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Effects of D-allulose on di (2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP)-induced toxicity in rats

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Abstract

Background: Oral exposure to high concentrations of DEHP and DBP causes testicular and hepatotoxicity in rodents. Phthalate metabolites such as mono (2-ethylhexyl) phthalate (MEHP) and mono-n-butyl phthalate (MBP) stimulate peroxisome proliferator-activated receptors and disrupts carbohydrate and lipid metabolism. The oxidative stress generated may be closely related to these toxicities.

Method: To clarify the effects of the rare sugar D-allulose, a potent free radical scavenger, on testicular and hepatotoxicity induced by DEHP and DBP, rats were fed DEHP or DBP containing diet and D-allulose water.

Result: Dietary exposure to DEHP and DBP induced a significant decrease in testicular weight and significant increase in liver weight. D-allulose treatment significantly inhibited the testicular weight loss. But D-allulose treatment did not significantly suppress the increase in liver weight. Plasma glucose levels were significantly lower in the DEHP- or DBP-only treated groups compared to controls, but were improved by D-allulose treatment. This suggests that D-allulose blocks DEHP- and DBP-induced glycemic suppression. Plasma lipid-related markers such as total cholesterol, high-density lipoprotein cholesterol, and triglycerides were lower than controls in all treatment groups on the DEHP and DBP diets, but showed a slight trend toward improvement with D-allulose.

Conclusion: D-allulose reduced DEHP- and DBP-induced testicular toxicity and blood glucose suppression in rats, but did not improve liver hypertrophy. This effect may be due to the strong oxidant scavenging ability of D-allulose.

Keywords: D-Allulose; DEHP; DBP; Testicular toxicity; Hepatotoxicity; Oxidative stress

1. Introduction

The major plasticizers di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) are known to be potent oxidative stressors in plants, animals and humans [1-10]. Oral exposure to high concentrations of DEHP and DBP causes testicular and hepatotoxicity in rodents [11-18]. Phthalate metabolites such as mono (2-ethylhexyl) phthalate (MEHP) and mono-n-butyl phthalate (MBP) stimulate peroxisome proliferator-activated receptors and increase carbohydrate and lipid metabolism. The oxidative stress generated may be closely related to these toxicities [19-21]. The current study shows the effect of the rare sugar D-allulose, which has strong radical scavenging ability [22, 23], on testicular and hepatotoxicity induced by DEHP and DBP.

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2. Materials and Methods

2.1. Chemicals and Animal Diet

Chemical purity> 97% DEHP and DBP were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The CE-2 diets (Clea, Tokyo, Japan) containing DEHP or DBP were prepared by Oriental Yeast Company (Chiba, Japan). D-allulose was provided by Kagawa Rare Sugar Research Center (Kagawa, Japan). The chemical purity of D-allulose was found to be >98%.

2.2. Animals and Ethics

Male Sprague-Dawley rats aged three-week-old purchased from Charles River (Kanagawa, Japan) 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. The experiment protocols had the approval by the Kagawa University Animal Committee.

2.3. Experimental Design

In the first experiment, four-week-old rats weighing 90-100g were divided into control and treatment groups consisting of at least 6 animals. The treatment group consumed 1% (w / w) DEHP diet and sugar free water (tap water) or 1% (w / w) D-glucose water or 1% (w / w) D-allulose water or 2% (w / w) D-allulose water for one week. At the end of the experiment, rats were sacrificed by ether anesthesia. The testis and liver were removed and weighed. Cardiac blood samples were collected in heparinized tubes and plasma was separated from whole blood by centrifugation at 1500 g and frozen at -40 °C until biochemical parameter measurements.

In the second experiment, four-week-old rats weighing 80-90 g were divided into control and treatment groups consisting of 6 animals. The treatment group consumed 2% (w / w) DBP diet and sugar free water (tap water) or 2% (w / w) D-allulose water for two weeks. At the end of the experiment, rats were sacrificed by ether anesthesia. The testis and liver were removed and weighed. Blood samples collected from the heart were collected into heparinized tubes and plasma was separated from whole blood by centrifugation at 1500 g and plasma were frozen at -40 °C until biochemical parameter measurement.

2.4. 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).

2.5. Statistical Analysis

Results were expressed as means ± standard deviations (SD). Statistical analysis was performed by one-way ANOVA test followed by Dunnett's postanalysis test for multiple comparisons. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. First experiment

Table 1 compares 1% (w/w) DEHP diet with sugar-free water or 1% (w/w) D-glucose water or 1% (w/w) D-allulose water or 2% (w/w) D-Allulose water for 1 week. The DEHP-only treated group showed a significant reduction in body weight gain, a significant decrease in testicular weigh and a significant increase in liver weight compared to controls, but D-allulose treatment significantly inhibited the decrease in testicular weight dose dependently (Figure 1). On the other hand, D-allulose treatment slightly inhibited liver weight increase in a dose-dependent manner, but the inhibitory effect was not significant.

Table 2 shows plasma biochemical parameters of the rats treated with DEHP diet plus sugar-free water or1% (w / w) D-glucose water or 1% (w / w) D-allulose water or 2% (w / w) D-allulose water for one week. Plasma glucose level was significantly lower in DEHP-only group compared to the control, but not significantly in the D-glucose and D-allulose treated groups to controls. Plasma lipid-related markers such as TCH, HDL-C, and TG of all treatment groups on the DEHP diet were significantly lower than controls, and were slightly improved by D-allulose treatment.

3.2. Second experiment

Table 3 shows body and organ weights, relative organ weights of the rats treated with 1% (w / w) DBP diet plus sugar-free water or 2% (w / w) D-allulose water for two weeks. The group treated with DBP alone showed a significant decrease in testicular weight and a significant increase in liver weight compared to controls. On the other hand, the group treated with DBP and D-allulose showed a significant inhibitory effect on testicular weight decrease and a slight inhibitory effect on liver weight increase. Table 4 shows plasma biochemical parameters of the rats treated with DBP diet plus sugar-free water or 2% (w / w) D-allulose water for two weeks. Plasma glucose level was significantly lower in DBP-only group compared to control, but not significantly in the D-allulose treated group to control. Plasma lipid-related markers such as TCH, HDL-C, and TG in all treatment groups on the DBP diet were significantly lower than those in the control group, but TCH and HDL-C showed a slight recovery with D-allulose treatment.

Table 1 Body and organ weights, relative organ weights (as a percentage of body weight) of the rats treated with 1% (w / w) DEHP diet plus sugar-free water or1% (w / w) D-glucose water or 1% (w / w) D-allulose water or 2% (w / w) D-allulose water for one week. *p < 0.05, **p < 0.01, ***p < 0.001as compared to control. #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to 1%DEHP-only group

group		**Control**		**DEHP**		**DEHP+ 1%Glucose**		**DEHP+ 1%Allulose**		**DEHP+ 2%Allulose**	
	n	**18**		**18**		**6**		**12**		**6**	
Initial body weight (g)	mean	97.1		94.3		94.0		95.1		92.3	
	SD	4.3		5.0		4.8		4.5		9.6	
Final body weight (g)	mean	159.2	###	141.3	***	136.2	***	137.0	***	132.6	***
	SD	9.1		8.4		5.9		4.6		4.6	
Testes (g)	mean	1.40	###	0.87	***	1.02	***	1.13	*** ###	1.23	###
	SD	0.11		0.20		0.24		0.22		0.12	
Relative testicular weight (%)	mean	0.88	###	0.62	***	0.75		0.82	###	0.93	###
	SD	0.05		0.15		0.15		0.16		0.09	
Liver (g)	mean	7.67	###	10.99	***	10.32	***	10.00	***	9.59	***
	SD	0.64		1.25		0.51		0.51		1.58	
Relative liver weight (%)	mean	4.82	###	7.77	***	7.59	***	7.31	***	7.23	***
	SD	0.24		0.69		0.47		0.45		1.18	

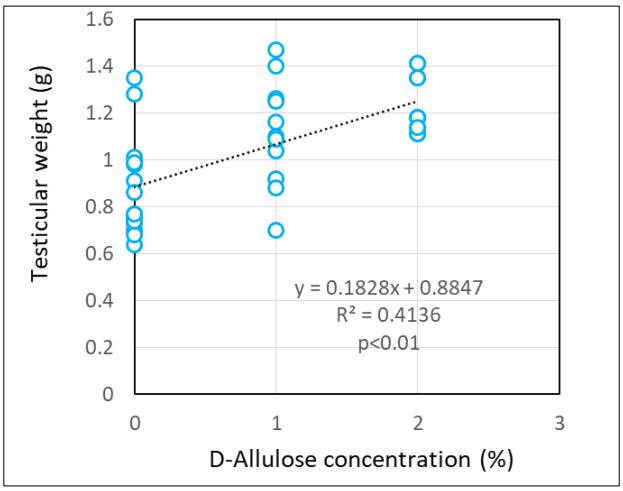

Figure 1 Relationship between testicular weight and D-allulose concentration in DEHP and D-allulose treatment groups

Table 2 Plasma Biochemical Parameters of the rats treated with 1% (w / w) DEHP diet plus sugar-free water or1% (w / w) D-glucose water or 1% (w / w) D-allulose water or 2% (w / w) D-allulose water for one week. *p < 0.05, **p < 0.01, ***p < 0.001as compared to Control. #p < 0.05, ##p < 0.01, ###p < 0.001as compared to 1%DEHP-only group

Group		**Controll**		**DEHP**		**DEHP+ 1%Glucose**		**DEHP+ 1%Allulose**		**DEHP+ 2%Allulose**	
	n	**18**		**18**		**6**		**12**		**6**	
Glucose (mg/dL)	mean	206.2	###	174.2	*	185.0		183.8		180.7	
	SD	56.7		27.8		15.8		23.7		6.3	
TC (mg/dL)	mean	90.1	###	65.2	***	65.8	***	66.1	***	69.7	
	SD	14.1		5.0		5.2		9.7		7.9	
HDL-C (mg/dL)	mean	36.4	###	26.4	***	24.5	***	26.3	***	28.5	
	SD	4.2		3.3		2.2		3.3		4.5	
TG (mg/dL)	mean	46.2	###	24.8	***	19.7	***	26.0	***	38.3	
	SD	21.5		9.5		4.3		8.5		12.9	

Table 3 Body and organ weights, relative organ weights (as a percentage of body weight) of the rats treated with 1% (w / w) DBP diet plus sugar-free water or 2% (w / w) D-allulose water for two weeks. *p < 0.05, **p < 0.01, ***p < 0.001as compared to Control. #p < 0.05, ##p < 0.01, ###p < 0.001as compared to 1%DBP-only group

Group		**Control**		**DBP**		**DBP+2%Allulose**	
	n	**6**		**6**		**6**	
Initial body weight (g)	mean	85.0		84.8		84.8	
	SD	2.8		2.8		3.8	
Final body weight (g)	mean	193.8		199.8		192.0	
	SD	9.1		5.6		8.2	
Testes (g)	mean	1.87	#	1.47	*	1.67	
	SD	0.25		0.35		0.09	
Relative testicular weight (%)	mean	0.97	#	0.73	*	0.87	
	SD	0.14		0.18		0.05	
Liver (g)	mean	9.26	###	12.59	***	12.17	**
	SD	1.19		0.73		1.20	
Relative liver weight (%)	mean	4.81	###	6.30	***	6.33	***
	SD	0.81		0.30		0.36	

Table 4 Plasma Biochemical Parameters of the rats treated with 1% (w / w) DBP diet plus sugar-free water or 2% (w / w) D-allulose water for two weeks. *p < 0.05, **p < 0.01, ***p < 0.001as compared to control. #p < 0.05, ##p < 0.01, ###p < 0.001as compared to 1%DBP-only group

Group		**Control**		**DBP**		**DBP+2%Allulose**	
	n	**6**		**6**		**6**	
Glucose (mg/dL)	mean	188.3	#	73.3	*	161.0	
	SD	51.9		36.0		100.6	
TC (mg/dL)	mean	86.8	##	66.0	**	71.7	**
	SD	11.5		5.7		4.3	
HDL-C (mg/dL)	mean	41.7	###	29.8	***	31.7	***
	SD	4.3		3.1		1.2	
TG (mg/dL)	mean	35.3		28.7		28.0	
	SD	12.5		11.3		11.7	

4. Discussion

Orally administered DEHP and DBP are rapidly metabolized to monophthalates (MEHP and MBP) by lipases in the gastrointestinal tract [24-26]. MEHP and MBP are known to be potent oxidative stressors and have been reported to disrupt Sertoli cell function and induce reproductive toxicity leading to germ cell apoptosis [27]. MEHP and MBP are also known to be PPAR agonists [7, 8] that induce peroxisome proliferation and cause liver hypertrophy [14, 15] and upregulation of glucose and lipid metabolism in rats [28, 29].

In this study, dietary exposure to DEHP and DBP induced a significant decrease in testicular weight and significant increase in liver weight. D-allulose significantly inhibited the testicular weight loss in dose dependent manner. But D-allulose treatment did not significantly suppress the increase in liver weight. Plasma TCH, HDL-C, and TG were slightly improved by D-allulose treatment, but not significantly. Plasma glucose levels were significantly lower in the DEHP- or DBP-only groups compared to controls, but not significantly in the D-allulose-treated groups versus controls. This suggests that D-allulose blocks DEHP- and DBP-induced glycemic suppression. MEHP- and MBP-induced PPAR-γ enhance lipid metabolism, whereas the generated reactive oxygen species can promote insulin secretion [30-32]. The antioxidant, D-allulose may regulate insulin hypersecretion and may also prevent tissue damage caused by hypoglycemia-induced oxidative stress [33, 34].

5. Conclusion

D-allulose reduced DEHP- and DBP-induced testicular toxicity and blood glucose suppression in rats, but did not improve liver hypertrophy. This effect may be due to the strong oxidant scavenging ability of D-allulose.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

Authors declare that there is no conflict of interests.

Statement of ethical approval

The experiment protocols had the approval by the Kagawa University Animal Committee.

References

[1] Hurst CH, Waxman DJ. Activation of PPARα and PPARγ by environmental phthalate monoesters. Toxicological Sciences. 2003; 74(2):297–308.

[2] Emiko Kasahara, Eisuke F Sato, Mami Miyoshi, Ryusei Konaka, Keiichi Hiramoto, Junzo Sasaki, Masaaki Tokuda, Yoshihisa Nakano, and Masayasu Inoue. Role of oxidative stress in germ cell apoptosis induced by di(2-ethylhexyl)phthalate. Biochem J. 2002 Aug 1; 365(Pt 3): 849–856.

[3] Exposure to phthalates DEHP and DINP May lead to oxidative damage and lipidomic disruptions in mouse kidney. Yue Gu Mei Gao Wenwen Zhang Lei Yan Fengmin Shao Jing Zhou Chemosphere. 2021 May 22; 271: 129740.

[4] Dangxia Zhou 1, Haixu Wang, Jing Zhang. Di-n-butyl phthalate (DBP) exposure induces oxidative stress in epididymis of adult rats. Toxicol Ind Health. 2011; 27(1): 65-71.

[5] Mao G, Liu H, Ding Y, Zhang W, Chen H, Zhao T, Feng W, Wu X, Yang L. Evaluation of combined developmental neurological toxicity of di (n-butyl) phthalates and lead using immature mice. Environ Sci Pollut Res Int. 2020 Mar; 27(9): 9318-9326.

[6] Källsten L, Almamoun R, Pierozan P, Nylander E, Sdougkou K, Martin JW, Karlsson O. Adult Exposure to Di-N-Butyl Phthalate (DBP) Induces Persistent Effects on Testicular Cell Markers and Testosterone Biosynthesis in Mice. Int J Mol Sci. 2022 Aug 5; 23(15): 8718.

[7] Moses T Bility, Jerry T Thompson, Richard H McKee, Raymond M David, John H Butala, John P Vanden Heuvel, Jeffrey M Peters. Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters. Toxicological Sciences. 2004; 82(1):170–182.

[8] Christopher H Hurst, David J Waxman. LE. Activation of PPARα and PPARγ by environmental phthalate monoesters. Toxicological Sciences. 2003; 74(2): 297–308.

[9] R Min Tang, Lei Zhang, Zheng Zhu, Ran Li, Shangqian Wang, Wei Wang, Zhiqiang Qin, Wei Zhang.Overexpression of miR-506-3p Aggravates DBP-Induced Testicular Oxidative Stress in Rats by Downregulating ANXA5 via Nrf2/HO-1 Signaling Pathway. Oxid Med Cell Longev. 2020; Nov 28: 4640605.

[10] Dangxia Zhou, Haixu Wang, Jing Zhang, Xiaoli Gao, Wenbao Zhao, Youli Zheng. Di-n-butyl phthalate (DBP) exposure induces oxidative damage in testes of adult rats. Syst Biol Reprod Med. 2010 Dec; 56(6): 413-419.

[11] Oishi, S., Hiraga, K., Effect of phthalic acid esters on mouse testes. Toxicol Lett. 1980 May; 5(6): 413-416.

[12] T J Gray, S D Gangolli. Aspects of the testicular toxicity of phthalate esters. Environ Health Perspect. 1986 Mar; 65: 229-235.

[13] Gray TJ, Butterworth KR. Testicular atrophy produced by phthalate esters. Arch Toxicol Suppl. 1980; 4: 452-455.

[14] T J Gray, J A Beamand, B G Lake, J R Foster, S D Gangolli, Peroxisome proliferation in cultured rat hepatocytes produced by clofibrate and phthalate ester metabolites. Toxicol Lett. 1982; 10(2-3):273-279.

[15] B G Lake, T J Gray, and S D Gangolli. Hepatic effects of phthalate esters and related compounds--in vivo and in vitro correlations. Environ Health Perspect. 1986 Aug; 67: 283–290.

[16] Takeshi Shono and Tomoaki Taguchi. Short-time exposure to mono-n-butyl phthalate (MBP)-induced oxidative stress associated with DNA damage and the atrophy of the testis in pubertal rats. Environmental Science and Pollution Research. 2014; 21: 3187–3190.

[17] Sander Kersten. Peroxisome proliferator activated receptors and lipoprotein metabolism. PPAR Res. 2008; 132960.

[18] Ágnes Lendvai, Manuel J Deutsch, Torsten Plösch, Regina Ensenauer. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. Am J Physiol Endocrinol Metab. 2016 May; 15; 310(10):E797-810.

[19] Mary C Sugden, Karen Bulmer, Geoffrey F Gibbons, Brian L Knight, and Mark J Holness. Peroxisome-proliferator-activated receptor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin. Biochem J. 2002 Jun 1; 364(Pt 2): 361–368.

[20] Xing Yuan, Shikai Yan, Jing Zhao, Duo Shi, Bin Yuan, Weixing Dai, Binghua Jiao, Weidong Zhang, Mingyong Miao. Lipid metabolism and peroxisome proliferator-activated receptor signaling pathways participate in late-phase liver regeneration. J Proteome Res. 2011 Mar 4; 10(3):1179-1190.

[21] S A Smith. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. Biochem Soc Trans. 2002 Nov; 30(Pt 6):1086-1090.

[22] Murata, A., Sekiya, K., Watanabe, Y., Yamaguchi, F., Hatano, N., Izumori, K., Tokuda, M. A novel inhibitory effect of d-allose on production of reactive oxygen species from neutrophils. J. Biosci.Bioeng. 2003; 96, 89–91.

[23] Suna S, Yamaguchi F, Kimura S. Tokuda M, Jitsunari F. Preventive effect of D-psicose, one of rare ketohexoses, on di(2-ethylhexyl) phthalate (DEHP)-induced testicular injury in rat. Toxicol Lett. 2007; 173: 107–117.

[24] Albro, P W and Moore B. Identification of the metabolites of simple phthalate diesters in rat urine. J. Chromatogr. 1974; 94: 209–218.

[25] Albro, P. W., Thomas, R., and Fishbein, L. Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of the urinary metabolites. J. Chromatogr. 1973; 76: 321–330.

[26] Manori J Silva, Ella Samandar, John A Reidy, Russ Hauser, Larry L Needham, Antonia M Calafat. Metabolite profiles of di-n-butyl phthalate in humans and rats, Environ Sci Techno. 2007 Nov 1; 41(21): 7576-7580.

[27] Cristian M. Sobarzo, Nogueira de Morais Rosana, Lustig Livia, Denduchis Berta, Helena F. Schteingart。Mono-(2-ethylhexyl) phthalate (MEHP) affects intercellular junctions of Sertoli cell: A potential role of oxidative stress. Reproductive Toxicology. 2015; 58: 203-212.

[28] S A Smith. Peroxisome proliferator-activated receptors and the regulation of mammalian lipid metabolism. Biochem Soc Trans. 2002 Nov;30(Pt 6):1086-1090.

[29] Xing Yuan, Shikai Yan, Jing Zhao, Duo Shi, Bin Yuan, Weixing Dai, Binghua Jiao, Weidong Zhang, Mingyong Miao. Lipid metabolism and peroxisome proliferator-activated receptor signaling pathways participate in late-phase liver regeneration. J Proteome Res. 2011 Mar 4; 10(3):1179-1190.

[30] Kai Zhang, Qingzhao Yuan, Jinyang Xie, Li Yuan, Yao Wang. PPAR-γ activation increases insulin secretion independent of CASK in INS-1 cells. Acta Biochimica et Biophysica Sinica. 2019; 51(7): 715–722.

[31] Kim HS, Hwang YC, Koo SH, Park KS, Lee MS, Kim KW, Lee MK. 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.

[32] Marylana Saadeh, Thomas C Ferrante, Ada Kane, Orian Shirihai, Barbara E Corkey, Jude T Deeney. Reactive oxygen species stimulate insulin secretion in rat pancreatic islets: studies using mono-oleoyl-glycerol. PLoS One 2012; 7(1): e30200.

[33] A Ra Kho, Bo Young Choi, Song Hee Lee, Dae Ki Hong, Jeong Hyun Jeong, Beom Seok Kang, Dong Hyeon Kang, Kyoung-Ha Park, Jae Bong Park, Sang Won Suh. The Effects of Sodium Dichloroacetate on Mitochondrial Dysfunction and Neuronal Death Following Hypoglycemia-Induced Injury. Cells. 2019 May 1; 8(5):405.

[34] Kim J H, Yoo B H, Won S J, Choi B Y, Lee B E, Kim I Y, Kho A, Lee S H, Sohn M, Suh S W. Melatonin Reduces Hypoglycemia-Induced Neuronal Death in Rats. Neuroendocrinology. 2015; 102:300–310.