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The Toxicity and Anti-cancer Activity of the Hexane Layer of *Melia azedarach* L. var. *japonica* Makino's Bark Extract

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In this study, the 4-week oral toxicity and anti-cancer activity of the hexane layer of *Melia azedarach* L. var. japonica Makino's bark extract were investigated. We carried out a hollow fiber (HF) assay and 28-day repeated toxicity study to confirm the anti-cancer effect and safety of the hexane layer. The HF assay was carried out using an A549 human adenocarcinoma cell via intraperitoneal (IP) site with or without cisplatin. In the result, the 200 mg/kg b.w of hexane layer with 4 mg/kg b.w of cisplatin treated group, showed the highest cytotoxicity aginst A549 carcinoma cells. For the 28-day repeated toxicity study, 6 groups of 10 male and female mice were given by gavage 200, 100, or 50 mg/kg b.w hexane layer with or without 4 mg/kg b.w of cisplatin against body weight, and were then sacrificed for blood and tissue sampling. The subacute oral toxicity study in mice with doses of 200, 100, and 50 mg/kg b.w hexane layer showed no significant changes in body weight gain and general behavior. The cisplatin-treated group significantly decreased in body weight compared to the control group but regained weight with 100 and 200 mg/kg b.w of hexane layer. The biochemical analysis showed significant increase in several parameters (ALT, total billirubin, AST, creatinine, and BUN) in cisplatin-treated groups. However, in the group given a co-treatment of hexane layer (200 mg/kg b.w), levels of these parameters decreased. In hematological analysis, cisplatin induced the reduction of WBCs and neutrophils but co-treatment with hexane layer (100 and 200 mg/kg b.w) improved these toxicities caused by cisplatin. The histological profile of the livers showed eosinophilic cell foci in central vein and portal triad in cisplatin treated mice. These results show that hexane layer might have an anti-cancer activity and could improve the toxicity of cisplatin.

***Key words*:** *Melia azedarach* L. var. *japonica* Makino, Hollow fiber (HF) assay, Cisplatin, Toxicity

INTRODUCTION

Several adult diseases including cancer are increasing because of change of dietary life and extension of life span. This activates the development of therapeutics (Baguley and Nash, 1981; Boyd, 1989; Geran *et al.*, 1977; Thayer *et al.*, 1971). Among them, cancer is the disease that causes high mortality worldwide second only to cardiovascular disease. It also takes up high mortality in Korea. For this reason, the screening of anti-cancer agents in foods and the investigation of cancer-causing materials are ongoing (Miyazaki and Nishijima, 1981). It has been reported that 200 kinds of medicinal herbs are prescribed to cancer patients in Korea (Cha, 1977; Hong, 1972). Also, it has been reported that several medicinal herbs (Hwang *et al.*, 1982), such as garlic (Son and Hwang, 1990), ginseng (Hwang and Oh, 1984), and balloon flower, have anti-cancer activity.

The stem or rhizome bark of *M. azedarach* may be found in Korea, China, and Japan. It has been reported that beta-dihydroagarofuran isolated from the bark of *M. azedarach* has anti-parasitic and pesticidal acitivities (Cespedes *et al.*, 2001). Moreover, the anti-malaria activity of hot-water and ethanol extracts from the bark of *M. azedarach*, the contraception activity of the extracts from the bark of *M. azedarach* in SD rats (Keshri *et al.*, 2003), and the extracts' antibacterial effect (Khan *et al.*, 2001) and anti-cancer activity (Itokawa *et al.*, 1995; Takeya *et al.*, 1996a, b) have been reported. Also, it has been known that the ethanol extracts from the bark of *M. azedarach* suppress the induction of iNOS. The beta-carboline alkaloid, in particular, suppresses the production of iNOS in Raw 264.7 cell line leaving an anti-inflammatory effect (Kwon *et al.*, 1999; Lee *et al.*, 2000). In addition, the bark of *M. azedarach* is used for treatment of taeniacide and malaria. In terms of anti-cancer activity, Takeya *et al*. (1996a, b) reported that trichilin-type and azadirachtin-type limonoid have a cytotoxicity in HeLa

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and P388 cell lines.

The antitumor drug cisplatin is used as a first-line chemotherapeutic modality in the treatment of epithelial malignancies, including lung, ovarian, testicular, and cervix cancer, among others (Gonzalez *et al.*, 2001). The principal mechanism of cisplatin-induced damage to tumors involves the interaction with DNA and activation of the mitogen-activated protein kinase (MAPK) signaling a pathway, which controls a wide spectrum of cellular processes including growth, differentiation, and apoptosis (Losa *et al.*, 2003). However, the efficiency of chemotherapy is so far from satisfactory due to its side effects and to the resistance of tumor cells.

In the previous study, we investigated the anti-cancer activity of the bark of *M. azedarach* through an MTT and HF assay in the HCT-15, A549, and SK-Hep1 cell lines (Kim and Kang, 2009). In this study, we investigated the anti-cancer activity and the improvement of toxicity caused by cisplatin with co-treatment of the hexane layer from the bark of *M. azedarach* using an HF assay and a 28-day repeated toxicity study.

MATERIALS AND METHODS

Cell culture and hollow fiber (HF) assay. A549 cells (purchased from ATCC; CCL-185) were grown at 37°C, 95% relative humidity, and 5% CO_2 atmosphere in a 75-cm^2 culture flask. Polyvinylidene fluoride (PVDF) HFs with a 1-mm internal diameter and a molecular weight cutoff point of 500 kDa (Spectrum Laboratories, Houston, TX, USA) were used (Itokawa *et al.*, 1995). Sterile HFs were flushed with normal growth media before being loaded with A549 cells at a density of 5×10^5 cells/m*l*. Each fiber was heat-sealed with preheated forceps every 1.5 cm along its length and cut into segments with 2-mm tails for ease of handling; at the highest seeding density, each was at 1.5-cm.

The HF segment contained approximately 10^5 cells. Loaded HFs were maintained under normal growth conditions *in vitro* for 24 hours before implantation. For *in vivo* implantation of HF, 6 week male nude mice were used. All animals were housed under 12 hourly light-dark cycles in an air-conditioned room and had unrestricted access to water *ad libitum* and food (Purina 5001 Rodent Chow; Purina, St. Louis, MO, USA). Mice were anesthetized by Zoletil and Rompun. HFs were implanted at i.p and incisions were closed using skin staples. After 2 days, 6 groups of 4 male mice were given by gavage 200, 100, or 50 mg/kg b.w hexane layer with or without 4 mg/kg b.w of cisplatin against body weight for 7 days. To analyze viable cell numbers within the HFs, the HFs were transferred to 0.5 m*l* of EDTA, cut in half (longitudinally) using a scalpel, and washed in EDTA solution for 3 minutes. The HFs were then washed in 0.5 m*l* of trypsin for 5 minutes, then washed again with media for 3 minutes. All washes were collected and pooled, and cells were harvested by centrifugation (500 g for 5 minutes). Numbers of viable cells were determined using a trypan blue exclusion assay.

Animals. Male and female ICR mice (20~25 g) were purchased from SLC Laboratories (Japan). The mice were housed in an animal facility and were kept on a 12-hr light/dark cycle at a temperature of 22 ± 2°C. Food (Purina 5001 Rodent Chow; Purina, St. Louis, MO, USA) and water were available *ad libitum*. Body weight and health conditions were monitored during acclimation. Then, the rats were randomly assigned to dosage groups. Groups of 10 mice were housed together in stainless steel cages in negative flow cage racks in a biohazards suite. This study was conducted in accordance with the Code of Ethics for Animal Experimentation of Semyung University. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Chemicals, extracts and dosing. The hexane layer of *M. azedarach* was fractionated from 80% EtOH crude extract. Dried bark of *M. azedrach* (2.0 kg; collected in Jeju, august, 2010) were extracted with 80% EtOH three times at room temperature. The resulting EtOH extract was subjected to successive solvent partitioning to produce n-hexane (22.6 g) and H2O (1.8 kg). The chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and 0.5% of carboxymethyl cellulose (CMC) was used as a vehicle. Appropriate quantities of hexane layer were dissolved in a sufficient vehicle on the day of oral administration. For the 28-day repeated toxicity study, each hexane layer with or without cisplatin dosage level was as follows: 50, 100, and 200 mg/kg b.w with or without cisplatin (4 mg/kg b.w). The control group received 0.5 m*l* of 0.5% of CMC. The solutions were administered as a bolus by gavage, using a curved, ball-tipped intubation needle affixed to a syringe. Cisplatin was injected intravenously every other day for 6 days and re-injected every other day for 6 days again 2 weeks from first injection.

28-day repeated toxicity study protocols. In the 28-day repeated toxicity study, 8 dosage levels of hexane layer (0, 50, 100, and 200 mg/kg b.w; 0, 50, 100, and 200 mg/kg b.w with cisplatin) were given daily by gavage to groups of 10 male and 10 female rats for 28 days.

Clinical observation, body weight, food intake, and water consumption. All animals were examined at least once a day after 1 to 4 hrs of administration for death and toxic symptoms during the experiment period of 28 days. Body weight, food intake, and water consumption of each animal were measured weekly.

Hematology. Hematological examinations were carried out on all animals after 28 days of treatment. Blood sam-

ples were collected from infraorbital plexus after 12 hrs of fasting. The following parameters were analyzed using a HEMAVET950 analyzer (Drew, USA): white blood cell (WBC) density, red blood cell (RBC) density, hemoglobin (Hb) density, hematocrit (HCT) percentage, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) density.

Clinical chemistry. At sacrifice, blood was collected via infraorbital plexus. Serum was prepared and stored at -70°C prior to analyses. Standard serum analyzer procedures were used to quantify the total protein (TPROT), albumin (ALB), total bilirubin (TBILI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol (CHOL), glucose (GLU), creatinine (CREAT), blood urea nitrogen (BUN), calcium (Ca), and phosphorus (P) levels in serum.

Necropsy and organ weight measurement. After 28 days of treatment, an entire necropsy was performed for all animals and the gross findings were recorded. The abdominal cavity of each animal was incised and the heart, liver, spleen, left/right kidney, large intestine, small intestine, left/right testis, left/right ovary, prostate, uterus, urinary bladder, stomach, lung, and brain were excised and weighed. Small pieces of tissue were placed into neutral buffered formalin for fixation.

Histopathology. After sacrifice, pieces of all excised tissues were individually placed into neutral buffered formalin for histological examination. The tissue specimens were routinely processed into paraffin; 5-μm thick sections were stained with hematoxylin and eosin (H&E). The slides were coded and examined in a single-blind fashion.

Statistical analyses. The statistical significance of hexane layer and cisplatin-induced changes in toxicity was assessed by a one-way analysis of variance. The significance of the apparent differences was evaluated by the Student-*t* test. The minimum level of significance selected for these tests was $p < 0.05$.

RESULTS

HF assay. All groups except the 50 mg/kg b.w of hexane layer treated group had significantly decreased cell viability compared to the control group. The 200 mg/kg b.w of hexane layer with cisplatin treated group showed significantly decreased cell viability compared to the cisplatin treated group (Fig. 1).

28-day repeated toxicity study. All treated groups did not show morbidity or abnormal clinical signs caused by

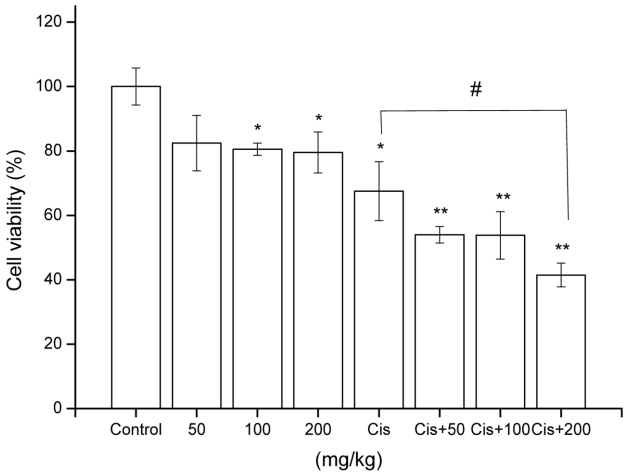

Fig. 1. Inhibitory effect on cell proliferation by hexane layer and cisplatin in A549 using HF assay. Data represented Mean ± SE, * significantly different from control group ($p < 0.05$); ** significantly different from control group ($p < 0.01$); # significantly different from cisplatin only ($p < 0.05$).

Table 1. Body weight change of male rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	0 weeks	1 week	2 weeks	3 weeks	4 weeks
Control	26.1 ± 2.60[a]	29.4 ± 2.55	31.4 ± 2.76	33.2 ± 2.70	35.1 ± 2.88
50	26.0 ± 3.50	29.4 ± 4.22	31.9 ± 3.90	33.8 ± 3.36	36.0 ± 3.74
100	26.3 ± 4.03	28.9 ± 3.63	31.9 ± 4.56	33.8 ± 4.66	36.3 ± 4.60
200	26.0 ± 4.99	28.9 ± 2.28	32.5 ± 3.95	33.8 ± 4.96	36.6 ± 3.86
Cisplatin[b]	25.9 ± 4.31	27.0 ± 2.45	30.5 ± 2.37	31.4 ± 3.44	32.6 ± 1.65[*]
Cisplatin + 50	25.8 ± 4.21	28.1 ± 4.98	28.2 ± 4.24	31.3 ± 2.83	31.9 ± 2.81[*]
Cisplatin + 100	25.0 ± 3.37	26.5 ± 3.63	30.6 ± 4.53	32.0 ± 3.89	33.4 ± 4.53
Cisplatin + 200	26.5 ± 3.92	28.9 ± 4.93	29.5 ± 3.60	36.0 ± 4.19[#]	36.9 ± 4.86[#]

[a]Values expressed are mean ± standard deviation, n = 10. [b]Cisplatin (4 mg/kg b.w).
[*]Significantly different from the control at $p < 0.05$.
[#]Significantly different from the cisplatin only at $p < 0.05$.

Table 2. Body weight change of female rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	0 weeks	1 week	2 weeks	3 weeks	4 weeks
Control	23.9 ± 2.33[a]	26.1 ± 2.60	28.3 ± 3.09	30.2 ± 2.74	31.3 ± 2.31
50	23.7 ± 3.43	27.6 ± 2.63	29.5 ± 3.34	31.3 ± 2.41	31.8 ± 2.39
100	23.9 ± 4.53	25.4 ± 3.13	27.7 ± 3.47	31.9 ± 3.35	32.9 ± 2.60
200	24.3 ± 4.79	25.4 ± 2.55	27.6 ± 3.06	30.2 ± 2.86	32.6 ± 3.06
Cisplatin	24.7 ± 3.89	25.3 ± 3.13	25.7 ± 1.49*	27.3 ± 2.95*	28.2 ± 1.75**
Cisplatin + 50	24.3 ± 3.47	25.0 ± 4.94	25.1 ± 3.21*	26.8 ± 3.85*	28.9 ± 4.95
Cisplatin + 100	24.9 ± 2.18	25.7 ± 4.81	25.8 ± 2.90	28.38 ± 4.32	28.6 ± 3.47
Cisplatin + 200	24.0 ± 1.94	24.5 ± 2.80	27.0 ± 4.29	28.1 ± 5.13	28.2 ± 4.02

[a]Values expressed are mean ± standard deviation, n = 10.
*Significantly different from the control at $p < 0.05$.
**Significantly different from the control at $p < 0.01$

Table 3. Food consumption of the male mice treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	1 week	2 weeks	3 weeks	4 weeks
Control	5.0 ± 0.21[a]	5.1 ± 0.17	5.2 ± 0.25	5.7 ± 0.10
50	6.0 ± 0.76	5.8 ± 0.42	5.7 ± 0.44	6.5 ± 0.35
100	7.9 ± 0.70	5.7 ± 0.27	5.4 ± 0.62	6.5 ± 0.64
200	7.5 ± 1.25	7.4 ± 0.49	5.9 ± 0.95	7.1 ± 0.95
Cisplatin	8.1 ± 1.57	5.9 ± 0.67	6.7 ± 1.27	6.3 ± 1.31
Cisplatin + 50	7.7 ± 2.05	7.5 ± 0.78	5.9 ± 1.69	5.8 ± 1.00
Cisplatin + 100	8.1 ± 1.40	6.5 ± 0.25	6.0 ± 0.87	6.6 ± 1.31
Cisplatin + 200	8.5 ± 0.90	6.5 ± 0.40	7.2 ± 0.80	6.1±1.00

[a]Values expressed are mean ± S.D., n = 10.

Table 4. Food consumption of the female mice treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	1 week	2 weeks	3 weeks	4 weeks
Control	4.5 ± 0.44[a]	4.5 ± 0.20	4.9 ± 0.15	5.0 ± 0.15
50	3.5 ± 0.21	4.7 ± 0.40	3.9 ± 0.17	5.6 ± 0.45
100	4.6 ± 0.76	4.4 ± 0.40	3.4 ± 0.40	5.2 ± 0.21
200	5.0 ± 0.81	4.9 ± 0.76	4.2 ± 0.87	6.4 ± 0.38
Cisplatin	4.8 ± 1.57	5.3 ± 0.81	5.5 ± 0.81	5.1 ± 0.36
Cisplatin + 50	6.0 ± 1.39	5.1 ± 1.57	7.4 ± 0.61	5.8 ± 0.60
Cisplatin + 100	6.1 ± 0.70	6.3 ± 1.34	8.0 ± 0.64	5.4 ± 0.99
Cisplatin + 200	5.7 ± 1.25	6.4 ± 0.70	7.8 ± 1.04	6.0 ± 1.51

[a]Values expressed are mean ± S.D., n = 10.

Table 5. Biochemical findings of the male mice treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	TPROT (g/d*l*)	ALB (U/*l*)	TBILI (mg/d*l*)	AST (U/*l*)	ALT (U/*l*)	Glu (mg/d*l*)	CREAT (mg/d*l*)	BUN (mg/d*l*)	Ca (mg/d*l*)	P (mg/d*l*)	Na (mEq/*l*)	Cl (mEq/*l*)
Control	8.3 ± 0.69	4.0 ± 0.81	2.5 ± 0.30	27.0 ± 9.26	16.2 ± 5.68	72.6 ± 9.15	1.0 ± 0.28	19.3 ± 6.69	8.4 ± 0.78	3.1 ± 1.30	163.2 ± 50.36	155.1 ± 31.78
50	8.6 ± 0.83	3.9 ± 1.06	2.6 ± 0.27	30.9 ± 6.20	19.7 ± 3.65	76.2 ± 10.06	0.9 ± 0.31	21.3 ± 7.45	8.7 ± 0.48	2.9 ± 0.90	211.5 ± 65.38	174.6 ± 54.18
100	8.9 ± 0.70	4.0 ± 0.82	2.8 ± 0.46	31.7 ± 8.85	19.0 ± 6.30	77.7 ± 12.15	0.9 ±0.32	21.1 ± 5.81	9.0 ± 0.74	3.1 ± 1.13	229.3 ± 85.60	208.1 ± 71.84
200	8.8 ± 0.48	4.3 ± 1.11	2.6 ± 0.51	32.9 ± 7.96	19.9 ± 8.01	79.6 ± 10.67	0.8 ± 0.32	24.4 ± 8.21	9.1 ± 1.04	3.0 ± 1.19	197.9 ± 38.76	212.9 ± 82.51
Cisplatin	8.8 ± 0.55	4.5 ± 1.32	2.6 ± 0.51	37.2 ± 7.68*	23.0 ± 3.50**	79.4 ± 10.88	1.4 ± 0.17**	29.4 ± 7.01**	9.1 ± 0.68	3.4 ± 1.28	228.2 ± 86.14	193.0 ± 55.90
Cisplatin + 50	8.8 ± 0.45	4.8 ± 1.50	2.9 ± 0.61	36.1 ± 4.17*	21.7 ± 5.30*	81.4 ± 12.26	1.4 ± 0.24**	29.2 ± 4.87**	9.0 ± 0.62	3.5 ± 1.23	222.3 ± 74.58	194.1 ± 48.47
Cisplatin + 100	8.8 ± 0.81	4.8 ± 0.82	2.7 ± 0.37	33.4 ± 3.72	21.0 ± 4.98	78.8 ± 5.03	1.2 ± 0.27#	28.6 ± 6.44*	8.9 ± 0.68	3.1 ± 0.76	230.8 ± 96.59	167.1 ± 62.37
Cisplatin + 200	8.8 ± 0.81	4.7 ± 1.08	2.6 ± 0.42	32.2 ± 6.77	20.0 ± 6.77	77.4 ± 6.58	0.8 ± 0.32##	24.0 ± 3.50#	9.0 ± 0.58	3.5 ± 0.64	204.4 ± 36.13	203.9 ± 67.40

*Significantly different from the control at $p < 0.05$.
**Significantly different from the control at $p < 0.01$.
#Significantly different from the cisplatin only at $p < 0.05$.
##Significantly different from the cisplatin only at $p < 0.01$.
TPROT, total protein; ALB, albumin; TBILI, total bilirubin.

hexane layer and cisplatin. The rates of body weight increase show significant differences compared to the control group in cisplatin and the 50 mg/kg b.w of hexane layer with cisplatin treated groups on 4 weeks. The rates increased significantly on the cisplatin treated male group in 200 mg/kg b.w of hexane on 3 and 4 weeks (Table 1). In the female groups, the body weight gain decreased significantly in the 50 mg/kg b.w of hexane with cisplatin group on 2 and 3 weeks (Table 2) compared to the control group in cisplatin treatment. There was no change of food consumption in all

Table 6. Biochemical findings of the female mice treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	TPROT (g/dl)	ALB (U/l)	TBILI (mg/dl)	AST (U/l)	ALT (U/l)	Glu (mg/dl)	CREAT (mg/dl)	BUN (mg/dl)	Ca (mg/dl)	P (mg/dl)	Na (mEq/l)	Cl (mEq/l)
Control	8.7 ± 0.64	5.0 ± 0.67	2.2 ± 0.53	28.0 ± 5.77	15.7 ± 4.69	80.6 ± 7.40	0.9 ± 0.09	11.2 ± 4.56	5.8 ± 0.94	5.3 ± 0.58	172.6 ± 56.06	144.9 ± 29.22
50	8.5 ± 0.58	5.1 ± 0.93	2.5 ± 0.58	31.0 ± 6.90	19.5 ± 3.62	80.9 ± 12.04	0.9 ± 0.10	10.1 ± 4.52	5.7 ± 0.97	5.1 ± 0.63	202.0 ± 54.49	162.1 ± 53.87
100	9.1 ± 0.63	5.5 ± 0.90	2.7 ± 0.82	29.5 ± 7.52	20.3 ± 5.80	83.1 ± 10.29	0.8 ± 0.29	12.8 ± 6.30	6.0 ± 0.70	5.4 ± 0.47	205.0 ± 68.26	183.6 ± 68.15
200	8.9 ± 0.79	5.4 ± 0.78	3.0 ± 1.02	33.8 ± 7.89	19.4 ± 3.65	88.3 ± 11.70	1.0 ± 0.09	15.7 ± 7.92	5.8 ± 0.65	5.7 ± 0.67	201.4 ± 57.00	184.2 ± 74.95
Cisplatin	9.3 ± 1.10	5.7 ± 0.88	2.7 ± 0.55	39.1 ± 4.82**	22.2 ± 4.81*	86.8 ± 6.96	1.3 ± 0.16**	22.0 ± 6.95**	5.8 ± 0.85	5.7 ± 0.53	216.9 ± 37.04	200.2 ± 86.02
Cisplatin + 50	9.4 ± 0.80	5.7 ± 0.78	2.7 ± 0.49	37.7 ± 4.14**	21.1 ± 3.44*	88.5 ± 9.65	1.2 ± 0.36*	21.3 ± 4.52**	6.0 ± 0.65	5.8 ± 0.56	215.2 ± 32.26	172.7 ± 36.12
Cisplatin + 100	9.3 ± 0.50	5.7 ± 0.83	2.7 ± 0.52	35.0 ± 6.60*	20.5 ± 3.67*	86.7 ± 5.97	1.1 ± 0.31	19.7 ± 2.59**	6.0 ± 0.76	5.8 ± 0.46	229.3 ± 80.15	160.3 ± 57.38
Cisplatin + 200	9.3 ± 0.81	5.7 ± 0.76	2.5 ± 0.31	30.5 ± 1.74##	19.6 ± 3.32	85.9 ± 8.24	1.0 ± 0.28	12.5 ± 2.82##	5.8 ± 0.75	5.7 ± 0.21	223.5 ± 72.56	180.8 ± 50.16

*Significantly different from the control at $p < 0.05$.
**Significantly different from the control at $p < 0.01$.
##Significantly different from the cisplatin only at $p < 0.01$.
TPROT, total protein; ALB, albumin; TBILI, total bilirubin.

Table 7. Hematological findings of the male rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	WBC (K/μl)	NE (K/μl)	LY (K/μl)	MO (K/μl)	EO (K/μl)	BA (K/μl)	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)	RBC (M/dl)	MCV (fL)	HCT (%)	PLT (K/μl)
Control	9.2 ± 2.22	2.3 ± 1.39	5.5 ± 1.89	0.8 ± 0.18	0.4 ± 0.10	0.1 ± 0.03	25.1 ± 11.83	59.9 ± 12.48	9.5 ± 2.64	4.9 ± 1.19	0.6 ± 0.31	9.6 ± 4.15	58.1 ± 7.39	54.5 ± 22.04	1037.1 ± 450.34
50	9.3 ± 2.96	2.7 ± 1.49	5.2 ± 2.43	0.9 ± 0.16	0.5 ± 0.08	0.1 ± 0.04	28.4 ± 13.21	55.1 ± 13.04	10.0 ± 3.09	5.9 ± 1.95	0.6 ± 0.43	9.5 ± 4.87	61.4 ± 7.62	58.1 ± 28.41	1246.7 ± 529.16
100	11.6 ± 3.82	3.7 ± 1.73	6.5 ± 3.33	0.9 ± 0.16	0.5 ± 0.04	0.1 ± 0.02	32.4 ± 14.98	54.5 ± 15.95	7.9 ± 2.08	4.8 ± 1.38	0.5 ± 0.22	7.9 ± 3.73	61.4 ± 4.67	48.1 ± 22.35	1044.6 ± 408.27
200	10.6 ± 3.14	4.1 ± 2.82	5.1 ± 2.90	0.9 ± 0.15	0.5 ± 0.06	0.0 ± 0.04	37.5 ± 20.24	47.8 ± 20.33	8.9 ± 3.35	5.3 ± 2.39	0.4 ± 0.30	7.2 ± 3.39	65.7 ± 8.93	45.5 ± 18.44	736.6 ± 347.5
Csplatin	7.1 ± 1.81*	1.2 ± 0.80*	4.7 ± 1.70	0.8 ± 0.16	0.4 ± 0.07	0.0 ± 0.01	16.46 ± 10.16	65.2 ± 12.06	11.8 ± 5.58	5.9 ± 1.93	0.6 ± 0.47	10.0 ± 4.42	63.3 ± 3.98	62.5 ± 26.57	1212.8 ± 544.49
Cisplatin + 50	6.1 ± 2.71*	1.1 ± 0.95*	3.8 ± 2.09	0.8 ± 0.15	0.4 ± 0.03	0.1 ± 0.02	16.1 ± 11.39	58.0 ± 15.72	16.0 ± 9.97	8.3 ± 4.95	1.0 ± 0.67	10.8 ± 2.96	65.2 ± 8.55	70.2 ± 21.98	944.0 ± 368.77
Cisplatin + 100	8.2 ± 3.45	2.5 ± 1.41#	4.4 ± 2.53	0.8 ± 0.15	0.4 ± 0.04	0.0 ± 0.01	29.8 ± 12.84#	52.4 ± 11.42#	11.4 ± 4.55	6.0 ± 2.53	0.4 ± 0.21	10.3 ± 3.72	64.7 ± 6.86	62.48 ± 26.57	1001.0 ± 379.33
Cisplatin + 200	11.2 ± 3.86#	4.3 ± 2.67##	5.5 ± 2.98	0.9 ± 0.19	0.4 ± 0.09	0.0 ± 0.01	36.2 ± 18.32#	49.9 ± 14.61#	8.8 ± 4.98	4.7 ± 2.67	0.4 ± 0.26	8.0 ± 3.43	64.0 ± 5.52	51.5 ± 22.90	1095.8 ± 545.15

*Significantly different from the control at $p < 0.05$.
#Significantly different from the cisplatin only at $p < 0.05$.
##Significantly different from the cisplatin only at $p < 0.01$.
NE, non-esterified; LY, lymphocytes; MO, monocytes; EO, eosinophils; BA, basophils.

Table 8. Hematological findings of the female rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	WBC (K/μl)	NE (K/μl)	LY (K/μl)	MO (K/μl)	EO (K/μl)	BA (K/μl)	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)	RBC (M/dl)	MCV (fL)	HCT (%)	PLT (K/μl)
Control	11.7 ± 3.87	3.9 ± 1.70	6.75 ± 3.29	0.7 ± 0.08	0.4 ± 0.18	0.1 ± 0.05	33.7 ± 11.14	54.8 ± 12.80	7.1 ± 3.05	3.8 ± 2.38	0.7 ± 0.46	9.6 ± 3.37	58.9 ± 8.11	57.0 ± 20.29	1020.3 ± 551.57
50	13.1 ± 5.71	5.0 ± 2.98	6.8 ± 4.25	0.7 ± 0.23	0.4 ± 0.16	0.1 ± 0.04	39.0 ± 13.23	49.0 ± 16.23	6.5 ± 5.28	4.9 ± 5.09	0.7 ± 0.63	10.8 ± 2.80	58.6 ± 7.46	63.2 ± 17.90	1122.0 ± 526.13
100	12.4 ± 3.89	5.1 ± 1.94	6.0 ± 2.99	0.7 ± 0.23	0.5 ± 0.17	0.1 ± 0.06	42.7 ± 12.01	46.5 ± 13.36	5.6 ± 2.20	4.4 ± 2.71	0.9 ± 0.72	7.8 ± 3.40	56.0 ± 6.23	43.5 ± 18.93	1351.5 ± 393.67
200	12.2 ± 3.11	5.2 ± 2.00	5.8 ± 2.37	0.7 ± 0.23	0.5 ± 0.14	0.1 ± 0.05	43.0 ± 15.48	46.3 ± 15.25	5.9 ± 2.52	4.0 ± 1.29	0.8 ± 0.43	8.0 ± 4.45	58.4 ± 6.96	45.6 ± 23.01	1084.8 ± 451.96
Csplatin	6.5 ± 2.56**	1.5 ± 1.37**	3.9 ± 1.71*	0.7 ± 0.22	0.4 ± 0.11	0.1 ± 0.03	20.5 ± 14.21*	59.8 ± 14.95	11.5 ± 8.11	7.2 ± 5.45	1.1 ± 0.52	10.9 ± 4.73	59.1 ± 10.88	62.8 ± 26.38	1056.9 ± 471.25
Cisplatin + 50	7.1 ± 3.47*	1.9 ± 1.35*	4.0 ± 2.36	0.7 ± 0.23	0.4 ± 0.11	0.1 ± 0.04	26.1 ± 11.70	55.6 ± 12.02	10.6 ± 6.11	6.3 ± 2.94	0.6 ± 0.43	9.8 ± 3.81	59.2 ± 8.35	56.5 ± 20.00	1142.3 ± 515.12
Cisplatin + 100	10.1 ± 2.68#	2.4 ± 1.73#	6.6 ± 2.41	0.6 ± 0.24	0.4 ± 0.11	0.0 ± 0.04	23.0 ± 15.37	65.8 ± 15.71	6.8 ± 3.38	3.9 ± 1.56	0.5 ± 0.60	12.0 ± 3.36	61.0 ± 10.12	71.6 ± 17.64	899.61 ± 475.51
Cisplatin + 200	11.1 ± 3.56##	5.1 ± 2.66##	5.0 ± 2.23	0.6 ± 0.23	0.4 ± 0.11	0.1 ± 0.03	44.0 ± 19.54#	45.7 ± 17.18	6.5 ± 4.74	3.4 ± 1.46#	0.5 ± 0.32#	9.6 ± 3.80	63.8 ± 11.73	59.0 ± 19.04	1139.1 ± 491.06

*Significantly different from the control at $p < 0.05$.
**Significantly different from the control at $p < 0.01$.
#Significantly different from the cisplatin only at $p < 0.05$.
##Significantly different from the cisplatin only at $p < 0.01$.
NE, non-esterified; LY, lymphocytes; MO, monocytes; EO, eosinophils; BA, basophils.

groups (Table 3 and 4).

In the serological analysis, the AST, ALT, CREAT, and BUN contents in the cisplatin and 50 mg/kg b.w of hexane layer with cisplatin treated male groups increased significantly compared to the control group. In the 100 mg/kg b.w of hexane layer with cisplatin treated group, the BUN increased significantly compared to the control group. The level of CREAT in 100 mg/kg b.w with cisplatin and the levels of CREAT and BUN decreased significantly compared to the cisplatin treated group (Table 5). There were significant increases in the AST, ALT, CREAT, and BUN levels in cisplatin and 50 mg/kg b.w of hexane layer with cisplatin treated female groups. In the 100 mg/kg b.w of hexane layer with cisplatin treated group, the AST, ALT, and BUN levels increased significantly compared to the control group. The 200 mg/kg b.w of hexane layer with cisplatin treated group showed significant decreases of AST and BUN compared to the cisplatin treated group (Table 6).

Hematological analysis is as follows: the WBC and neutrophil contents in the cisplatin and 50 mg/kg b.w of hexane layer with cisplatin treated male groups decreased significantly compared to the control group. In the 100 mg/kg b.w of hexane layer with cisplatin treated group, neutrophil increased significantly compared to the cisplatin treated group. The contents of WBC and neutrophil in the 200 mg/kg b.w with cisplatin group increased significantly compared to the cisplatin treated group (Table 7). There were significant decreases in the WBC, neutrophil, and lymphocyte in the cisplatin treated group, and WBC and neutorophil in the 50 mg/kg b.w of hexane layer with cisplatin treated female group. In the 100 and 200 mg/kg b.w of hexane layer with cisplatin treated group, WBC and neutrophil contents increased significantly compared to the cisplatin treated group (Table 8). Absolute and relative organ weight did not changed compared to the control group (Table 9, 10, 11 and 12).

In the histopathological analysis, the eosinophilic cell foci present in the early proliferative pathogen in hepatocyte were found in the cisplatin treated group (Fig. 2). No other histological changes were seen in other organs at all hexane layer and cisplatin treatments.

DISCUSSION

In this study, we investigated the anti-cancer activity and the improvement of toxicity caused by cisplatin with the addition of the hexane layer from the bark of *Melia azedarach* L. var. japonica Makino using an HF assay and a 28-day repeated toxicity study.

In the HF assay, the hexane layer decreased cell viability further compared to the cisplatin treated group. This result is consistent with the previous reported study (Itokawa *et al.*, 1995; Takeya *et al.*, 1996a, b).

Our results showed that hexane layer did not cause the toxicity but cisplatin increased the levels of major biomarkers that indicate damage of the liver and kidneys, such as ALT, AST, CREAT, and BUN by serological analysis. Also, cisplatin-induced hematological side effects were improved

Table 9. Absolute organ weights of the male rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	Liver (g)	Lung (g)	Kidney(L) (g)	Kidney(R) (g)	Adrenal(L) (g)	Adrenal(R) (g)	Testis/ Ovary(L) (g)	Testis/ Ovary(R) (g)	Trachea (g)	Brain (g)	Stomach (g)	Spleen (g)	Heart (g)	Prostate gland (g)
Control	1.5 ± 0.42	0.3 ± 0.05	0.4 ± 0.06	0.4 ± 0.07	0.02 ± 0.01	0.0 ± 0.00	0.2 ± 0.05	0.2 ± 0.03	0.0 ± 0.01	0.4 ± 0.06	0.5 ± 0.09	0.2 ± 0.07	0.3 ± 0.09	0.4 ± 0.07
50	2.1 ± 0.68	0.3 ± 0.05	0.5 ± 0.07	0.4 ± 0.04	0.0 ± 0.01	0.0 ± 0.00	0.2 ± 0.04	0.2 ± 0.05	0.0 ± 0.01	0.5 ± 0.04	0.5 ± 0.05	0.2 ± 0.06	0.3 ± 0.11	0.4 ± 0.08
100	2.1 ± 0.77	0.3 ± 0.05	0.4 ± 0.06	0.4 ± 0.09	0.0 ± 0.01	0.0 ± 0.00	0.2 ± 0.04	0.2 ± 0.02	0.0 ± 0.01	0.5 ± 0.04	0.5 ± 0.09	0.2 ± 0.08	0.3 ± 0.11	0.5 ± 0.11
200	2.4 ± 1.01	0.3 ± 0.05	0.5 ± 0.08	0.4 ± 0.10	0.0 ± 0.00	0.0 ± 0.01	0.2 ± 0.04	0.1 ± 0.05	0.0 ± 0.01	0.5 ± 0.06	0.5 ± 0.08	0.2 ± 0.08	0.3 ± 0.11	0.5 ± 0.10
Cisplatin	2.1 ± 0.29	0.3 ± 0.04	0.4 ± 0.09	0.4 ± 0.08	0.0 ± 0.01	0.0 ± 0.00	0.2 ± 0.07	0.1 ± 0.04	0.0 ± 0.01	0.4 ± 0.04	0.5 ± 0.08	0.2 ± 0.06	0.3 ± 0.09	0.4 ± 0.08
Cisplatin + 50	2.2 ± 0.73	0.3 ± 0.04	0.5 ± 0.09	0.4 ± 0.07	0.0 ± 0.01	0.0 ± 0.01	0.2 ± 0.05	0.2 ± 0.03	0.0 ± 0.01	0.5 ± 0.04	0.5 ± 0.08	0.2 ± 0.07	0.3 ± 0.11	0.4 ± 0.10
Cisplatin + 100	2.4 ± 0.91	0.3 ± 0.04	0.5 ± 0.08	0.4 ± 0.10	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.05	0.1 ± 0.03	0.0 ± 0.01	0.5 ± 0.05	0.5 ± 0.09	0.3 ± 0.10	0.3 ± 0.11	0.4 ± 0.10
Cisplatin + 200	2.6 ± 0.95	0.3 ± 0.03	0.5 ± 0.11	0.4 ± 0.11	0.0 ± 0.01	0.0 ± 0.01	0.2 ± 0.04	0.2 ± 0.06	0.0 ± 0.01	0.5 ± 0.06	0.5 ± 0.08	0.3 ± 0.11	0.3 ± 0.11	0.4 ± 0.08

Values expressed are mean ± S.D., n= 10.

Table 10. Relative organ weights of the male rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	Liver (g)	Lung (g)	Kidney(L) (g)	Kidney(R) (g)	Adrenal(L) (g)	Adrenal(R) (g)	Testis/ Ovary(L) (g)	Testis/ Ovary(R) (g)	Trachea (g)	Brain (g)	Stomach (g)	Spleen (g)	Heart (g)	Prostate gland (g)
Control	5.8 ± 1.10	0.8 ± 0.16	1.1 ± 0.20	1.0 ± 0.23	0.1 ± 0.01	0.0 ± 0.01	0.4 ± 0.12	0.4 ± 0.12	0.1 ± 0.02	1.2 ± 0.20	1.3 ± 0.27	0.5 ± 0.19	0.8 ± 0.29	1.1 ± 0.22
50	5.8 ±1.98	0.9 ± 0.20	1.3 ± 0.28	1.1 ± 0.19	0.0 ± 0.02	0.0 ± 0.01	0.5 ± 0.12	0.4 ± 0.17	0.1 ± 0.04	1.3 ± 0.18	1.4 ± 0.21	0.5 ± 0.20	0.9 ± 0.30	1.2 ± 0.25
100	5.9 ± 2.65	0.8 ± 0.18	1.2 ± 0.26	1.2 ± 0.36	0.1 ± 0.02	0.0 ± 0.01	0.5 ± 0.15	0.4 ± 0.10	0.1 ± 0.04	1.3 ± 0.26	1.5 ± 0.35	0.7 ± 0.27	0.8 ± 0.28	1.3 ± 0.39
200	6.6 ± 3.27	0.9 ± 0.19	1.3 ± 0.26	1.2 ± 0.37	0.1 ± 0.01	0.0 ± 0.02	0.4 ± 0.13	0.4 ± 0.16	0.1 ± 0.04	1.4 ± 0.25	1.5 ± 0.30	0.7 ± 0.24	0.8 ± 0.27	1.3 ± 0.36
Cisplatin	6.5 ± 0.98	0.9 ± 0.12	1.3 ± 0.28	1.2 ± 0.29	0.1 ± 0.02	0.0 ± 0.01	0.5 ± 0.21	0.4 ± 0.14	0.1 ± 0.02	1.4 ± 0.16	1.5 ± 0.27	0.6 ± 0.20	0.9 ± 0.27	1.2 ± 0.26
Cisplatin + 50	6.9 ± 2.33	0.9 ± 0.13	1.3 ± 0.22	1.2 ± 0.16	0.1 ± 0.02	0.1 ± 0.02	0.5 ± 0.16	0.5 ± 0.11	0.1 ± 0.03	1.4 ± 0.12	1.5 ± 0.23	0.7 ± 0.20	1.0 ± 0.31	1.3 ± 0.26
Cisplatin + 100	7.1 ± 2.47	0.9 ± 0.22	1.3 ± 0.26	1.2 ± 0.22	0.0 ± 0.02	0.0 ± 0.02	0.5 ± 0.12	0.4 ± 0.09	0.1 ± 0.05	1.4 ± 0.28	1.6 ± 0.36	0.7 ± 0.29	0.9 ± 0.40	1.2 ± 0.18
Cisplatin + 200	7.1 ± 2.82	0.9 ± 0.14	1.2 ± 0.19	1.2 ± 0.32	0.0 ± 0.01	0.0 ± 0.01	0.5 ± 0.14	0.4 ± 0.18	0.1 ± 0.03	1.3 ± 0.20	1.4 ± 0.31	0.7 ± 0.31	0.9 ± 0.36	1.2 ± 0.28

Values expressed are mean ± S.D., n = 10.

Table 11. Absolute organ weights of the female rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	Liver (g)	Lung (g)	Kidney(L) (g)	Kidney(R) (g)	Adrenal(L) (g)	Adrenal(R) (g)	Testis/ Ovary(L) (g)	Testis/ Ovary(R) (g)	Trachea (g)	Brain (g)	Stomach (g)	Spleen (g)	Heart (g)	Uterus (g)
Control	1.5 ± 0.32	0.2 ± 0.10	0.3 ± 0.02	0.2 ± 0.03	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.03	0.4 ± 0.10	0.1 ± 0.04	0.2 ± 0.06	0.1 ± 0.01
50	1.9 ± 0.57	0.3 ± 0.08	0.2 ± 0.02	0.2 ± 0.05	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.05	0.4 ± 0.07	0.2 ± 0.04	0.2 ± 0.08	0.1 ± 0.01
100	1.9 ± 0.48	0.3 ± 0.05	0.2 ± 0.02	0.3 ± 0.07	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.07	0.4 ± 0.11	0.1 ± 0.05	0.3 ± 0.09	0.1 ± 0.01
200	2.0 ± 0.79	0.3 ± 0.09	0.2 ± 0.02	0.2 ± 0.05	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.080	0.4 ± 0.10	0.1 ± 0.04	0.2 ± 0.09	0.1 ± 0.02
Cisplatin	1.7 ± 0.44	0.2 ± 0.08	0.2 ± 0.02	0.2 ± 0.04	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.05	0.4 ± 0.08	0.1 ± 0.04	0.2 ± 0.08	0.1 ± 0.01
Cisplatin + 50	1.9 ± 0.56	0.3 ± 0.09	0.2 ± 0.02	0.2 ± 0.05	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.05	0.4 ± 0.11	0.1 ± 0.05	0.3 ± 0.09	0.1 ± 0.01
Cisplatin + 100	1.9 ± 0.50	0.3 ± 0.07	0.2 ± 0.03	0.3 ± 0.07	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.09	0.4 ± 0.11	0.1 ± 0.06	0.3 ± 0.08	0.1 ± 0.01
Cisplatin + 200	2.0 ± 0.86	0.3 ± 0.11	0.2 ± 0.03	0.3 ± 0.07	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.4 ± 0.11	0.5 ± 0.07	0.1 ± 0.03	0.3 ± 0.10	0.1 ± 0.01

Values expressed are mean ± S.D., n = 10.

Table 12. Relative organ weights of the female rats treated with hexane layer and cisplatin for 4 weeks by PO

Dose (mg/kg b.w)	Liver (g)	Lung (g)	Kidney(L) (g)	Kidney(R) (g)	Adrenal(L) (g)	Adrenal(R) (g)	Testis/ Ovary(L) (g)	Testis/ Ovary(R) (g)	Trachea (g)	Brain (g)	Stomach (g)	Spleen (g)	Heart (g)	Uterus (%)
Control	4.8 ± 0.91	0.7 ± 0.33	0.8 ± 0.07	0.7 ± 0.06	0.0 ± 0.01	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	1.2 ± 0.16	1.2 ± 0.34	0.4 ± 0.13	0.6 ± 0.19	0.4 ± 0.04
50	6.1 ± 1.86	0.8 ± 0.26	0.7 ± 0.08	0.7 ± 0.17	0.1 ± 0.02	0.0 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	1.2 ± 0.20	1.4 ± 0.21	0.5 ± 0.11	0.7 ± 0.24	0.4 ± 0.04
100	5.7 ± 1.59	0.9 ± 0.18	0.7 ± 0.10	0.7 ± 0.21	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	1.2 ± 0.25	1.3 ± 0.34	0.4 ± 0.12	0.8 ± 0.28	0.4 ± 0.03
200	6.1 ± 2.01	0.9 ± 0.25	0.8 ± 0.02	0.8 ± 0.14	0.0 ± 0.01	0.0 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	1.3 ± 0.28	1.4 ± 0.28	0.4 ± 0.15	0.7 ± 0.23	0.4 ± 0.06
Cisplatin	5.9 ± 1.37	0.8 ± 0.30	0.9 ± 0.07	0.7 ± 0.14	0.1 ± 0.02	0.0 ± 0.02	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.03	1.3 ± 0.18	1.5 ± 0.32	0.5 ± 0.12	0.8 ± 0.28	0.4 ± 0.04
Cisplatin + 50	6.1 ± 1.87	1.0 ± 0.37	0.8 ± 0.13	0.7 ± 0.15	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.06	0.1 ± 0.03	0.1 ± 0.03	1.3 ± 0.30	1.5 ± 0.57	0.5 ± 0.20	0.9 ± 0.39	0.4 ± 0.06
Cisplatin + 100	5.8 ± 0.85	1.0 ± 0.23	0.8 ± 0.15	0.8 ± 0.27	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.04	0.1 ± 0.03	0.1 ± 0.03	1.4 ± 0.31	1.5 ± 0.28	0.5 ± 0.23	0.8 ± 0.28	0.4 ± 0.07
Cisplatin + 200	7.1 ± 3.12	1.0 ± 0.23	0.8 ± 0.12	0.8 ± 0.20	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05	1.4 ± 0.35	1.5 ± 0.24	0.5 ± 0.11	0.8 ± 0.21	0.4 ± 0.08

Values expressed are mean ± S.D., n = 10.

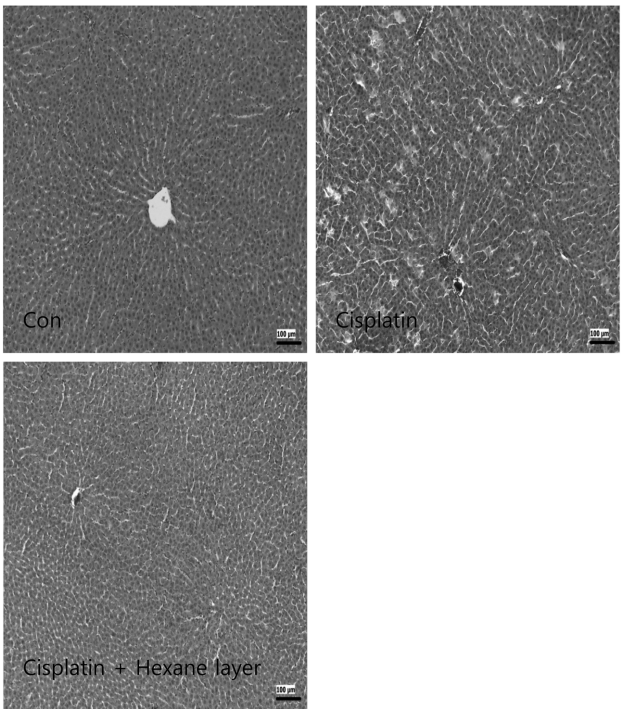

Fig. 2. Histopathological examination of the liver. The eosinophilic foci were found in cisplatin-treated mice (H&E stain, ×200).

by hexane layer administration.

The histopathological analysis showed that hexane layer from bark of *Melia azedarach* L. var. *japonica* Makino improves the hepatotoxicity caused by cisplatin. With these results, it is suggested that hexane layer from the bark of *Melia azedarach* L. var. *japonica* Makino might act as adjuvant chemotherapeutics by having anti-cancer activity and improving cisplatin-induced toxicity. According to previous our study, *M. azedarch* was induced autophagy system, however, further study of autophagy inducing mechanisms of *M. azedarch* for confirmation is needed.

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