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A 90 Day Repeated Oral Toxicity Study on Plantamajoside Concentrate from *Plantago asiatica*

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Plantago asiatica **is distributed widely in East Asia. Since ancient times it has been used as a diuretic to treat acute urinary infections, and as an antiinflammatory, antiasthmatic, antioxidant, antibacterial, antihyperlipidemic and antihepatitis drug. The major compound, plantamajoside from *P. asiatica*, which is used as a marker compound in chemotaxonomic studies, was reported to have antibacterial activity, inhibition activity against cAMP phosphodiesterase and 5-lipoxygenase and antioxidant activity. However, there are no reports on the safety of plantamajoside. This study assessed the toxic effects of plantamajoside concentrate (PC), the purity of which was above 80%, in rats following administration at dose levels of 0, 500, 1000 and 2000 mg/kg body weight/day for 13 weeks, as recommended by the OECD guidelines. The results showed that there were no differences in body weight, food intake, water consumption, relative organ weight or the hematological and serum biochemical values among the different dosage groups. No death or abnormal clinical signs were observed during the experimental period. Therefore, the results suggested that no observed adverse effect level (NOAEL) of the PC in rats after oral administration is considered to be greater than 2000 mg/kg in rats under the conditions employed in this study. Copyright © 2007 John Wiley & Sons, Ltd.**

Keywords: Plantago asiatica; plantamajoside; toxicity; NOAEL; phytochemicals; Plantaginaceae.

INTRODUCTION

Plantago asiatica is a member of the Plantaginaceae family, and is widely distributed in Korea, China and Japan. It is a virile plant used in Chinese medicine, where its leaf is called *Plantaginis Herba* and its seed *Plantaginis Semen* (Bae and Kim, 2004), and is also commercialized as forms of dietary supplement or extract. Since ancient times it has been used as a diuretic to treat acute urinary infection, and as an antiinflammatory, antiasthmatic, antioxidant, antibacterial, antihyperlipidemic and antihepatitis drug (Liu *et al.*, 2002; Samuelsen, 2000). The plantaginin, plantamajoside, acetoside and 6-hydroxyluteolin 7-glucoside isolated from *P. asiatica* were the chief compounds (Nishibe, 2002; Murai *et al.*, 1995; Komoda *et al.*, 1989), and they had various biological activities (Ko *et al.*, 2004; Chiang *et al.*, 2003). The plantamajoside, in particular, was reported to show a minimum inhibitory concentration of 2.0 mg/mL against *Staphylococcus aureus* 502A (Ravn and Brimer, 1988), inhibition activity against cAMP phosphodiesterase [$IC_{50}(\times 10^{-5}$ M): 16.0] and 5-lipoxygenase [$IC_{50}(\times 10^{-5}$ M): 3.73] (Ravn *et al.*, 1990), and antioxidant activity on ADP + NADPH-induced lipid peroxidation in rat liver microsome [IC_{50} (μM): 0.64] (Miyase *et al.*, 1991). Also, the plantamajoside is used as a marker compound in chemotaxonomic studies, as are other members of the dihydroxyphenylethyl glycoside family (Mølgaard, 1986; Andary *et al.*, 1998). However, to our knowledge, there are no reports on the safety of plantamajoside. Therefore, the objective of present study was to investigate the chronic toxicity (90 days) of the plantamajoside concentrate (PC) having at least above 80% purity. The experiment was conducted largely in agreement with the recently updated OECD guideline 407 (OECD, 1995).

MATERIALS AND METHODS

Plant material. *P. asiatica* was purchased from a local commercial market (Kyungdong Herb-Market, Seoul, Korea), was identified by Professor B. W. Kang (College of Life Sciences and Biotechnology, Korea University). Voucher specimens were deposited in the Herbarium of the College of Life Sciences and Biotechnology, Korea University, register number H-212.

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Preparation of plantamajoside. The plantamajoside was isolated from *P. asiatica* using a previously described method (Andary *et al.*, 1988). Due to the limitation of the amount needed to perform a chronic toxicological study of plantamajoside, it was decided to use the PC, containing plantamajoside, which was estimated to be at least above 80% by HPLC (Fig. 1). The lyophilized PC was weighed according to dosage, dissolved in distilled water according to dosage (500, 1000 and 2000 mg/kg body weight), and then stored at 4 °C. The HPLC apparatus consisted of a pump (ProStar, Varian) equipped with a UV-VIS detector at 335 nm. The column was a SymmetryPrep™ C18 (7.8 × 300 mm i.d. 7 μm) (Waters, USA). Elution was performed using methanol as a linear gradient.

Animals. Male and female Sprague-Dawley rats (5 weeks old, ten animals per group in both genders) weighing 160–170 g were purchased from Samtako Bio Korea (Gyeonggi, Korea) and quarantined for a week. All rats were kept under controlled conditions of temperature (22 ± 1 °C) and humidity (60 ± 5%). They were given pellet food (Samtako Bio Korea) and drinking water *ad libitum*. The experimental protocol met the National Guidelines on the Proper Care and Use of Animals in Laboratory Research, and was approved by the Institutional Animal Ethics Committee.

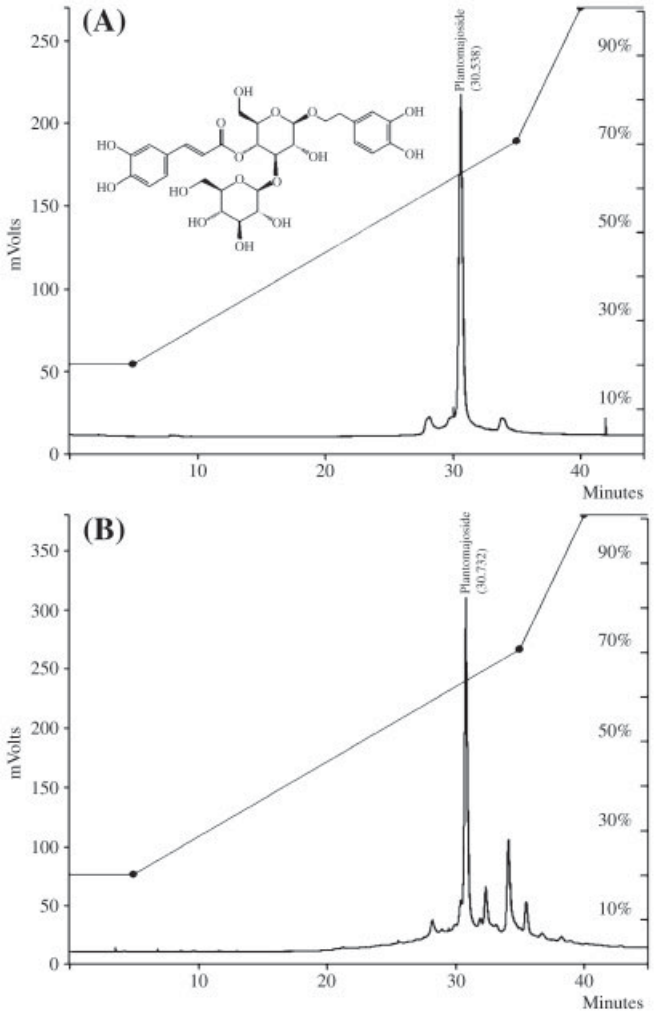

Figure 1. HPLC profiles of the authethic plantomajoside of *Plantago asiatica* (A) and the plantomajoside concentrate (PC) (B).

Chronic toxicity in rats – 90 days with repeated doses. The rats were randomly assigned to the following experimental and control groups: Group 0, animals designated as the normal untreated controls received water vehicle orally once a day throughout the entire experiment (13 weeks); Group 500, 1000 and 2000, animals received orally 500, 1000 and 2000 mg of PC/kg body weight, respectively. The animals were weighed individually on day 1, and twice every week thereafter. The water consumption and food intake of the rats were measured weekly. Animals were killed at the end of 13 weeks, and all animals were fasted overnight prior to killing.

Hematological and serum biochemical parameters. The hematology parameters including white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCt), mean corpuscular hemoglobin (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) count were determined (Rodrigues *et al.*, 1998). The serum biochemistry parameters including total protein (TP), albumin (ALB), glucose (GLU), cholesterol (CHOL), total bilirubin (TBIL), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP) were determined (Oliveira *et al.*, 2005).

Morphological study. The organ weights of thymus, lung, heart, kidney, liver, spleen, and testis or ovary were measured at the end of the study. A complete necropsy was performed on the treated and control animals which were killed. After fixation in 10% phosphate buffered formalin, liver and kidney were processed in routine manner, embedded in paraffin, and sectioned. Then to perform light microscopic evaluation, the liver was stained with hematoxylin and eosin (H&E), and kidney was stained with periodic acid schiff (PAS).

Statistical analysis. The biochemical data were analysed with SigmaStat V 3.5 (Jandel Scientific, San Rafeal, CA). Data are expressed as mean ± standard error of mean. Statistical analysis was done using one-way analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range. Values were considered statistically significant when $p < 0.05$.

RESULTS

Chronic toxicity

To examine the chronic oral toxicity of the PC from *P. asiatica* male and female SD rats were used. The PC was administered at doses of 0, 500, 1000 and 2000 mg/kg intragastrically. There were no deaths among the rats treated with the PC during the 90 day observation period (data not shown). The dose (2000 mg/kg) of PC used in this study was the highest that could be prepared for oral administration in rats. Therefore, the oral no observed adverse effect level (NOAEL) of the PC, although it could not be determined precisely, was greater than 2000 mg/kg. All rats appeared to be entirely healthy and normal during the period (data not shown).

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Table 1. Daily food consumption (g) of SD rats during treatment with plantamajoside solution for 90 days

	Male (mg/kg)				Female (mg/kg)			
Week	0	500	1000	2000	0	500	1000	2000
1	29.0 ± 2.6	29.5 ± 1.3	28.5 ± 1.4	27.8 ± 1.8	23.8 ± 1.8	20.3 ± 1.0	24.2 ± 2.1	22.5 ± 1.1
2	30.3 ± 6.5	30.0 ± 1.3	34.0 ± 2.6	31.5 ± 4.2	23.7 ± 1.8	21.5 ± 1.6	26.2 ± 2.7	24.5 ± 1.6
3	29.6 ± 2.3	30.5 ± 9.0	33.2 ± 7.3	33.7 ± 1.7	20.0 ± 2.3	21.2 ± 2.8	17.5 ± 3.2	17.2 ± 2.6
4	35.0 ± 5.9	28.3 ± 1.7	31.2 ± 2.9	32.3 ± 3.1	20.4 ± 1.6	20.5 ± 1.8	28.8 ± 1.7	23.2 ± 1.6
5	35.5 ± 5.2	33.5 ± 2.2	36.8 ± 5.5	38.3 ± 1.3	23.2 ± 2.5	21.5 ± 2.9	23.0 ± 4.3	18.3 ± 4.2
6	28.8 ± 2.4	31.8 ± 1.2	30.7 ± 1.3	32.0 ± 1.9	19.0 ± 3.9	18.8 ± 3.5	22.7 ± 2.2	25.0 ± 2.9
7	29.0 ± 2.7	24.0 ± 0.4	27.3 ± 2.3	29.5 ± 3.1	17.7 ± 1.6	18.2 ± 0.7	19.8 ± 1.6	14.0 ± 0.8
8	33.3 ± 2.1	35.8 ± 5.5	31.7 ± 4.8	36.7 ± 3.8	17.2 ± 0.9	19.5 ± 3.3	21.2 ± 1.5	24.0 ± 5.7
9	27.0 ± 1.8	29.0 ± 1.9	31.8 ± 1.9	32.0 ± 1.7	16.3 ± 1.2	16.5 ± 31.3	23.0 ± 2.4	16.0 ± 1.6
10	27.3 ± 1.5	27.8 ± 0.7	33.7 ± 7.9	26.2 ± 1.6	13.8 ± 3.9	18.8 ± 1.6	19.0 ± 1.6	19.2 ± 1.8
11	29.2 ± 2.6	33.3 ± 1.5	31.3 ± 1.9	34.2 ± 1.8	11.2 ± 0.6	16.5 ± 1.3	20.0 ± 1.4	19.0 ± 2.8
12	27.5 ± 2.8	26.3 ± 1.4	27.2 ± 1.6	30.5 ± 1.2	18.2 ± 2.1	14.8 ± 1.7	18.8 ± 1.3	17.6 ± 2.6
13	29.5 ± 1.3	27.5 ± 1.7	27.3 ± 1.6	27.5 ± 1.2	17.7 ± 1.3	15.2 ± 1.4	18.8 ± 0.7	17.6 ± 2.2

Values are presented as mean ± SEM (n = 5).

Table 2. Daily water consumption (mL) of SD rats during treatment with plantamajoside solution for 90 days

	Male (mg/kg)				Female (mg/kg)			
Week	0	500	1000	2000	0	500	1000	2000
1	45.0 ± 4.8	42.5 ± 2.1	44.2 ± 3.5	45.8 ± 2.4	48.3 ± 3.3	43.3 ± 2.8	49.2 ± 1.5	46.7 ± 2.1
2	44.2 ± 3.0	40.0 ± 3.4	53.5 ± 3.3	60.0 ± 7.9	42.5 ± 4.4	39.2 ± 1.5	51.7 ± 5.6	57.5 ± 6.2
3	42.5 ± 2.8	37.5 ± 2.5	46.7 ± 3.1	53.3 ± 5.4	31.2 ± 2.4	43.3 ± 4.9	47.5 ± 8.1	25.0 ± 8.5
4	48.3 ± 6.7	43.3 ± 5.1	51.7 ± 4.8	50.8 ± 3.5	43.0 ± 4.0	43.3 ± 3.1	50.8 ± 6.9	46.7 ± 4.2
5	46.7 ± 4.0	55.8 ± 7.1	53.3 ± 7.6	51.7 ± 2.8	39.2 ± 2.7	45.0 ± 3.7	38.3 ± 8.0	33.3 ± 4.9
6	53.8 ± 7.6	46.7 ± 4.8	45.0 ± 4.8	53.3 ± 2.1	40.0 ± 4.3	40.0 ± 4.8	30.0 ± 5.8	47.5 ± 2.1
7	50.8 ± 3.3	48.3 ± 1.7	51.7 ± 3.1	60.8 ± 6.5	56.7 ± 3.1	46.7 ± 5.4	43.3 ± 3.8	34.2 ± 8.0
8	41.7 ± 3.3	40.8 ± 2.0	31.7 ± 4.8	51.7 ± 5.4	40.0 ± 5.5	34.2 ± 5.2	32.5 ± 3.8	40.0 ± 2.2
9	35.0 ± 2.6	42.5 ± 3.1	46.7 ± 4.2	43.3 ± 2.1	36.7 ± 3.6	32.5 ± 4.4	41.0 ± 6.6	36.7 ± 2.5
10	36.7 ± 3.1	39.2 ± 5.1	41.7 ± 3.8	36.7 ± 4.0	38.3 ± 3.8	38.3 ± 4.2	32.0 ± 2.0	36.7 ± 3.6
11	40.0 ± 2.9	40.8 ± 3.5	42.5 ± 3.1	48.3 ± 3.1	43.3 ± 5.1	35.0 ± 3.2	35.0 ± 4.5	37.5 ± 2.1
12	40.0 ± 1.3	39.2 ± 3.3	40.0 ± 4.5	39.2 ± 4.0	35.8 ± 3.0	34.2 ± 1.5	39.0 ± 3.7	44.2 ± 2.0
13	40.8 ± 1.5	40.0 ± 2.6	40.8 ± 3.5	40.8 ± 3.5	37.5 ± 2.1	33.1 ± 1.1	37.0 ± 3.4	40.0 ± 2.2

Values are presented as mean ± SEM (n = 5).

The water consumption and food intake of the rats were monitored for 90 days (Tables 1 and 2). There was no change in water consumption for both the male and female rats regardless of the dose of PC used. The body weights of the rats were also monitored for 90 days (Fig. 2). Mean body weights increased slightly on the day following treatment in both the male and female rats, and the final mean body weights in the male rats were higher than that in the female rats. Overall, the changes in body weight among groups within both the male and female rats were not statistically significant.

Hematological and serum biochemical parameters

The hematological profiles of the control and treated groups are presented in Table 3. The repeated oral treatment for 90 days did not cause significant changes in all of the hematological parameters. Table 4 showed the

Table 3. Effect of the plantamajoside concentrate (PC) by oral route on hematological parameters in SD rats treated for 90 consecutive days

	Male (mg/kg)				Female (mg/kg)			
Parameter	0	500	1000	2000	0	500	1000	2000
WBC (10^3/mm^3)	6.0 ± 0.4	5.5 ± 1.0	6.0 ± 0.9	6.2 ± 0.7	3.3 ± 0.3	3.2 ± 0.3	3.0 ± 0.6	3.0 ± 0.4
RBC (10^6/mm^3)	9.1 ± 0.2	9.0 ± 0.1	9.3 ± 0.3	8.9 ± 0.2	8.5 ± 0.1	8.7 ± 0.2	8.6 ± 0.42	8.6 ± 0.3
Hb (g/dL)	17.3 ± 0.2	16.5 ± 0.1	16.7 ± 0.3	16.0 ± 0.2	16.4 ± 0.3	16.7 ± 0.4	16.3 ± 0.3	16.7 ± 0.7
HCt (%)	52.5 ± 0.8	52.3 ± 0.3	53.2 ± 1.2	50.7 ± 0.8	49.0 ± 0.6	51.6 ± 1.1	50.6 ± 0.8	51.9 ± 2.4
MCV (μ^3)	57.7 ± 0.6	58.2 ± 0.4	57.3 ± 0.5	56.8 ± 1.3	57.6 ± 0.8	59.6 ± 0.6	59.2 ± 0.5	57.8 ± 0.9
MCH (pg)	19.2 ± 0.3	18.4 ± 0.1	18.0 ± 0.2	17.8 ± 0.5	19.2 ± 0.3	19.3 ± 0.2	19.1 ± 0.2	18.6 ± 0.3
MCHC (g/dL)	33.3 ± 0.5	31.5 ± 0.2	31.4 ± 0.2	31.4 ± 0.2	33.4 ± 0.3	32.4 ± 0.2	32.3 ± 0.1	32.1 ± 0.2
PLT (10^3/mm^3)	937.4 ± 35.4	884.4 ± 20.0	860.0 ± 34.6	959.3 ± 11.9	886.0 ± 28.1	854.7 ± 27.6	919.8 ± 17.2	912.0 ± 38.4

Values are presented the mean ± SEM (n = 5).

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Table 4. Effect of the PC by oral route on serum biochemical parameters in SD rats treated for 90 consecutive days

	Male (mg/kg)				Female (mg/kg)			
Parameter	0	500	1000	2000	0	500	1000	2000
TP (g/dL)	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.2	6.1 ± 0.1	6.1 ± 0.1	6.5 ± 0.2	6.2 ± 0.1	6.7 ± 0.2
ALB (g/dL)	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.8 ± 0.0	3.9 ± 0.1	3.9 ± 0.0	4.2 ± 0.1
GLU (g/dL)	190.7 ± 13.3	183.0 ± 9.9	187.2 ± 5.1	184.6 ± 8.9	145.4 ± 15.8	143.8 ± 10.1	141.8 ± 11.6	147.3 ± 14.5
CHOL (mg/dL)	61.7 ± 5.1	61.5 ± 1.7	60.2 ± 5.3	62.6 ± 9.1	52.5 ± 2.0	57.0 ± 4.5	58.8 ± 2.8	55.0 ± 1.7
TBIL (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
GPT (IU/L)	31.3 ± 2.7	27.5 ± 1.5	26.8 ± 2.0	26.2 ± 2.8	25.7 ± 2.1	22.5 ± 1.5	25.0 ± 2.2	26.0 ± 0.6
GOT (IU/L)	106.3 ± 11.7	96.5 ± 14.9	97.8 ± 8.2	93.2 ± 9.3	110.1 ± 7.3	111.3 ± 11.0	115.5 ± 7.7	109.0 ± 3.8
ALP (IU/L)	76.7 ± 3.3	78.0 ± 4.5	78.8 ± 7.9	72.8 ± 4.5	60.0 ± 8.1	55.2 ± 4.3	56.6 ± 6.7	58.3 ± 5.7

Values are presented the mean ± SEM (n = 5).

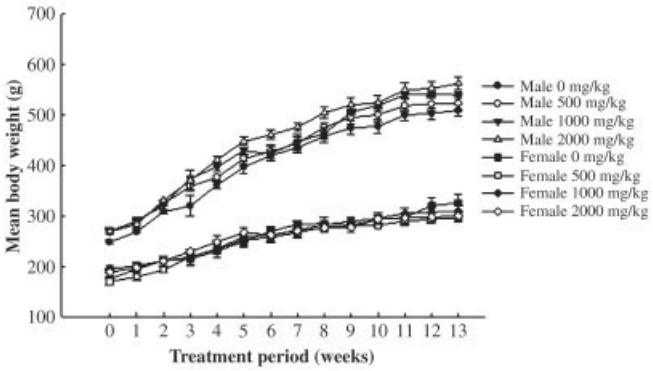

Figure 2. Change in body weight of SD rats during treatment with PC for 90 days. Values are presented the mean ± SEM (n = 5).

serum biochemical results of the 90 days chronic toxicity study. There was no difference in the serum biochemical parameters among the control and treated groups, regardless of the dose of PC used.

Gross pathological finding

At the end of the observation period all rats were killed and autopsied. All major organs including thymus, lung, heart, kidney, liver, spleen and testis or ovary were examined grossly. There were no abnormal lesions in either male and female rats, regardless of the PC dose used (Table 5). Small pieces of liver and kidneys were fixed in 10% neutral buffered formalin, and cut for observation under a light microscope (Figs 3, 4). The histological observations of liver and kidney were no different compared with the control group in both male and female rats, regardless of the dose of PC used.

DISCUSSION

The analysis of the results obtained for the subchronic phase, following treatments of orally administered PC at doses of 500, 1000 and 2000 mg/kg, showed no significant difference compared with the control groups for hematological, biochemical, morphological and histological data.

Studies on the toxicity of the Plantaginaceae family are scarce. In one study, the oral administration of an aqueous extract of *Plantago major*, which is closely related to *P. asiatica*, in a daily dose of 2000 mg/kg for a total of 40 days, did not result in any mortality (Garcia *et al.*, 2003). That study, however, did not perform clinical toxicological evaluations of chemical, hematological and biochemical blood parameters, but did report minor changes in behavior such as diminutions in the reflection of alignment and reaction alarm. Findings on the genotoxicity of *P. major* extract are not consistent. For example, a water extract of *P. major* showed recombinagenic toxicity in somatic cells of *Drosophila melanogaster* (Pimenta and Nepomuceno, 2005) in contrast, an alcohol extract had no toxicity on *Aspergillus nidulans* D-30 (Samuelsen, 2000). In addition, *P. major* extract was negative for mutagenicity in *S. typhimurium* TA100 and TA98 strains (Basaran *et al.*, 1996), and using a reversion assay it was found that PC did not

Table 5. Effect of the PC by oral route on relative organ weight in SD rats treated for 90 consecutive days

	Male (mg/kg)				Female (mg/kg)			
Parameter	0	500	1000	2000	0	500	1000	2000
Body weight (g)	509.0 ± 11.5	523.0 ± 15.4	539.2 ± 5.8	562.2 ± 12.2	325.8 ± 16.8	296.0 ± 7.9	309.2 ± 9.6	300.2 ± 9.0
Thymus (%) (g/BP)	0.15 ± 0.021	0.12 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	0.13 ± 0.02
Lung (%) (g/BP)	0.40 ± 0.01	0.41 ± 0.01	0.40 ± 0.01	0.41 ± 0.03	0.47 ± 0.01	0.56 ± 0.01	0.51 ± 0.01	0.51 ± 0.01
Heart (%) (g/BP)	0.31 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.36 ± 0.02	0.33 ± 0.01	0.32 ± 0.01
Kidney (%) (g/BP)	0.76 ± 0.04	0.71 ± 0.01	0.66 ± 0.02	0.69 ± 0.04	0.67 ± 0.02	0.72 ± 0.02	0.66 ± 0.02	0.64 ± 0.02
Liver (%) (g/BP)	2.57 ± 0.06	2.70 ± 0.12	2.41 ± 0.04	2.48 ± 0.19	2.16 ± 0.12	2.44 ± 0.08	2.35 ± 0.04	2.39 ± 0.06
Spleen (%) (g/BP)	0.17 ± 0.01	0.15 ± 0.01	0.15 ± 0.00	0.16 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.17 ± 0.01
Testis (%) (g/BP)	0.77 ± 0.03	0.76 ± 0.01	0.74 ± 0.02	0.74 ± 0.02	–	–	–	–
Ovary (%) (g/BP)	–	–	–	–	0.08 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.00

Values are presented the mean ± SEM (n = 5).

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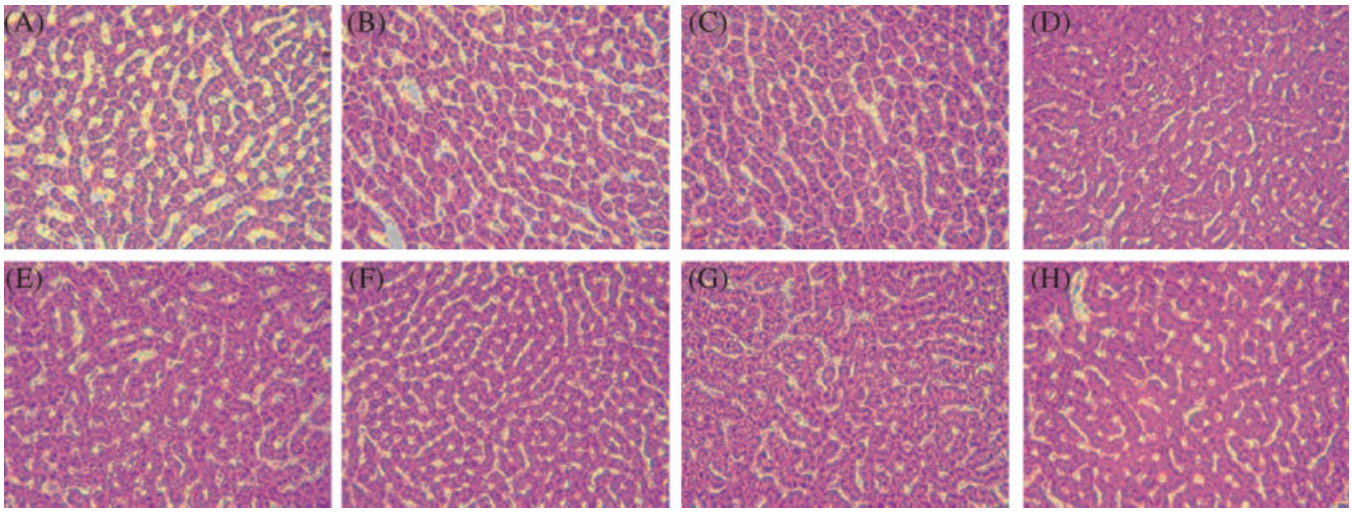

Figure 3. Representative microscopic findings of the liver of SD rats treated orally with the PC for 90 days. Male (A) 0 mg/kg (B) 500 mg/kg (C) 1000 mg/kg, (D) 2000 mg/kg, female (E) 0 mg/kg, (F) 500 mg/kg (G) 1000 mg/kg, and (H) 2000 mg/kg (hematoxylin-eosin stain, × 200).

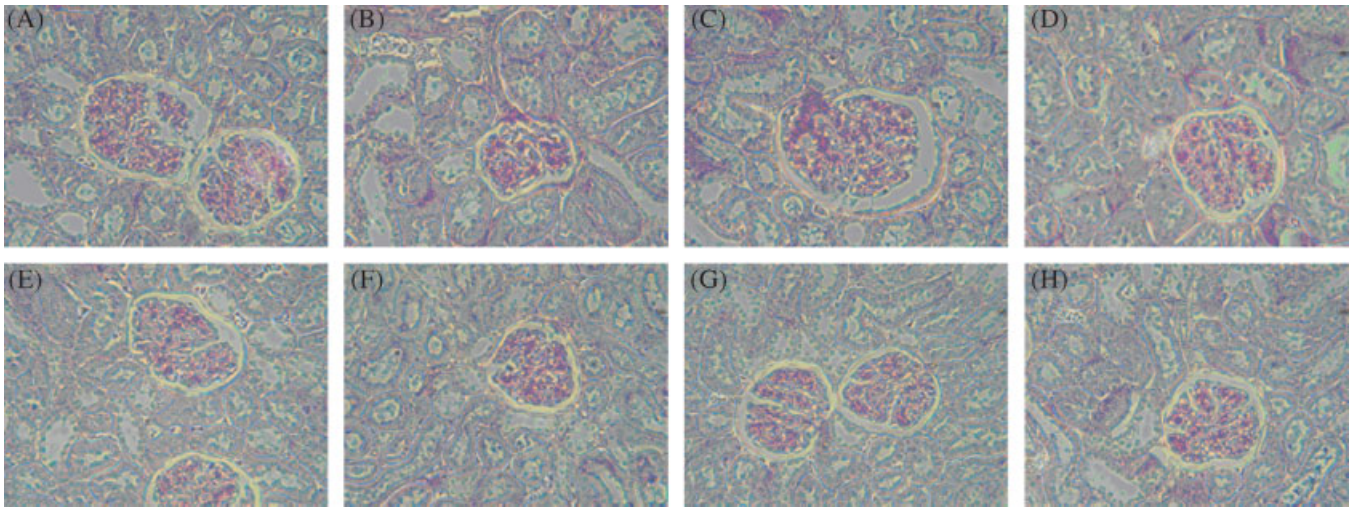

Figure 4. Representative microscopic findings of the kidney of SD rats treated orally with the PC for 90 days. Male (A) 0 mg/kg (B) 500 mg/kg (C) 1000 mg/kg, (D) 2000 mg/kg, female (E) 0 mg/kg, (F) 500 mg/kg (G) 1000 mg/kg, and (H) 2000 mg/kg (periodic acid schiff stain, × 200).

induce mutagenicity in *S. typhimurium* TA98, TA100 and TA1535 (unpublished data). It has been suggested that some components of *P. major*, such as oxalic acid, nitrates, and erucic acid, are genotoxic (Pimenta and Nepomuceno, 2005). However, in our study, these agents are expected to have been removed during the preparation of PC.

No mortality or abnormal signs in behavior, breathing, cutaneous effects, sensory nervous system responses, or gastrointestinal effects were found in the rats treated with PC at a 2000 mg/kg dose, which was the highest dose that could be prepared for oral administration. According to these results, the no observed adverse effect level (NOAEL) of PC was greater than 2000 mg/kg. Some plants used in traditional medicine are shown to produce secondary metabolites, or phytochemicals, having beneficial health effects. Although many natural plant extracts used traditionally have passed the test of time in terms of toxicity or adverse effects, the safety of the active phytochemicals from these plants must precede their pharmaceutical use. In our study, no chronic toxicity was observed for plantamajoside concentrate even at a dose of 2000 mg/kg intragastrically. Therefore, plantamajoside may be a candidate phytochemical agent for health products. However, further studies such as cytogenetic experiments that include reversion, chromosomal aberration, and micronucleus assays are necessary to confirm this evidence.

Acknowledgements

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