Effects of D-allose and D-allulose on DEHP toxicities in rats

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ABSTRACT - Background: Oral exposure to high concentrations of di-(2-ethylhexyl) phthalate (DEHP) is known to cause testicular and hepatotoxicity in rodents. These toxicities have been shown to be related to oxidative stress generated by DEHP metabolites such as mono-(2-ethylhexyl) phthalate (MEHP). On the other hand, rare sugars, such as D-allose and D-allulose are known to show strong anti-oxidative activity.

Method: To clarify the effects of D-allose and D-allulose on DEHP toxicities, rats were exposed to 1% (w / w) DEHP diet plus sugar-free water or 1% (w / w) D-allose or1% (w / w) D-allulose water. One week after treatment, organ weights, plasma and testicular MEHP concentrations, plasma biochemical parameters were measured. To reveal the protective potency of D-allose and D-allulose against DEHP-induced oxidative stress in the testes, rats pre-treated with D-allose or D-allulose water at a concentration of 4% (w / w) received a single dose of 2 g/kg of DEHP in corn oil by oral gavages. After 24 hours, testicular malondialdehyde (MDA) levels were measured.

Result: All DEHP diet-treated groups showed a significant decrease in testicular weight and a significant increase in liver weight compared to the Control group, while D-allose and D-allulose water treatment suppressed both testicular weight loss and liver weight gain. A significant negative correlation between relative testicular weight and plasma or testicular MEHP concentration was found among rats treated with DEHP-free diet (Control) and DEHP diet alone. Most of the data plots for the DEHP diet plus D-allose or D-allulose group were scattered above the regression line. A significant positive correlation between relative liver weight and plasma MEHP concentration was found among rats treated with DEHP-free diet and DEHP diet alone. Most of the data plots for the DEHP diet plus D-allose or D-allulose group were scattered below the regression line. Plasma alanine transaminase (ALT) levels of the DEHP diet plus D-allose or D-allulose group were significantly lower than DEHP diet alone group. Pre-treatment with D-allose or D-allulose water at a concentration of 4% resulted in almost complete suppression of testicular MDA production among DEHP-administered rats.

Conclusion: These results indicate that D-allose and D-allulose can reduce testicular and hepatotoxicity induced by DEHP.

Keywords: DEHP; testicular toxicity; hepatotoxicity; D-allose; D-allulose; anti-oxidative effect

1. INTRODUCTION

Di-2-ethylhexyl phthalate (DEHP) is widely used as a plasticizer for plastics such as polyvinyl chloride. Oral exposure to high concentrations of DEHP is known to cause testicular and hepatotoxicity in rodents [1-3]. These toxicities have been shown to be related to oxidative stress generated by DEHP metabolites such as mono-(2-ethylhexyl) phthalate (MEHP) [4-6]. In recent years, in Kagawa, Japan, a method for mass-producing rare sugars such as D-allose, D-allulose, and D-tagatose has been established [7-9], and research on rare sugars has revealed antioxidant and anti-apoptotic properties. In particular, D-allose and D-allulose show stronger scavenging activity than other rare sugars [10-13].

Present study shows the effects of rare sugars, D-allose and D-allulose on testicular and hepatotoxicity induced by DEHP.

2. MATERIALS AND METHODS

2.1. Chemicals and Animal Diet

Chemical purity> 97% DEHP was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The CE-2 diets (Clea, Tokyo, Japan) containing DEHP were prepared by Oriental Yeast Company (Chiba, Japan). D-allose and D-allulose were provided by Kagawa Rare Sugar Research Center (Kagawa, Japan). The chemical purity of D-allose and D-allulose was found to be >98%. Chemical purity> 90% MEHP was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

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 (protocol no. 132/2007).

2.3. Experimental Design

In the first experiment, four-week-old rats weighing 90-110g were divided into control and treatment groups consisting of 12 animals. The treatment group consumed 1% (w / w) DEHP diet and sugar free water (tap water) or 1% (w / w) D-allose water or 1% (w / w) D-allose water for one week.

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. Testes and plasma were frozen at -40 ° C until MEHP and biochemical parameter measurement.

In the second experiment, 3-week-old SD rats, weighing 45-55g were divided into control and treatment group. The control group received a normal diet and sugar free water for five days. The treatment groups received a normal diet and sugar free water or 4% D-allose or 4％D-allulose water for five days. After treatment, treatment groups were given a single dose of 2 g/kg of DEHP in corn oil by oral gavages. The control group received only corn oil. Rats were sacrificed by decapitation at 24 hour after DEHP administration. The testes were removed immediately and frozen in liquid nitrogen, and maintained at −80 °C until the malondialdehyde (MDA) assay.

2.4. Plasma and Testicular MEHP Measurement

The concentrations of MEHP in plasma and testis were measured by high performance liquid chromatography. Analytical procedure and equipment are as reported [14].

2.5. Plasma Biochemical Parameter Measurements

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

2.6. Testicular MDA Assay

Testicular MDA levels from the second experiment were estimated by colorimetry using trichloroacetic acid (TCA) and thiobarbituric acid (TBA).

2.7. Statistical Analysis

SPSS 12.0 software package and Excel 2016 were used for statistical analyses and regression 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

As shown in Table 1, all DEHP diet-treated groups showed a significant decrease in testicular weight and a significant increase in liver weight compared to the Control group, while D-allose and D-allulose water treatment suppressed both testicular weight loss and liver weight gain. There were no significant differences in plasma and testicular MEHP levels between the DEHP diet treatment groups. A significant negative correlation between relative testicular weight (as a percentage of body weight) and plasma or testicular MEHP concentration was found among rats treated with DEHP-free diet (Control) and DEHP diet alone. Most of the data plots for the DEHP diet plus D-allose or D-allulose group were scattered above the regression line (Figure 1). A significant positive correlation between relative liver weight (as a percentage of body weight) and plasma MEHP concentration was found among rats treated with DEHP-free diet and DEHP diet alone. Most of the data plots for the DEHP diet plus D-allose or D-allulose groups were scattered below the regression line (Figure 2).

Table 2 shows plasma biochemical parameters of the rats treated with DEHP diet plus sugar-free water or D-allose water or D-allulose water. Plasma lipid-related markers such as total cholesterol (TCH), high density lipoprotein cholesterol (HDL-C) triglyceride (TG) of all treatment groups on the DEHP diet were significantly lower than Control. Plasma alanine transaminase (ALT) levels in the DEHP diet plus D-allose or D-allulose treatment groups were significantly lower than in the DEHP diet alone group.

Table 1. Body and organ weights, relative organ weights (as a percentage of body weight), plasma and testicular MEHP levels of the rats treated with 1% (w / w) DEHP diet plus sugar-free water or 1% (w / w) D-allose water or 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 Control.

	Control	1%DEHP	1%DEHP+1%Allose	1%DEHP+1%Allulose
n	12	12	12	12
Body weight (g)	164.6 ± 4.6 ###	140.6 ± 9.3 ***	135.0 ±10.6 ***	137.0 ± 4.6 ***
Testes (g)	1.45 ± 0.07 ###	0.83 ± 0.18 ***	0.97 ± 0.25 ***, #	1.13 ± 0.22***, ##
Relative testicular weight (%)	0.88 ± 0.05 ###	0.59 ± 0.13 ***	0.73 ± 0.18 *, #	0.82 ± 0.16 ###
Liver (g)	7.99 ± 0.50 ###	11.32± 1.23 ***	9.37 ± 0.90 ***, ###	10.00 ± 0.51 ***, ##
Relative liver weight (%)	4.85 ± 0.26 ###	8.05 ±0.64 ***	6.95 ± 0.35 ***. ###	7.31 ± 0.45 ***, ###
Plasma MEHP (µg / ml)	-	42.2 ± 10.8	46.6 ± 18.0	46.8 ± 17.1
Testicular MEHP (µg / g)	-	3.75 ± 1.39	4,29 ± 1.93	3.52 ± 2.20

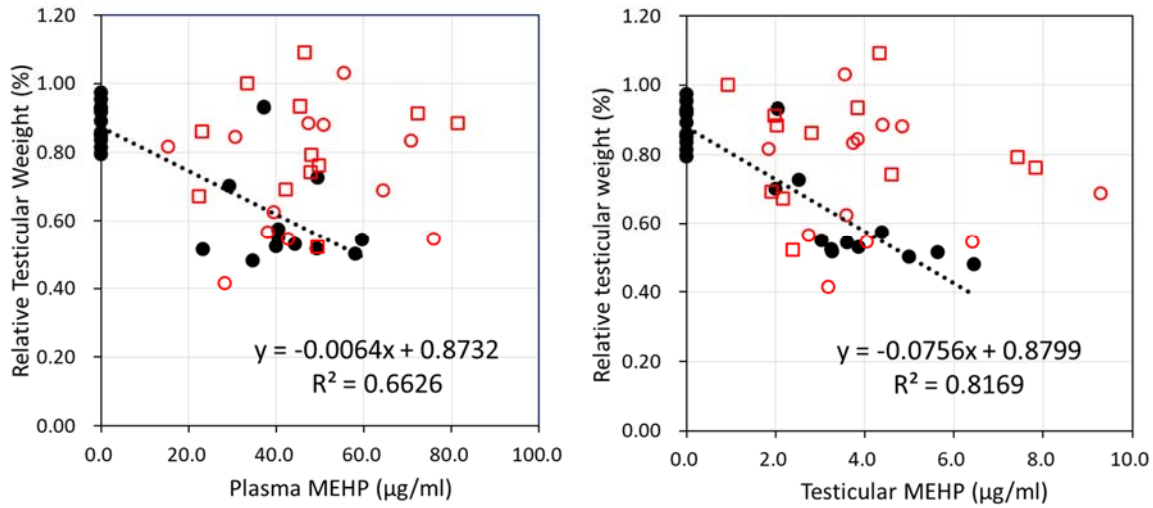

Figure 1. Relationship between relative testicular weight (as a percentage of body weight) and plasma or testicular MEHP concentration. Closed circle; rats given the DEHP-free diet (Control) or 1% (w/w) DEHP diet. Open circle; rats given the 1% (w/w) DEHP diet plus 1% (w/w) D-allose water. Open square; rats given the 1% (w/w) DEHP diet plus 1% (w/w) D-allulose water. Regression lines and equations are calculated from the Control group and the 1% (w/w) DEHP diet group.

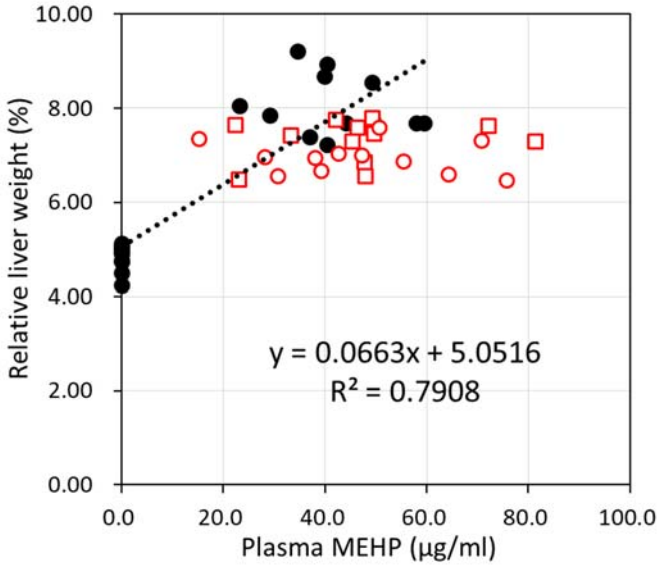

Figure 2. Relationship between relative liver weight (as a percentage of body weight) and plasma MEHP concentration. Closed circle; rats given the DEHP-free diet (Control) or 1% (w/w) DEHP diet. Open circle; rats given the 1% (w/w) DEHP diet plus 1% (w/w) D-allose water. Open square; rats given the 1% (w/w) DEHP diet plus 1% (w/w) D-allulose water. Regression line and equation are calculated from the Control group and the 1% (w/w) DEHP diet group.

Table 2. Plasma Biochemical Parameters of the rats treated with 1% (w / w) DEHP diet and sugar-free water or 1% (w / w) D-allose or1% (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 Control

	Control	1%DEHP	1%DEHP+1%Allose	1%DEHP+1%Allulose
n	12	12	12	12
AST (U/L)	146.0 ± 39.9	174.8 ± 102.1	138.8 ±107.3	121.6 ± 48.5
ALT (U/L)	51.5 ± 7.5	57.7 ± 19.2	41.5 ± 7.7 ##	43.9 ± 8.6 ##
TC (mg/dL)	90.1± 14.2	64.8 ± 4.9 ***	70.8 ± 8.2 ***	66.1 ± 9.7 ***
HDL-C (mg/dL)	38.0 ± 3.6	26.5± 3.1 ***	28.3 ± 4.0 ***	26.3 ± 3.3 ***
TG (mg/dL)	58.8 ± 25.7 ###	25.3 ± 10.1 ***	31.0 ± 12.9 ***	26.0 ± 8.5 ***

3.2. Second experiment

As shown in Table 3, testicular MDA levels at 24 hours after the oral administration of 2g/kg of DEHP were significantly increased in the group pre-treated with sugar-free water, in comparison with the control group. However, pre-treatment with 4% D-allose or 4% D-allulose significantly suppressed the production of MDA.

Table 3. Malondialdehyde (MDA) levels in the testes of control and DEHP-administered rats pre-treated with 4% D-allose or 4% D-allulose. *p < 0.05, **p < 0.01, ***p < 0.001as compared to Control. #p < 0.05, ##p < 0.01, ###p < 0.001as compared to DEHP alone.

	Control	DEHP	DEHP+4%Allose	DEHP+4%Allulose
n	6	12	7	7
MDA (nmol / g wet tissue)	33.8 ± 3.4###	51.2 ± 9.8***	38.3 ± 5.5##	38.4 ±6.2##

4. DISCUSSION

As shown in Figure 3, orally administered DEHP is rapidly metabolized to mono-(2-ethylhexyl) phthalate (MEHP) by lipase in the gastrointestinal tract [15]. MEHP is known as a peroxisome proliferator [4], [16, 17], has significant dose-related induction of CYP4A1 and lauric acid ω-hydroxylase activity in rat liver [18-20], and is suspected to be a hepatocarcinogen. MEHP has also been reported to be a reproductive toxicant that disrupts the function of Sertoli cells [21], resulting in germ cell apoptosis, and it has been shown that MEHP increases the amount of Fas ligand secreted by Sertoli cells to initiate the apoptosis of Fas-positive testicular germ cells [5]. On the other hand, it has been shown that MEHP induces oxidative stress in germ cells and causes apoptosis of spermatocytes as the direct action of MEHP on germ cells [6]. In any case, oxidative stress is definitely a trigger for injury.

In this study, all DEHP diet-treated groups showed a significant decrease in testicular weight and a significant increase in liver weight compared to the Control group, while D-allose and D-allulose water treatment suppressed both testicular weight loss and liver weight gain. And plasma alanine transaminase (ALT) levels in the DEHP diet plus D-allose or D-allulose treatment groups were significantly lower than in the DEHP diet alone group. Pre-treatment with D-allose or D-allulose water at a concentration of 4% resulted in almost complete suppression of testicular MDA production among DEHP-administered rats. These results indicate that D-allose and D-allulose can reduce DEHP-induced testicular and hepatotoxicity by suppressing oxidative stress [22, 23].

Plasma lipid-related markers such as total cholesterol (TCH), high density lipoprotein cholesterol (HDL-C) triglyceride (TG) of all treatment groups on the DEHP diet were significantly lower than Control. DEHP has been found to inhibit lipid and sterol synthesis in rats [24, 25], but there was no significant improvement by D-allose and D-allulose administration. The relationship between sterol synthesis and oxidative stress is unclear.

5. CONCLCUSION

The present study indicates that D-allose and D-allulose can reduce testicular and hepatotoxicity induced by DEHP.

6. ACKNOWLEDGMENTS

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The authors report no conflicts of interest in this work.

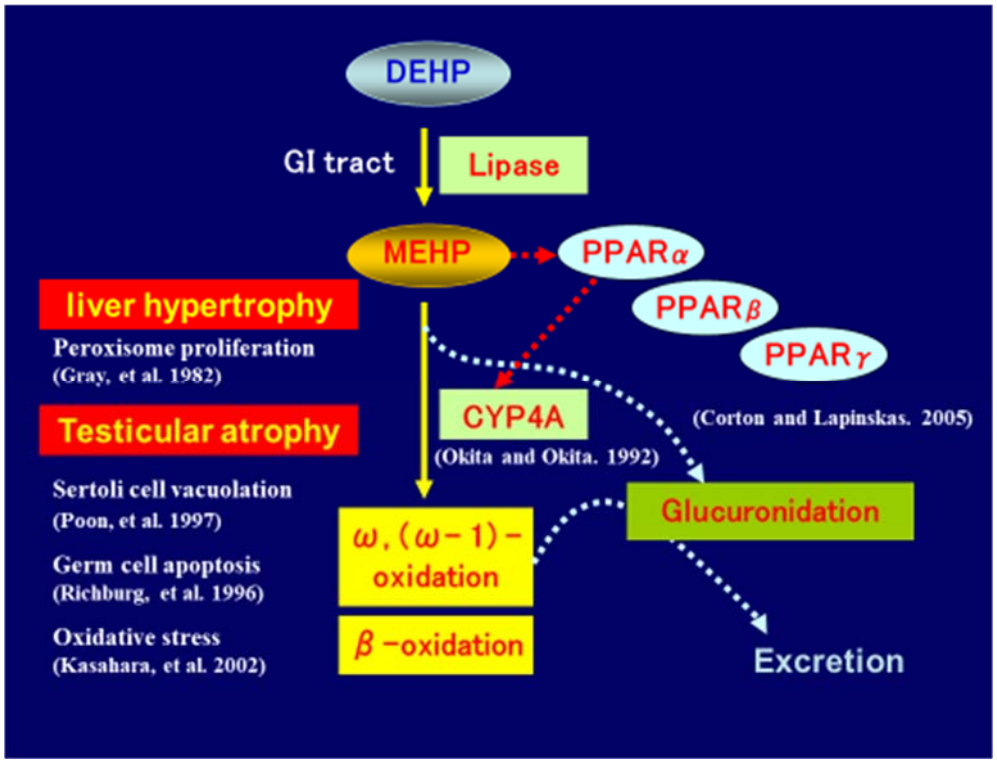

Figure 3. DEHP metabolism and toxicities in rats

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