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The effect of phytosterol protects rats against 4-nitrophenol-induced liver damage

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ABSTRACT

We investigated the effect of phytosterol (PS) in regard to liver damage induced by 4-nitrophenol (PNP). Twenty rats were randomly divided into four groups (Control, PS, PNP, and PNP + PS). The PS and PNP + PS groups were pretreated with PS for one week. The PNP and PNP + PS groups were injected subcutaneously with PNP for 28 days. The control group received a basal diet and was injected with vehicle alone. Treatment with PS prevented the elevation of the total bilirubin levels, as well as an increase in serum alkaline transaminase and aspartate transaminase, which are typically caused by PNP-induced liver damage. Histopathologically showed that liver damage was significantly mitigated by PS treatment. However, there was no significant change in antioxidant enzyme activities, and the Nrf2-antioxidant system was not activated after treatment with PS. These results suggest that PS could mitigate liver damage induced by PNP, but does not enhance antioxidant capacity.

1. Introduction

4-Nitrophenol (PNP) is extensively used in agriculture and industry and is commonly found in water and soil (Bhushan et al., 2000). A nitrophenol derivative, PNP, has been isolated from diesel exhaust particles (DEP) (Noya et al., 2008). It is also a degradation product of the insecticide parathion (Kim et al., 2006), which is used as an acaricide, soil disinfectant, and pre-harvest foliage treatment for a wide variety of indoor and outdoor crops. The opportunities for humans, wild animals, and livestock to be exposed to parathion may be elevated in both rural and residential environments. PNP has been detected in human urine, possibly due to exposure to OP pesticides (Hryhorczuk et al., 2002). Studies have shown that PNP has endocrine-disrupting activities (Hass et al., 2012). As an environmental endocrine disruptor, it is not only a vasodilator (Mori et al., 2003) but exhibits estrogenic and anti-androgenic activities (Li et al., 2006). In addition, PNP effects the hypothalamus-pituitary-gonadal axis (Li et al., 2009) through disrupted hormonal balances and differentially modulated AR, ER-α and -β expression in male rat testes (Zhang et al., 2013). Moreover, PNP is resistant to biodegradation (Call et al., 1980); as a result, increasing attention has been focused on understanding the toxicology of this class of compounds. Due to the large-scale production, applications, and potential toxicity of PNP, the U.S. Environmental Protection Agency has classified PNP as both a hazardous waste and a priority toxic pollutant.

Oxidative stress, an imbalance that favors pro-oxidants over antioxidants, can potentially lead to biomolecule damage (Sies, 1993). As the production of reactive oxidative species (arising either from the mitochondrial electron transport chain or excessive stimulation of NAD(P)H) exceeds the capacity of cellular antioxidant defenses to remove these toxic species, the result is oxidative stress. Cells are endowed with a defense pathway for the prevention and/or treatment of oxidative stress that reduces the production of reactive metabolites by raising the levels of endogenous antioxidant activities for superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) (Aruoma, 1994). The NF-E2-related factor (Nrf2) pathway is the major regulator of the antioxidant response (Hybertson et al., 2011; Sahin et al., 2010).

Abbreviations: AKP, aryl hydrocarbon receptor; ALT, alkaline transaminase; AST, aspartate transaminase; CAT, catalase; GCLC, glutamate cysteine ligase catalytic subunit; GSH-PX, glutathione peroxidase; HEh, ematoxylin and eosin; HO-1, heme oxygenase-1; MDA, malondialdehyde; NQO1, NADPH oxidoreductase1; Nrf2, nuclear factor erythroid 2-related factor 2; PNP, 4-nitrophenol; PS, phytosterol; PBS, phosphate-buffered saline; SD, Sprague–Dawley; SOD, superoxide dismutase; TBIL, total bilirubin.

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In the basal, inactive state, Nrf2 is associated with Keap1 in the cytoplasm. In response to oxidative stress, Nrf2 dissociates from Keap1; this rescues Nrf2 from proteasomal degradation and enables Nrf2 to enter the nucleus, resulting in a response of cellular protection that is characterized by the modulation of the transcription of antioxidants and phase II detoxifying enzymes (e.g., glutathione S-transferase (GST), NADPH: quinine oxidoreductase (NQO1)) (Itoh et al., 1997).

The liver is the major organ of xenobiotic metabolism and excretion. PNP is inevitably absorbed into humans and animals as a xenobiotic due its widespread prevalence. PNP induced liver damage has been reported by using zebrafish as a model (Lam et al., 2013). Phytosterol has been found to be abundant in plant material (Garcia-Llatas and Rodriguez-Estrada, 2011). Stigmasterol, campesterol, and β-sitosterol are the most common phytosterols in the human diet (Calpe-Berdiel et al., 2009). Phytosterol has positive health effects, including being strongly associated with improvements in serum lipid profiles and reductions of the risk for cardiovascular disease (Blair et al., 2000; Kendall and Jenkins, 2004) and breast cancer (Llaverias et al., 2013). Meanwhile, other effects, such as those regarding endothelial function, oxidative stress and antioxidant status, coagulation and platelet aggregation, inflammation, neurocognitive function, eye disease and anti-osteoarthritic, have been found. Therefore, the objective of this study was to evaluate the protective effect of phytosterol on PNP-induced liver damage.

2. Materials and methods

2.1. Chemicals

PNP monomer dry crystals ($C_6H_5NO_3$, >99.9% purity, CAS 100-02-7) were purchased from Chengdu Kelong Chemical Reagent Factory, China. Commercial grade PS (mixture of β-sitosterol, stigmasterol, and campesterol, >90% purity, CAS 83-46-5) was purchased from the Jiangsu Chunzhigu Biological products company.

2.2. Animals

Twenty-one-day-old male Sprague-Dawley (SD) rats were purchased from the QingLongShan Laboratory Animal Company (NanJing, China). The weanling weight showed no significant differences ($P > 0.05$) between the rats. Rats were maintained under a 12 h light, 12 h dark schedule, with feed and sterile distilled water available ad libitum. Rats were allowed to adapt to the new surroundings for one week before the formal experiment. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

2.3. Experimental design

After 1 week of acclimatization, twenty Sprague–Dawley rats were divided into four groups (Control, PS, PNP, and PNP + PS) (Table 1). The choice of PNP and PS doses was based on the results of our pilot study and other studies (Li et al., 2006; Nieminen et al., 2010). The PS and PNP + PS groups were pretreated with PS (50 mg/kg in basal diet) for one week. The PNP and PNP + PS groups were injected subcutaneously with PNP (100 mg/kg body weight/day) for 28 days at 9 AM. Meanwhile, the control group received a basal diet and was injected with the vehicle alone (PBS containing 0.05% Tween80). The PNP was dissolved in the vehicle, which was sterile phosphate-buffered saline (PBS) containing 0.05% Tween80; stored under refrigeration (approximately 4 °C); and replaced once per week to maintain the stability of the dosing solutions. Twenty-four hours after the final injection, rats were weighed, anesthetized with ethylic ether, and killed.

Table 1
Agents administered to the difference experiment groups.

Group	Treatment
Control	Basal diet and injected subcutaneously with vehicle alone (PBS containing 0.05% Tween80)
PS	PS (50 mg/kg in basal diet)
PNP	Basal diet and injected subcutaneously with PNP (100 mg/kg body weight/day)
PNP + PS	Injected subcutaneously with PNP (100 mg/kg body weight/day) and PS (50 mg/kg in basal diet)

2.4. Sample collection

Blood samples were collected in plastic tubes and centrifuged at 3500 rpm for 15 min at 4 °C. The serum was separated and stored at −20 °C until it was assayed for alanine transaminase, aspartate transaminase, and alkaline phosphatase activities, as well as the total bilirubin level. The liver was excised and immediately weighed after killing. An aliquot of the liver was fixed in 4% paraformaldehyde and processed for histological examination; a second portion of the liver was stored at −20 °C until it was assayed for superoxide dismutase, catalase, and glutathione peroxidase activities, as well as the malondialdehyde level; the remainder of the liver was stored in liquid nitrogen for RT-PCR.

2.5. Assay for serum TBIL and marker enzymes

Blood samples were centrifuged at 3500 rpm for 15 min at 4 °C, and then, serum was collected. The TBIL concentration and ALT, AST, and AKP activities were assayed using commercial reagent kits obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China), according to the manufacturer's protocols.

2.6. Assay for hepatic antioxidant activities

A 0.15-g piece of liver tissue was placed in a bottle and diluted 1:9 (wt:vol) with ice-cold normal saline. Liver tissue was then homogenized and centrifuged at 3800 rpm for 15 min. The supernatant was collected and used for measurements. The activities of MDA, SOD, CAT, and GSH-Px were assayed using commercial reagent kits obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China), according to the manufacturer's protocol.

2.7. Histology

An aliquot of the livers were removed and fixed in 4% buffered formalin. After 24 h, the liver samples were dehydrated in alcohol, embedded in paraffin, and sectioned at 5–7 μm. For histological assessment, sections were stained with hematoxylin and eosin. The stained sections were mounted and examined under light microscopy. Photographs of each slide were taken at 100× magnification.

2.8. RNA extraction, reverse transcription (RT) and quantitative PCR

Total RNA was extracted from the tissue using an RNeasy Mini kit® (Qiagen, China) according to the manufacturer's instructions. The concentration and purity of the isolated total RNA was determined spectrophotometrically at 260 and 280 nm with a Nanodrop® 8000 (Thermo Fisher Scientific, Wilmington, USA). Total RNA (1 μg) was reverse transcribed to cDNA with an Omniscript® Reverse Transcription kit (Takara) using Oligo-dT