2.** Catalase immunoreactivity in Control (A), Se supplementation (B), Se deficient (C), DEHP (D), DEHP/SeS (E), DEHP/SeD (F). Strong immunoreactivity is seen in DEPH, DEHP/SeS ve DEHP/SeD groups. (Indirect immunoperoxidase X400)