World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(REVIEW ARTICLE)

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Protective potency of free radical scavengers against DEHP-induced testicular atrophy: A mini-review

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World Journal of Biology Pharmacy and Health Sciences, 2023, 15(02), 114–121

Publication history: Received on 28 June 2023; revised on 06 August 2023; accepted on 09 August 2023

Article DOI: https://doi.org/10.30574/wjbphs.2023.15.2.0345

Abstract

Contamination with the plasticizer di(2-ethylhexyl) phthalate (DEHP) is widespread worldwide. DEHP has been found to have testicular toxicity in animal experiments, and is suspected to be reproductively toxic to mammals, including humans. Therefore, it is important to explore the effects of prophylaxis on DEHP-induced reproductive toxicity, such as testicular atrophy. The DEHP metabolite mono(2-ethylhexyl) phthalate (MEHP) is a potent oxidative stress factor and is thought to be an active toxicant involved in testicular damage. For this reason, free radical scavengers that protect the testis from inflammation and generation of reactive oxygen species are considered to be effective prophylactic agent. The authors discuss the efficacy of several free radical scavengers in preventing testicular atrophy. The efficacy of these free radical scavengers was revealed in a DEHP mixed diet exposure experiment. Examples include nicotinic acid, caffeine, ethanol, and the rare sugar D-allulose.

Keywords: Di(2-ethylhexyl) phthalate (DEHP); Testicular atrophy; Free radical scavenger; D-allulose

1. Introduction

Di(2-ethylhexyl) phthalate (DEHP), the most widely used polyvinyl chloride (PVC) plasticizer, is a global contaminant with suspected endocrine disrupting effects [1-3]. DEHP is known to be a reproductive toxicant in laboratory animals [4], and previous studies have shown toxicity to male reproductive organs [5-9]. Although the toxicity of DEHP in humans is not clear, several epidemiological studies [10-17] suggest an association between exposure to phthalates and reproductive toxicity. Therefore, it is important to explore the effects of prophylaxis on DEHP-induced reproductive toxicity, such as testicular atrophy.

The DEHP metabolite mono(2-ethylhexyl) phthalate (MEHP) is a potent oxidative stress factor and is thought to be an active toxicant involved in testicular damage [18-22]. For this reason, free-radical scavenging agents which protects the testis against inflammation and reactive oxygen species generation are considered to be effective as prophylactic agent. The authors discuss the effectiveness of several free radical scavengers in preventing testicular atrophy as revealed in DEHP-mixed diet exposure experiments.

2. DEHP-induced testicular atrophy in rats

It has been reported that testicular atrophy is usually induced by daily gavage of rats with 1-2 g/kg DEHP or by ingesting 1-2% DEHP in the diet [4-9]. In recent years, at 5000 ppm (0.5%) DEHP in the diet, vacuolization of testicular Sertoli cells and decreased testicular body weight ratio were observed [23]. This has raised concerns about the effects of low DEHP exposure.

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Most orally administered DEHP is hydrolyzed in the gastrointestinal tract of rats [24] and distributed as MEHP in the body. MEHP, a potent oxidative stressor, damages Sertoli cells after reaching the testis and induces germ cell apoptosis [18-23]. This results in testicular atrophy. Therefore, MEHP is considered to be a substance directly involved in testicular toxicity [18-20]. In our studies, plasma or testicular MEHP concentrations were closely associated with the degree of testicular atrophy [25, 26, 45–47]. In addition, 4-week-old rats showed higher susceptibility than 5-week-old rats. Moreover, after oral administration of 2 g/kg DEHP, MEHP in plasma reached its maximum concentration at 24 hours and disappeared after 48 hours, and testicular malondialdehyde (MDA) production also changed in parallel with plasma MEHP [46]. This indicates that MEHP is closely involved in oxidative stress generation in the testis. Based on the above, free radical scavengers that protect testes from inflammation and reactive oxygen species are considered to be effective as prophylactic agents.

3. Protective potency of free radical scavengers taken in through food and drink

An antioxidant vitamin, nicotinic acid, caffeine and ethanol, which are hydroxyl radical scavengers [27-34], are ingested in relatively large amounts through food and drink on a daily basis. Therefore, it is important to clarify the relationship between DEHP toxicity and these free radical scavengers.

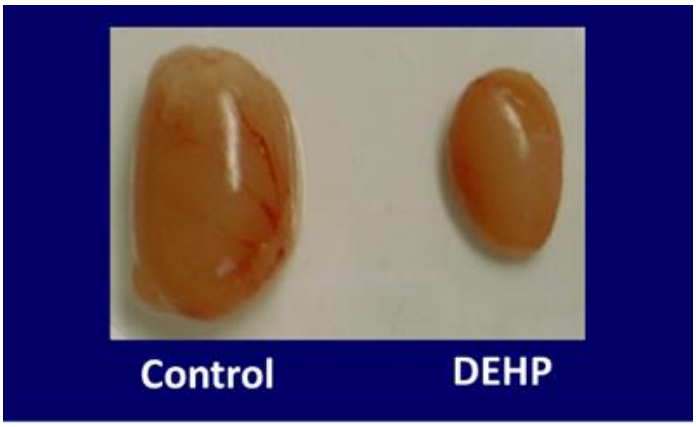

Figure 1 Testicular atrophy caused by DEHP diet

In the experiment, 4-week-old SD rats were given drinking water containing nicotinic acid (0.5 w/w %), caffeine (0.05 w/w %), and ethanol (2.5 or 5 v/v %) with DEHP (1 w/w %) diet [35, 36]. After one week of experimental administration, a significant decrease in testis weight was observed in the DEHP-only administration group (Figure 1), and testicular MEHP concentration in the control group and DEHP–only administered group showed a strong negative correlation with the relative testicular weight (as a percentage of body weight). This indicates that there is a close relationship between the tissue MEHP concentration and testicular atrophy in the target organ, the testis. On the other hand, treatment with nicotinic acid, caffeine, and ethanol significantly suppressed testicular weight loss. In addition, plots of relative testicular weight versus testicular MEHP for the nicotinic acid, caffeine, and ethanol groups were clearly above the regression line obtained from the relationship between relative testicular weight and testicular MEHP concentration for the control and DEHP-only groups. (Figure 2). Furthermore, it was suggested that combined use of ethanol and caffeine had a combined effect of reducing testicular atrophy. These findings suggest that nicotinic acid, caffeine and ethanol may inhibit DEHP-induced testicular atrophy. But, protective effect of these antioxidants was not observed in a two weeks experiment. These experimental results suggest that nicotinic acid, caffeine and ethanol have a weak protective effect against toxicity caused by high-concentration DEHP contamination. However, we cannot ignore the DEHP toxicity-preventing effect of antioxidants that we consume daily through food and drink.

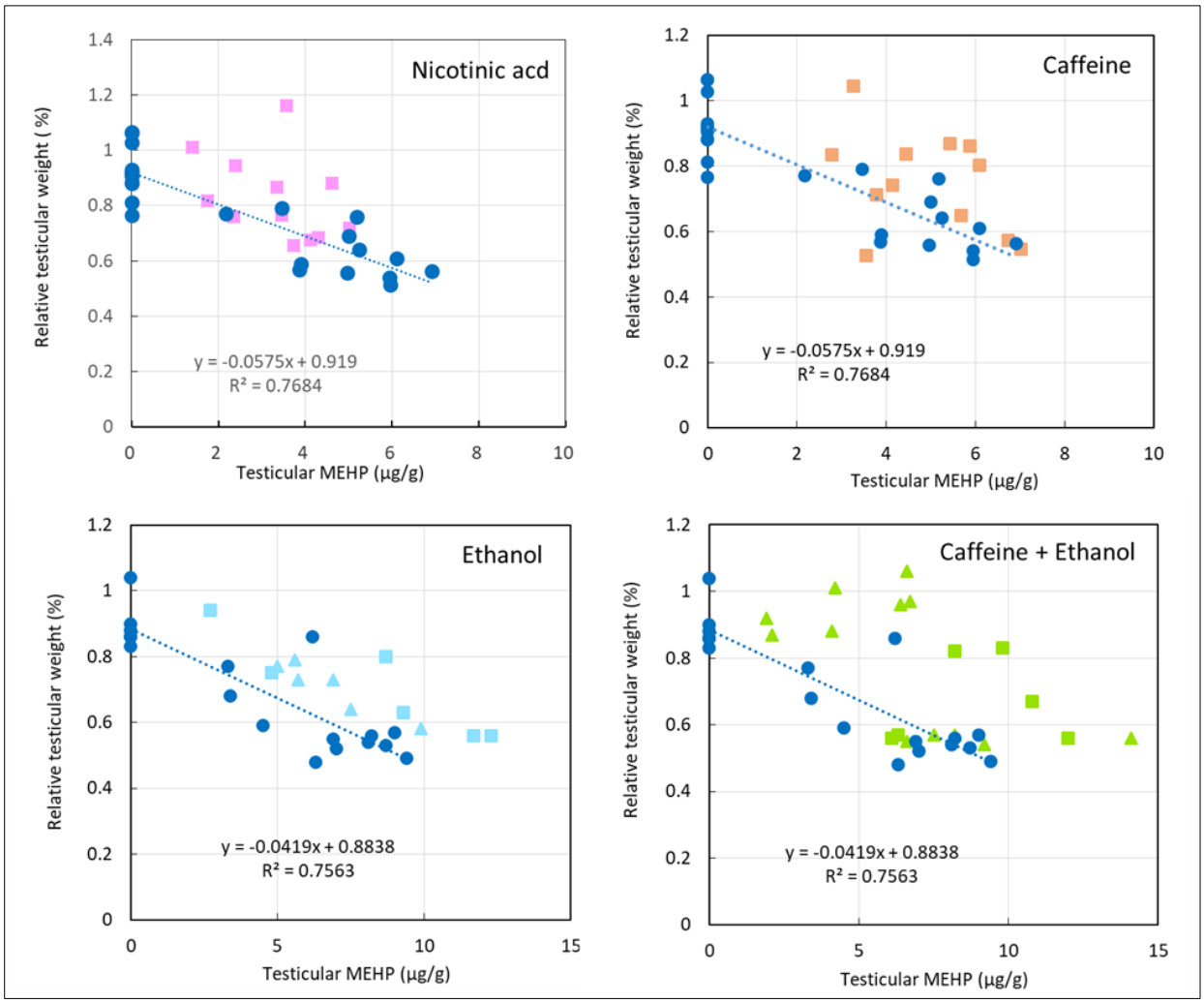

Figure 2 Relationship between relative testicular weight (testes/body %) and testicular MEHP concentration in DEHP and free radical scavengers treated groups. Filled circles; Control and DEHP-only treated rats. Filled squares nicotinic acid, caffeine and ethanol (2.5%) treated rats. Filled triangles; ethanol (5%) treated rats

4. Protective potency of D-allulose

Known as an artificial sweetener, D-allulose (also known as D-psicose) is a simple sugar that is about 70% as sweet as sucrose but has 1/10th the calories of sucrose. It also contains small amounts of figs, raisins, molasses, maple syrup, etc., and is also called "rare sugar" in Japan due to its small amount. D-allulose is recognized as GRAS (Generally Recognized As Safe) by the U.S. Food and Drug Administration. Since mass production of rare sugars by enzymatic isomerization of sugars was established by Ken Izumori at Kagawa University [37-39] in Japan, evoked the rare sugar studies revealing their anti-oxidative and anti-apoptotic properties [40-44]. Notably, D-allulose exhibits stronger scavenging activity than other rare sugars [43]. The authors investigated the protective effect of rare sugars on DEHP (1 w/w %) diet-induced testicular atrophy and found that D-allulose water and D-allose water have potent protective capacity [45]. Furthermore, testicular MDA production after oral administration of 2 g/kg DEHP was almost suppressed by pretreatment with 4% D-allulose water or 4% D-allose water (Figure 3). Furthermore, D-allulose water with a concentration of 2% or more showed almost complete protection against DEHP (1 w/w %) diet -induced testicular atrophy for up to 2 weeks (Figure 4, 5) [25, 45-47]. As shown in Figure 6, testis sections from DEHP-only given rats showed degeneration of the seminiferous tubules and sloughing of germ cells into the tubular lumen. On the other hand, testicular sections from rats given a DEHP diet and 4% D-allulose water showed normal spermatogenesis with normal cell arrangement within the seminiferous tubules.

These results suggest that D-allulose has a very high protective capacity against DEHP-induced testicular atrophy and may be the best prophylactic agent for DEHP–induced testicular atrophy we have explored.

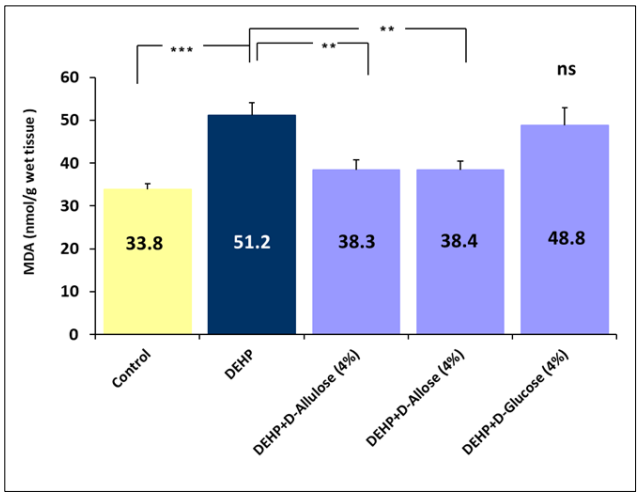

Figure 3 Testicular MDA levels 24 hours after oral administration of 2 g/kg DEHP. Pretreatment with 4% D-allose or 4% D-allulose almost suppressed the formation of MDA. **P < 0.01, ***P < 0.001 as compared to DEHP-only administered group

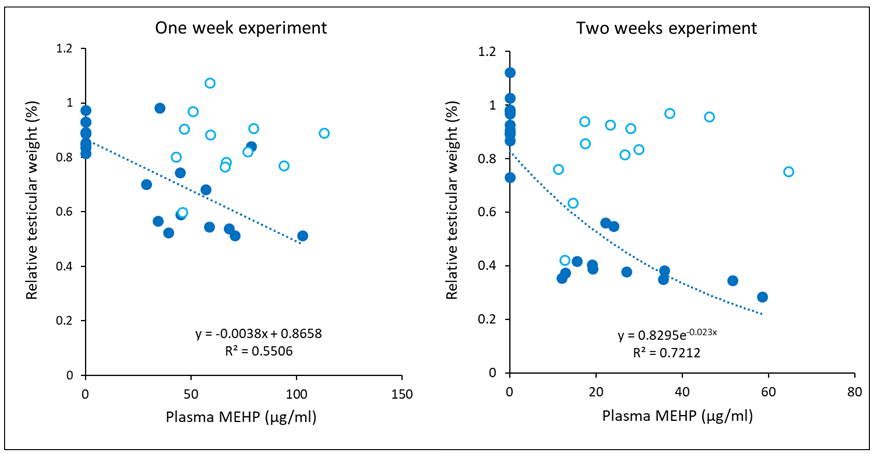

Figure 4 Relationship between plasma MEHP levels and relative testicular weights of control and treatment rats receiving the diets for one or two weeks. Filled circles; Control and DEHP-only treated rats. Open circles; rats given the DEHP diet plus 2% D-allulose water

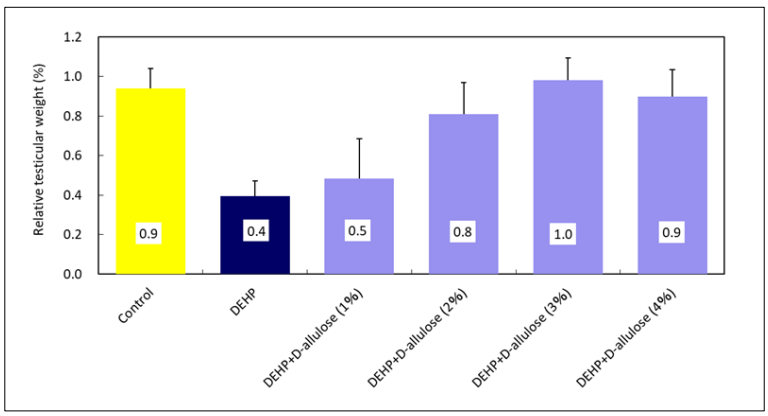

Figure 5 Relative testicular weights of the rats treated with DEHP and D-allulose for two weeks

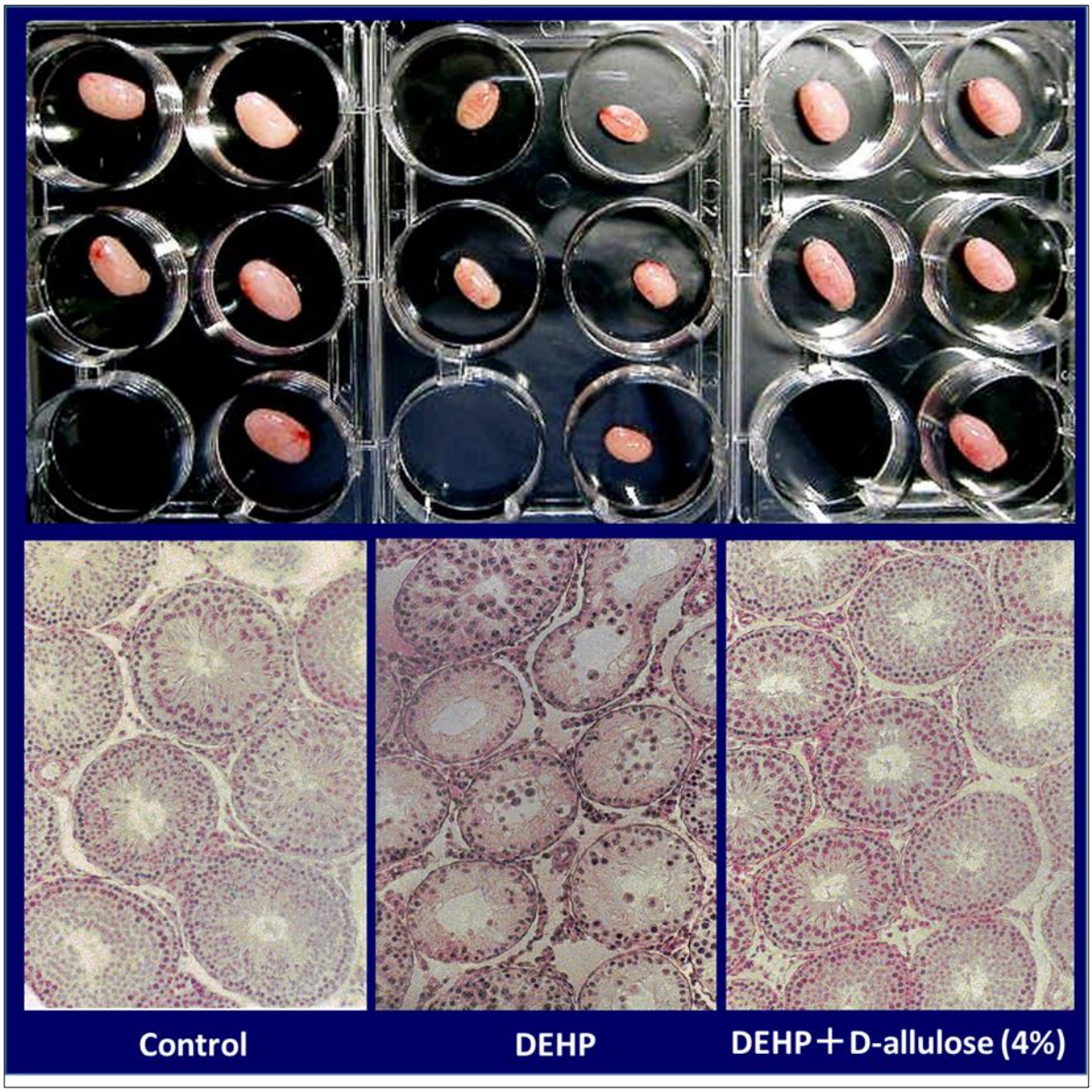

Figure 6 Rat testes and seminiferous tubules after two weeks of treatment with DEHP and D-allulose

5. Conclusion

The authors discussed the effectiveness of several free-radical scavengers in preventing DEHP–induced testicular atrophy, which was revealed in DEHP mixed diet exposure experiments. Although the reproductive toxicity of DEHP in humans remains unclear, D-allulose was suggested to be most useful as a prophylactic agent against oxidant-mediated testicular injury in mammals, including humans.

Compliance with ethical standards

Acknowledgments

The author appreciates the help of colleagues in the laboratory.

Disclosure of conflict of interest

There is no conflict of interest in this work.

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