Bulletin of Pharmaceutical Research 2025;15(1):194
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)
DOI: 10.21276/bpr.2025.15.1.2

RESEARCH PAPER

EFFECTS OF NICOTINIC ACID (NA) ADMINISTRATION ON DI(2-ETHYLHEXYL)PHTHALATE TOXICITY IN RATS

Shigeru Suna[1]* and Fumihiko Jitsunari[2]

[1]*Private Health Research Laboratory, 14-22 Shinkita-machi, Takamatsu-shi, Kagawa, 760-0001, Japan*
[2]*Professor Emeritus, Kagawa University, Takamatsu, Kagawa, Japan.*

**E-mail*: jpsuna0218@yahoo.co.jp
Tel.: +81-87-832-1000.

Received: Dec 04, 2024 / Revised: Apr 28, 2025 / Accepted: Apr 29, 2025

Nicotinic acid (NA), which is relatively abundant in foods that people consume daily, was administered together with di(2-ethylhexyl) phthalate (DEHP) to rats to examine its effects on DEHP toxicity. Four-week-old male SD rats were divided into control, DEHP, NA, and DEHP+NA groups (6 rats per group). The treatment groups were fed 1% (w/w) DEHP diets and/or 0.5% (w/w) NA water for one week. Obvious testicular atrophy was observed in the DEHP group. On the other hand, the testis weight of the DEHP+NA group was significantly higher than that of the DEHP alone group. In the liver, both the DEHP alone and DEHP + NA groups showed similar levels of hypertrophy. Blood biochemical tests showed that the blood glucose level in the DEHP-alone group was significantly lower than that in the control group, while the blood glucose level in the DEHP+NA group was not different from that in the control group. Plasma total cholesterol and HDL cholesterol were significantly lower in the DEHP alone and DEHP+NA groups than in the control group. However, HDL cholesterol in the DEHP + NA group was slightly higher than in the DEHP group. NA was found to prevent testicular atrophy and hypoglycemia caused by DEHP.

Key words: Nicotinic acid, DEHP toxicity, Testis, Hypoglycemia, Testicular atrophy.

INTRODUCTION

Polyvinyl chloride products containing di(2-ethylhexyl) phthalate (DEHP) are used in an extremely wide range of applications, including construction materials, household goods, toys and medical devices, and so the opportunities for DEHP exposure in daily life are high. DEHP has been shown in animal studies to adversely affect the testes, liver, kidney, and endocrine system [1-11], and there is concern that environmental exposure may adversely affect human health [12, 13]. Although the mechanism of toxicity is not fully understood, it is thought to be closely related to the fact that phthalate metabolites such as mono(2-ethylhexyl) phthalate (MEHP) stimulates peroxisome proliferator-activated receptors and disrupts carbohydrate and lipid metabolic systems, generating oxidative stress [14-19]. On the other hand, many nutrients are ingested through the gastrointestinal tract via food, beverages, etc., in which the presence of substances that may influence DEHP toxicity expression is considered.

Nicotinic acid (NA), which is relatively abundant in daily food intake, is essential for energy metabolism and DNA synthesis, and is also known for its potent antioxidant action and inhibition of apoptosis [20, 21].

Therefore, an ameliorative effect of DEHP and its metabolites on the load on the lipid metabolic system and inhibition of oxidative stress are expected. In present study, the effects of concurrent consumption of drinking water containing nicotinic acid, a hydroxyl radical scavenger, on toxicity in rats exposed to dietary DEHP, are reported.

MATERIALS AND METHODS

Chemicals and animal diet

Chemical purity was >97%. DEHP was purchased from Wako Pure Chemical Industries, Ltd. (Osaka). The CE-2 diets (Clea, Tokyo, Japan) containing DEHP was prepared by Oriental Yeast Company (Chiba). MEHP was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo). All other chemicals were of the highest grade available from commercial sources.

Animals and ethics

Male Sprague-Dawley rats aged three-week-old purchased from Charles River (Kanagawa) were housed at the Laboratory Animal Center of Kagawa University. They were acclimated at 22-24 °C and 50-60% relative humidity with a 12 h light/dark cycle. Experiment protocols had the approval by University Animal Committee.

Experimental design

Four-week-old rats weighing 123.8 ± 2.8 g were divided into four groups of six rats each. The treatment groups were fed diets containing 1% (*w/w*) DEHP and/or 0.5% (*w/w*) NA water for one week. The control group was fed normal feed and tap water. At the end, the rats were sacrificed. Organs were removed and weighed. Plasma and organs were frozen at -40°C until MEHP/biochemical parameter measurements.

MEHP analysis in plasma and testis

The MEHP levels in organs and plasma were determined by HPLC [22].

Metal analysis in testis

Metals in wet-digested testes were analyzed by the previously reported flame atomic absorption spectrometry [23].

Plasma biochemical parameter measurements

Plasma levels of glucose, total cholesterol, high density lipoprotein cholesterol and triglyceride were measured using an automated biochemical analyzer, Hitachi 7600 (Hitachi, Japan).

Statistical analysis

Results were expressed as means±standard deviations (SD). A one-way ANOVA test followed by Tukey's multiple comparison test and multiple linear regression analysis was performed to compare treatment groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Body weight and organ weight are shown in **Table 1**. Body and testes weight in the NA group did not differ from the control group; in the DEHP group, body weight was little different from the control group, but testes weight was significantly lower than in the control group; in the DEHP+NA group, body weight was little different from control group, but testes weight or relative testicular weight (% of body weight) was significantly higher than in the DEHP group. In the liver, both the DEHP alone and DEHP + NA groups showed similar levels of hypertrophy.

Table 1. Body and organs weight DEHP and nicotinic acid (NA) treated rats.*p<0.05, **p<0.01, ***p<0.001, as compared to control, #p<0.05, ##p<0.01, ###p<0.001, as compared to DEHP

Group	Control (n=6)	NA (n=6)	DEHP (n=6)	DEHP+NA (n=6)
Initial body weight(g)	123.8±2.8	119.5±3.7	123.5±3.3	122.3±4.4
Final body weight (g)	183.3±9.2	187.6±4.9	181.7±8.0	177.2±13.8
Testes (g)	1.71±0.12###	1.81±0.08###	1.11±0.15***	1.53±0.35
Relative testicular weight (%)	0.94±0.06###	0.97±0.05###	0.61±0.09***	0.86±0.19
Kidneys (g)	1.86±0.10	1.91±0.12	1.84±0.11	1.99±0.18
Relative kidney weight (%)	1.01±0.06	1.02±0.06	1.01±0.05	1.12±0.06
Liver (g)	9.03±0.88###	8.51±0.45###	13.95±1.07***	13.27±2.08***
Relative liver weight (%)	4.93±0.42###	4.54±0.31###	7.68±0.49***	7.46±0.62***

Table 2. Plasma and testicular MEHP concentrations

	Control (n=6)	NA (n=6)	DEHP (n=6)	DEHP+NA (n=6)
Plasma MEHP (μg/ml)	BDL>	BDL>	43.1±8.6	33.9±7.3
Testis MEHP (μg/g)	BDL>	BDL>	5.4±1.2	4.6±1.6

*BDL: Below the detection limit

As shown in **Table 2**, MEHP concentrations in plasma and testis in the DEHP+NA group were slightly lower than in the DEHP group. As shown in **Figure 1**, the relative testicular weights of the

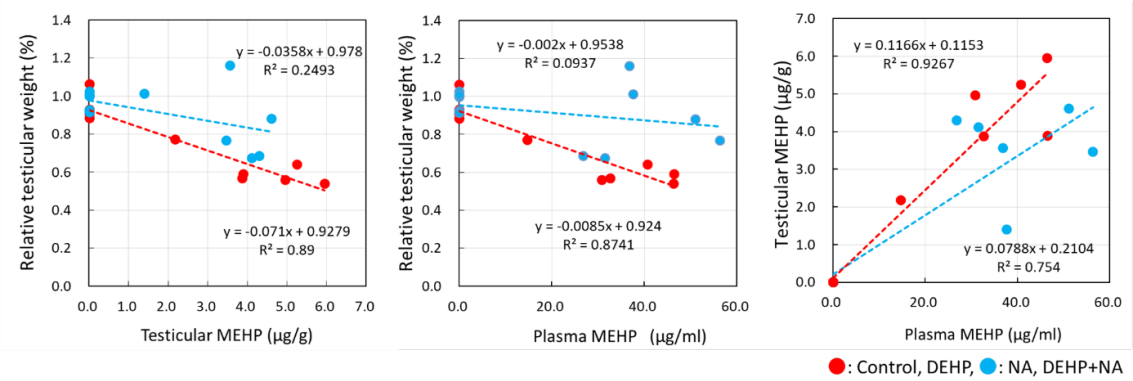

Fig. 1. Relationship between relative testicular weight, plasma MEHP and testicular MEHP

control and DEHP groups showed a strong negative correlation with plasma MEHP concentration and testicular MEHP concentration, and a strong positive correlation was shown between plasma MEHP concentration and testicular MEHP concentration. In contrast, the relative testicular weights of the NA and DEHP + NA groups showed weak negative correlations with plasma MEHP concentration and testicular MEHP concentration, and the slope of the regression line was more moderate than that of the control and DEHP groups. Plasma MEHP concentration and testicular MEHP concentration showed a strong positive correlation, but the slope of the regression line was more moderate than that of the control and di(2-ethylhexyl) phthalate (DEHP) groups.

Table 3. Testicular metal concentration of the rats treated with DEHP diet and nicotinic acid (NA) water for one week. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to Control. ##p < 0.05, ###p < 0.01, ####p < 0.001 as compared to 1% DEHP alone

Group	Control (n=6)	NA (n=6)	DEHP (n=6)	DEHP+NA (n=6)
Zn (ppm)	20.2±2.8	18.9±2.1	17.8±2.4	20.4±2.1
Fe (ppm)	13.8±1.8##	14.2±1.3##	29.9±2.2**	15.8±4.3
Cu (ppm)	1.5±0.1##	1.4±0.2##	2.2±0.2**	1.9±0.4**
Ca (ppm)	12.1±1.7##	12.3±1.8##	17.3±3.7**	14.9±4.8*

Testicular metal concentrations are shown in **Table 3**. Metal concentrations in the NA group did not differ from the control group; in the DEHP group, Zn concentration was slightly lower than in the control group, but Fe, Cu and Ca were significantly higher than in the control group; in the DEHP+NA group, only Cu was significantly higher than in the control group. As shown in **Figure 2**, testicular Fe, Cu and Ca were closely correlated with relative testicular weight, but Zn was not. A stepwise multiple regression analysis using the testicular metal concentration as the objective variable and relative testicular weight, testicular MEHP concentration, and NA treatment as explanatory variables showed that relative testicular weight and testicular MEHP were significant independent variables of testicular Cu concentration (**Figure 3, Table 4**). Plasma biochemical parameter (**Table 5**) showed that the plasma glucose level in the DEHP-alone group was significantly lower than that in the control group, while the plasma glucose level in the DEHP+NA group was not different from that in the control group.

Table 4. Stepwise multiple regression analysis with testicular Cu concentration as the objective variable and relative testicular weight, testicular MEHP concentration, and NA treatment as explanatory variables

Independent variables	Estimated regression coefficient β	95% confidence interval		Partial regression coefficient β	*p*-value	Cumulative R^2
		Lower bound	Upper bound			
Intercept	2.748	2.156	3.339	-	0.000	-
Relative testicular weight	-1.388	-1.995	-0.780	-0.572	0.000	0.780
Testicular MEHP	0.082	0.033	0.130	0.422	0.002	0.862

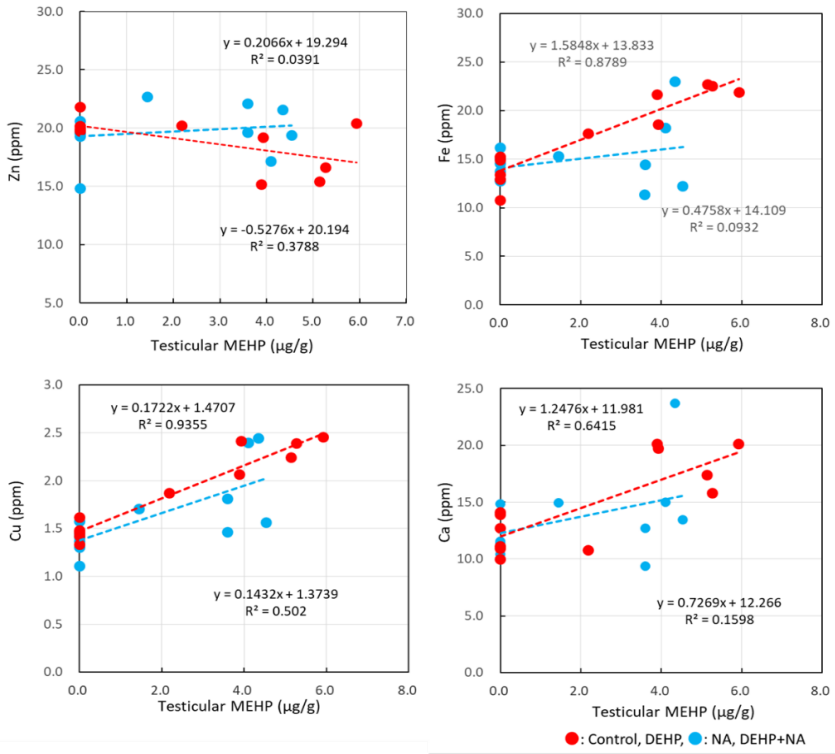

Fig. 2. Relationship between testicular metal concentrations and testicular MEHP

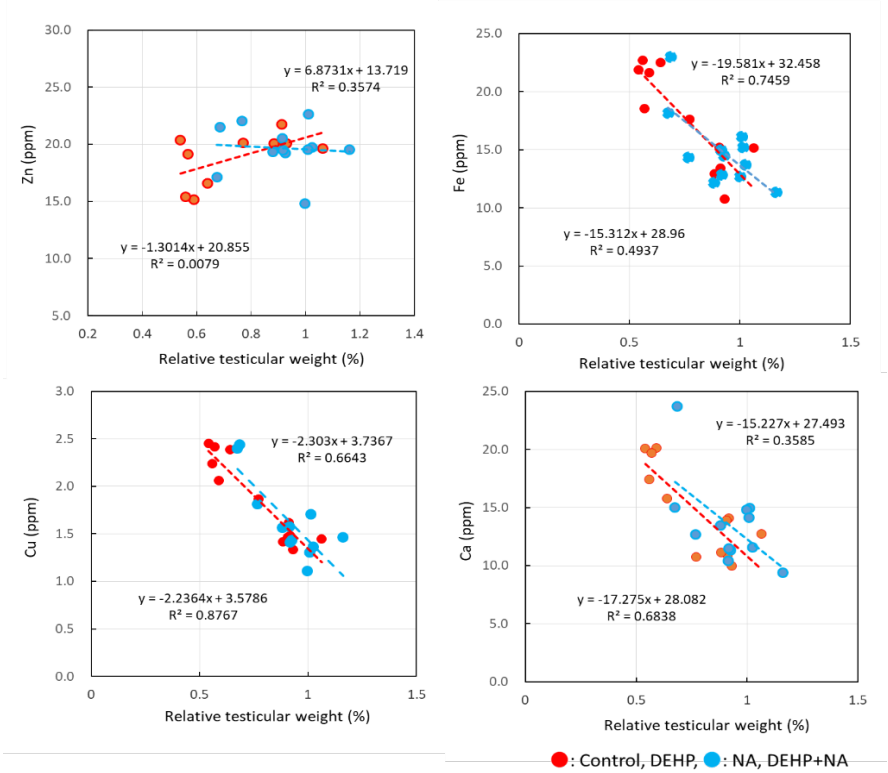

Fig. 3. Relationship between testicular metal concentrations and relative testicular weight

Plasma total cholesterol and HDL cholesterol were significantly lower in the DEHP alone and DEHP+NA groups than in the control group. However, HDL cholesterol in the DEHP + NA group was slightly higher than in the DEHP group. Triglycerides were slightly lower in the DEHP alone and DEHP+NA groups than in the control group.

Table 5. Plasma biochemical parameters of the rats treated with DEHP diet and nicotinic acid (NA) water for one week. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to Control. #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to 1% DEHP alone

	Control (n=6)	NA (n=6)	DEHP (n=6)	DEHP+NA (n=6)
Glucose (mg/dl)	171.5±46.8##	180.8±41.2##	72.7±34.3**	162.2±19.4##
Total cholesterol(mg/dl)	90.0±9.6#	77.0±4.4*	77.5±6.2*	58.8±6.5***
HDL cholesterol (mg/dl)	28.2±2.5##	27.5±2.8##	20.7±4.4**	23.2±2.1*
Triglycerides (mg/dl)	22.5±12.8	25.3±8.8	17.0±2.4	16.5±4.8

DISCUSSION

DEHP is known to be a potent oxidative stressor in mammals [24-26]. Oral exposure to high concentrations of DEHP causes testicular and hepatotoxicity in rodents [1-8]. MEHP, the active metabolite of DEHP, stimulates peroxisome proliferator-activated receptors and increases carbohydrate and lipid metabolism [14-19]. The oxidative stress generated may be closely related to DEHP toxicity. In the present study, we examined the effect of NA, a potent radical scavenger, on DEHP-induced testicular and hepatotoxicity, and found that NA significantly inhibited the decrease in DEHP testicular weight. In the control and DEHP-only groups, there was a strong negative correlation between plasma MEHP concentration or testicular tissue MEHP concentration and testicular relative weight, indicating a close relationship between MEHP and testicular atrophy, but the association between MEHP and testicular weight in the NA-treated group was weaker and the slope of the regression line was less. Distribution ratio of MEHP between plasma and testicular tissue showed a suppressive trend with NA treatment. In the DEHP group, testicular Zn concentration was slightly lower than in the control group, but Fe, Cu and Ca were significantly higher than in the control group; in the DEHP+NA group, only Cu was significantly higher than in the control group. In addition, increased concentrations of Fe, Cu, and Ca in the testes were found to be closely related to decreased relative testicular weight. Furthermore, multiple regression analysis suggested that MEHP concentration in the testis was significantly related to increased Cu concentration in the testis. This suggests that the oxidative stress of MEHP may induce the SOD enzyme [26, 27]; SOD contains a large amount of Cu, which may be a factor in the induction of Cu in the testes. In addition, administration of NA slightly improved zinc levels, which may indicate that it prevented germ cell apoptosis [28, 29].

NA did not improve plasma TCH, HDL-C and TG, but prevented DEHP-induced hypoglycemia; it has been reported that MEHP-activated PPAR-γ promotes lipid metabolism, while the reactive oxygen species produced promote insulin secretion [30-32]. The antioxidant NA may regulate insulin hypersecretion and protect against tissue damage caused by hypoglycemia-induced oxidative stress [33, 34].

CONCLUSION

The effect of co-administration of NA on toxicity induced by DEHP, known as an endocrine disrupting chemical, in rats was investigated. NA was found to have a significant effect in preventing testicular atrophy and hypoglycemia.

REFERENCES

1. Oishi S, Hiraga K. Testicular atrophy induced by phthalate acid esters: Effect on testosterone and zinc concentrations. *Toxicol Appl Pharmacol*. 1980;53:35-41. doi:10.1016/0041-008x(80)90378-6
2. Gray TJ, Butterworth KR. Testicular atrophy produced by phthalate esters. *Arch Toxicol Suppl*. 1980;4:452-5. doi:10.1007/978-3-642-67729-8_106
3. Sjoberg P, Lindqvist NG, Ploen L. Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. *Environ Health Perspect*. 1986;65:237-42. doi:10.1289/ehp.65-1474698
4. Barber ED, Topping DC. Subchronic 90-day oral toxicology of di(2-ethylhexyl) terephthalate in the rat. *Food Chem Toxicol*. 1995;33:971-8. doi:10.1289/ehp.65-1474698
5. Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Subchronic oral toxicity of di-*n*-octyl phthalate and di(2-ethylhexyl) phthalate in the rat. *Food Chem Toxicol*. 1997;35:225-39. doi:10.1016/s0278-6915(96)0006 4-6
6. Seth PK. Hepatic effects of phthalate esters. *Environ Health Perspect*. 1982;45:27-34. doi:10.1289/ehp.824527
7. Lake BG, Gray TJ, Gangolli SD. Hepatic effects of phthalate esters and related compounds - In vivo and in vitro correlations. *Environ Health Perspect*. 1986;67:283-90. doi:10.1289/ehp.824527
8. Zhao ZB, Ji K, Shen XY, Zhang WW, Wang R, Xu WP, et al. Di(2-ethylhexyl) phthalate promotes hepatic fibrosis by regulation of oxidative stress and inflammation responses in rats. *Environ Toxicol Pharmacol*. 2019;68:109-19. doi:10.1016/j.etap.2019.03.008
9. Rowdhwal SSS, Chen J. Toxic effects of di-2-ethylhexyl phthalate: An overview. *Biomed Res Int*. 2018;2018:1750368. doi:10.1155/2018/1750368

10. Kim MJ, Kim HH, Song YS, Kim OH, Choi K, Kim S, et al. DEHP down-regulates Tshr gene expression in rat thyroid tissues and FRTL-5 rat thyrocytes: A potential mechanism of thyroid disruption. *Endocrinol Metab (Seoul)*. 2021;36(2):447-54. doi:10.3803/EnM.2020.920
11. Sherif NAE, El-Banna A, Abdel-Moneim RA, Sobh ZK, Balah MIF. The possible thyroid disruptive effect of di-(2-ethylhexyl) phthalate and the potential protective role of selenium and curcumin nanoparticles: A toxicological and histological study. *Toxicol Res (Camb)*. 2021;11(1):108-21. doi:10.1093/toxres/tfab122
12. Benjamin S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *J Hazard Mater*. 2017;340:360-83. doi:10.1016/j.jhazmat.2017.06.036
13. Wang Y, Qian H. Phthalates and their impacts on human health. *Healthcare (Basel)*. 2021;9(5):603. doi:10.3390/healthcare9050603
14. Gray TJ, Beamand JA, Lake BG, Foster JR, Gangolli SD. Peroxisome proliferation in cultured rat hepatocytes produced by clofibrate and phthalate ester metabolites. *Toxicol Lett*. 1982;10(2-3):273-9. doi:10.1016/0378-4274(82)90087-x
15. Kersten S. Peroxisome proliferator activated receptors and lipoprotein metabolism. *PPAR Res*. 2008;2008:132960. doi:10.1155/2008/132960
16. Lendvai A, Deutsch MJ, Plösch T, Ensenauer R. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. *Am J Physiol Endocrinol Metab*. 2016;310(10):E797-810. doi:10.1152/ajpendo.00372.2015
17. Sugden MC, Bulmer K, Gibbons GF, Knight BL, Holness MJ. Peroxisome-proliferator-activated receptor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin. *Biochem J*. 2002;364(Pt 2):361-8. doi:10.1042/BJ20011699
18. Yuan X, Yan S, Zhao J, Shi D, Yuan B, Dai W, et al. Lipid metabolism and peroxisome proliferator-activated receptor signaling pathways participate in late-phase liver regeneration. *J Proteome Res*. 2011;10(3):1179-90. doi:10.1021/pr100960h
19. Smith SA. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. *Biochem Soc Trans*. 2002;30(Pt 6):1086-90.
20. Hageman GJ, Stierum RH. Niacin, poly(ADP-ribose) polymerase-1 and genomic stability. *Mutat Res*. 2001;475:45-56. doi:10.1016/s0027-5107(01)00078-1
21. Kamat JP, Devasagayam TP. Nicotinamide (vitamin B3) as an effective antioxidant against oxidative damage in rat brain mitochondria. *Redox Rep*. 1999;4:179-84. doi:10.1179/135100099101534882
22. Suna S, Jitsunari F. Blood and organ distribution of mono(2-ethylhexyl) phthalate (MEHP) in rats exposed to di(2-ethylhexyl) phthalate (DEHP) via diet. *World J Biol Pharm Health Sci*. 2023;13(3):19-25. doi:10.30574/wjbphs.2023.13.3.0116
23. Suna S, Jitsunari F. Effect of caffeine and ethanol intake on di(2-ethylhexyl) phthalate (DEHP)-induced testicular atrophy. *World J Biol Pharm Health Sci*. 2023;13(03):252-61. doi:10.30574/wjbphs.2023.13.3.0138
24. Kasahara E, Sato EF, Miyoshi M, Konaka R, Hiramoto K, Sasaki J, et al. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. *Biochem J*. 2002;365(Pt 3):849-56. doi:10.1042/BJ20020254
25. Poitou K, Rogez-Florent T, Dirninger A, Corbière C, Monteil C. Effects of DEHP, DEHT and DINP alone or in a mixture on cell viability and mitochondrial metabolism of endothelial cells in vitro. *Toxics*. 2022;10(7):373. doi:10.3390/toxics10070373
26. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol*. 2018;217(6):1915-28. doi:10.1083/jcb.201708007
27. Niwa Y, Ishimoto K, Kanoh T. Induction of superoxide dismutase in leukocytes by paraquat: correlation with age and possible predictor of longevity. *Blood*. 1990;76(4):835-41.
28. Yamaguchi S, Miura C, Kikuchi K, Celino FT, Agusa T, Tanabe S, et al. Zinc is an essential trace element for spermatogenesis. *Proc Natl Acad Sci U S A*. 2009;106(26):10859-64. doi:10.1073/pnas.0900602106
29. Downey AM, Hales BF, Robaire B. Zinc transport differs in rat spermatogenic cell types and is affected by treatment with cyclophosphamide. *Biol Reprod*. 2016;95(1):22. doi:10.1095/biolreprod.116.140558
30. Zhang K, Yuan Q, Xie J, Yuan L, Wang Y. PPAR-γ activation increases insulin secretion independent of CASK in INS-1 cells. *Acta Biochim Biophys Sin (Shanghai)*. 2019;51(7):715-22. doi:10.1093/abbs/gmz052
31. Kim HS, Hwang YC, Koo SH, Park KS, Lee MS, Kim KW, et al. PPAR-γ activation increases insulin secretion through the up-regulation of the free fatty acid receptor GPR40 in pancreatic β-cells. *PLoS One*. 2013;8:e50128. doi:10.1371/journal.pone.0050128
32. Saadeh M, Ferrante TC, Kane A, Shirihai O, Corkey BE, Deeney JT. Reactive oxygen species stimulate insulin secretion in rat pancreatic islets: Studies using mono-oleoyl-glycerol. *PLoS One*. 2012;7(1):e30200. doi:10.1371/journal.pone.0030200
33. Kho AR, Choi BY, Lee SH, Hong DK, Jeong JH, Kang BS, et al. The effects of sodium dichloroacetate on mitochondrial dysfunction and neuronal death following hypoglycemia-induced injury. *Cells*. 2019;8(5):405. doi:10.3390/cells8050405
34. Kim JH, Yoo BH, Won SJ, Choi BY, Lee BE, Kim IY, et al. Melatonin reduces hypoglycemia-induced neuronal death in rats. *Neuroendocrinology*. 2015;102(4):300-10. doi:10.1159/000434722

*****