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Toxicologic assessment of *Paecilomyces tenuipes* in rats: Renal toxicity and mutagenic potential

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ABSTRACT

Paecilomyces tenuipes is entomogenous fungus that is called snow-flake Dongchunghacho in Korea. Although it is widely used in traditional medicines, its safety has not yet been comprehensively investigated. Therefore, the aim of this study was to evaluate the genotoxicity, acute and subchronic toxicity of *P. tenuipes*. The acute oral LD_{50} of *P. tenuipes* extract in rats was estimated to be greater than 2000 mg/kg of body weight. In the subchronic study, the oral treatment of rats with 500, 1000 or 2000 mg/kg *P. tenuipes* extract daily for 13 weeks did not induce any dose-related changes (body weight, food consumption, clinical observation, urinalysis, hematology, clinical chemistry and organ weight). In contrast, histopathological observation revealed that *P. tenuipes* extract induced karyomegaly in outer medulla of kidney in all treated rats. Importantly, *P. tenuipes* extract exerted the mutagenic potential in Ames assay. Since karyomegalic alterations have been known to be associated with carcinogenicity, our finding on the mutagenicity of *P. tenuipes* extract supports the possibility on the potential involvement of *P. tenuipes* in carcinogenicity at least partially. In conclusion, the subchronic oral exposure of *P. tenuipes* may induce kidney abnormality at the concentration higher than 500 mg/kg body weight, although further studies using other animal models are needed to identify the toxicity of *P. tenuipes*.

1. Introduction

Paecilomyces tenuipes, an entomogenous fungus on the lepidopteran larvae, pupae, and adults, is called snow-flake Dongchunghacho in Korea because of its appearance (Nam et al., 2001). This entomogenous fungus has long been widely used as health food ingredients and traditional nutritious medicines, especially for allergic diseases, asthma, cancer and tuberculosis in Asian countries (Zhu et al., 1998a,b). In addition, the *P. tenuipes* has been known to contain novel ingredients that induce cellular differentiation and inhibit cell growth in various malignant cell lines (Shim et al., 2000, 2001) and to induce apoptosis in a human leukemic cell line (Park et al., 2000). The *P. tenuipes* has also been reported to improve lipid profiles in rats fed a high fat diet (Koh and Choi, 2003). And, Schmidt et al. (2003) demonstrated that the *P. tenuipes* could play an important role in aging process through the modulation of monoamine oxidase inhibitory activity and subsequent contribution to oxidative stress.

Several reports on toxicity of *Paecilomyces species* were published previously. Two-week oral treatment with complex powder

Abbreviations: SD, Sprague Dawley; OECD, Organization for Economic Co-operation and Development; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; TC, total cholesterol; TP, total protein; TB, total bilirubin; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; TG, triglyceride; CHL, Chinese hamster lung; FBS, fetal bovine serum; PCE, polychromatic erythrocytes.

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suspension of *P. sinclairii* and larvae of its host *Bombyx mori* did not induce any toxicological effects in adult Sprague Dawley (SD) rats (Kwack and Lee, 2009). The acute oral LD_{50} of this complex was found to be greater than 5 g/kg of body weight in rats and dogs (Kim et al., 1996). And, the results of a battery of *in vitro* and *in vivo* genotoxicity tests indicated that this complex does not possess any mutagenic or genotoxic potential (Ahn et al., 2004a). However, there has been controversy over whether *Paecilomyces species* is beneficial or toxic to kidney function. Jeong et al. (2012) revealed that *P. sinclairii* exposed orally induced kidney cell damage and filtration dysfunction. The complex of *P. sinclairii* fruiting body and silkworm larvae was also known to induce tubular cell abnormalities, including tubular edemas and tubular destruction, in kidney (Ahn et al., 2004b). In contrast, there was another report on beneficial effect of *Paecilomyces species* on kidney functions. Zhu et al. (1998b) identified that *Cordyceps* mushrooms supported kidney functions through an increase in 17-hydroxycorticosteroid and 17-ketosteroid levels. For these reasons, further studies are required to elucidate the effect of *Paecilomyces species* on kidney functions. Especially, among *Paecilomyces species*, the toxicity of the *P. tenuipes* and underlying mechanism remains unclear yet. Therefore, we performed the genotoxicity, acute and subchronic oral toxicity to investigate the potential hazards and safety concerns associated with the *P. tenuipes*.

2. Materials and methods

2.1. Test substance and animals

The *P. tenuipes* extract was kindly provided by Korea Food Research Institute (Seongnam, Korea). In brief, fruiting bodies of *P. tenuipes* were collected, dried and homogenized into powder-form. 100 g of power was macerated with 1 liter of water at 110 °C for 10 h. The suspension was filtered through filter paper for 5 h. After centrifugation at 6000 rpm for 20 min, the supernatant of the extract was freeze–dried. It was resuspended with distilled water. SD rats and ICR mice were obtained from Orient bio (Seongnam, Korea) and housed in an environmentally-conditioned room (22 ± 2 °C, 40–60% humidity, and 12 h light cycle). The animals were allowed free access to rodent diet (Purina, Seoul, Korea) and tap water. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Biomedical Research Institute at Seoul National University Hospital. And, this study was performed in compliance with the Good Laboratory Practices for toxicity test guidance issued by the Korea Food and Drug Administration (KFDA, 2005).

2.2. Acute oral toxicity study

The study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline 420 (OECD, 2001). After quarantine and acclimatization, healthy rats of either sex were divided into two groups of 10 animals each (5 males and 5 females). The rats were fasted for 16 h prior to conducting the experiment but had free access to water. The rats were orally administered with the *P. tenuipes* extract at a dose of 0 or 2000 mg/kg of body weight. On the day of dosing, all rats were observed for mortality and signs of toxicity for several hours after dosing and once daily thereafter for 14 days. Body weights were recorded on the day of treatment and on test day 1, 7 and 14. At the end of the study, all surviving rats were anesthetized with isoflurane, and blood was collected *via* the posterior vena cava from anesthetized animals.

2.3. Subchronic oral toxicity study

The study was performed according to the OECD test guideline 408 (OECD, 1997a). Groups of 10 rats of each sex were orally treated with 0, 500, 1000, 2000 mg/kg *P. tenuipes* extract daily for 13 weeks. The rats were observed daily for clinical signs and mortality, and body weights were measured every week during the study period. At the last week of treatment, urinalysis of 10 rats (5 males and 5 females) per group was performed using fresh urine to determine pH, specific gravity, leukocyte, nitrite, protein, ketone body, urobilinogen, bilirubin, glucose, and occult blood using urine analyzer (Miditron Junior II, Roche, Mannheim, Germany). At the end of the study, all rats were anesthetized with isoflurane, and blood was collected from posterior vena cava.

Blood samples collected in an EDTA blood collection tube for hematology analysis were assayed using an automatic hematology analyzer ADVIA 2120i (Siemens Diagnostics, Tarrytown, NY, USA) for the following parameters: total white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential WBC.

In serum biochemistry analysis, serum was analyzed using an automatic chemistry analyzer 7070 (Hitachi, Tokyo, Japan) for blood urea nitrogen (BUN), creatinine, total cholesterol (TC), total protein (TP), albumin, total bilirubin (TB), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), glucose, K, Cl, Ca, and P.

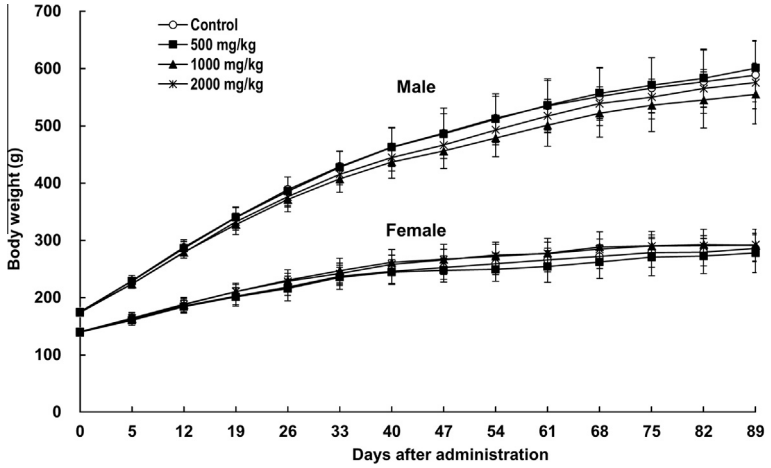

Fig. 1. Growth curves for male and female SD rats orally administered with *P. tenuipes* for 13 weeks. Data expressed as means ± SD.