# The novel preventive effect of a Japanese ethical Kampo extract formulation TJ-90 (Seihaito) against cisplatin-induced nephrotoxicity

Short title: Preventive Effect of TJ-90 against cisplatin-induced nephrotoxicity

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**Abstract**

Background and purpose: Chinese herbal medicine has been developed as the traditional Japanese Kampo medicine, and it has been widely used to cure various symptoms in clinical practice. However, only a few studies are currently available on the effect of the Kampo medicine on renal disease. Nephrotoxicity is one of major side effect of cisplatin, the first metal-based anticancer drug. In the present study, we examined the effect of the Kampo medicine against cisplatin-induced nephrotoxicity (CIN).

Methods: First, we screened the ethical Kampo extract formulation having positive effect against CIN using HK-2 cells. Next, we examined the preventive action of the selected ethical Kampo extract formulation against CIN *in vivo* using a mouse model.

Results: Cisplatin-induced cell death was significantly suppressed by TJ-43 (Rikkunshito) and TJ-90 (Seihaito); however, cisplatin-induced cleaved caspase-3 expression was inhibited only by TJ-90. In *an in vivo* mouse model of cisplatin-induced kidney injury with dysfunction and increased inflammatory cytokine expression, TJ-90 showed amelioration of these damaging effects. Cisplatin-induced apoptosis and superoxide production were inhibited by treatment with TJ-90. The expression of cleaved

caspase-3, 4-hydroxynonenal, and MAPK phosphorylation increased after cisplatin administration, but decreased after the administration of TJ-90. Among 16 crude drug extracts present in *Seihaito, Bamboo* Culm (Chikujo in Japanese) inhibited cisplatin-induced cell death and cleaved caspase-3 expression in HK-2 cells. Moreover, the anti-tumor effect of cisplatin was not affected by TJ-90 co-treatment in cancer cell lines.

Conclusion: TJ-90 might have a novel preventive action against CIN through the suppression of inflammation, apoptosis, and oxidative stress without interfering with the anti-tumor effect of cisplatin. Collectively, these findings might contribute to innovations in supportive care for cancer treatment-related side effects.

**Keywords:** cisplatin, nephrotoxicity, Seihaito, inflammation, oxidative stress, apoptosis

## Introduction

Cisplatin is widely used as an antitumor agent for the treatment of various malignancies in clinical practice. Despite its efficacy, cisplatin use is associated with severe side effects, such as bone marrow suppression, peripheral neuropathy, ototoxicity, anaphylaxis, and particularly nephrotoxicity (cisplatin-induced nephrotoxicity; CIN). CIN has been reported in approximately 25% of cancer patients undergoing cisplatin chemotherapy (Campbell et al., 1983). A recent study also demonstrated that the incidence of CIN was 20 % in a case-noncase study of a pharmacovigilance database (Pierson-Marchandise et al., 2017). CIN develops in pediatric and adult patients (McMahon et al., 2020).

CIN manifests mainly as renal proximal tubule damage due to uptake of cisplatin by the tubular cells (Ciarimboli et al., 2005). Cisplatin-induced renal tubular cell injury and death induce a robust inflammatory response, further exacerbating renal tissue damage. Cisplatin may injure the renal vasculature and cause reduced blood flow and ischemic injury, contributing to the decline in glomerular filtration rate. Collectively, these occurrences account for CIN, triggering acute kidney injury (AKI) (Pabla and Dong, 2008). Moreover, patients with recurrent AKI or long-term renal dysfunction are at a high

risk of developing chronic kidney disease (CKD) (Chawla et al., 2014; Sears and Siskind, 2021). The use of cisplatin is limited in patients with CIN-induced AKI or CKD, which negatively affects cancer treatment.

The molecular mechanisms underlying CIN involve inflammation, apoptosis, oxidative stress, DNA damage, and mitochondrial dysfunction (Ozkok and Edelstein, 2014). Although many studies have attempted to develop preventive methods or agents against CIN for many years, no treatment strategies are currently available for the prevention of CIN; only hydration therapy with the administration of diuretics, such as furosemide or mannitol, is used as a preventive measure (Li et al., 2021; Santoso et al., 2003). As described earlier, the incidence of CIN remained unchanged for three decades.

The Kampo medicine is a traditional Japanese herbal medicine derived from the ancient Chinese medicine, which has been developed into a personalized medicine adapting the health of the Japanese people for many years. It is prescribed as a formula of natural herbs according to symptom-based diagnosis (Fuyuno, 2011). Recently, the ethical Kampo extract formulations has been used for palliative and supportive care of cancer patients with chemotherapy-induced peripheral neuropathy and diarrhea (Yamakawa et al., 2013), surgical stress, or disease-related cachexia (Okumi and Koyama,

2014). For example, Rikkunshito improves nausea, vomiting, and anorexia in patients treated with cisplatin (Ohnishi et al., 2017), which might be attributed to the maintenance of ghrelin receptor expression and ghrelin secretion in the hypothalamus by antagonizing the serotonin receptors (Tominaga et al., 2011; Yakabi et al., 2010a; Yakabi et al., 2010b). Rikkunshito also suppressed renal inflammation in mice with angiotensin II-induced renal injury (Azushima et al., 2019) and body weight loss in mice with unilateral ureter obstruction-induced renal fibrosis (Wakui et al., 2020); however, it failed to mitigate renal fibrosis or renal dysfunction in the above model. Juzentaihotou is also efficacious against general fatigue in patients with cancer (Motoo and Cameron, 2022) and alleviates renal fibrosis and inflammation in mice with adenine-induced CKD (Ito et al., 2022). Thus, the protective effects of the ethical Kampo extract formulations on the kidney remains unclear.

In the present study, we examined whether the ethical Kampo extract formulation can exert a preventive effect against the kidney injury and dysfunction using a CIN model.

## Materials and Methods

The ethical Kampo extract formulations (TJ-41; Hochuekkito, TJ-43; Rikkunshito, TJ-90; Seihaito, and TJ-114; Saireito) and 3D-HPLC-based profiles (Figure S1) were provided by Tsumura & Co. (Tokyo, Japan). Single crude drug extracts were gifted from the INM deposited WAKANYAKU library, Institute of Natural Medicine, University of Toyama. Cisplatin (Landa™) was purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan). The following commercially available antibodies were used: anti-4-hydroxynonenal (4-HNE; MHN-100P, Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd., Shizuoka, Japan), anti-cleaved caspase-3 (Asp175) (9661), anti-caspase-3 (9665), anti-phospho-SAPK/JNK (Thr183/Tyr185) (9251), anti-total SAPK/JNK (9252), anti-phospho-p44/42 MAPK (Extracellular Signal-regulated Kinase 1/2 - ERK1/2) (9101), anti-total p44/42 MAPK (ERK1/2) (9102), anti-phospho-p38 MAPK (4551), anti-total p38 MAPK (9221) (Cell Signaling Technology, Danvers, MA), and anti-β-actin (sc-47778) (Santa Cruz Biotechnology, Inc., Dallas, TX).

*Cell culture*

HK-2 (Human proximal tubule cells) were obtained from the American Type Culture Collection (Virginia, USA) (Hamano et al., 2021). The cells were cultured in Dulbecco's modified Eagle medium containing 10% fetal bovine serum (FBS), and grown to confluence for approximately 24 h; the cells were then incubated with culture medium containing 0.5% FBS for 24 h. Subsequently, the cells were pre-treated with 100 μg/mL of the ethical Kampo extract formulations, single crude drug extracts, or vehicle for 1 h before stimulation with 50 μM cisplatin or vehicle, and examined 24 h later. 3LL cells (mouse Lewis lung cancer) and colon-26 cells (mouse rectal adenocarcinoma) were maintained and sub-cultured in RPMI 1640 containing 10% FBS. Cancer cell lines were also pre-treated with vehicle or the ethical Kampo extract formulation and then treated with cisplatin, as described above. All cancer cell lines were obtained from the Japanese Collection of Research Bioresources Cell Bank. The dose of ethical Kampo extract formulation used in this study was based on previous studies (Ikarashi et al., 2012; Yagi et al., 2020).

*Cell death assay*

Cell death was assessed using the CellTiter 96 $AQ_{ueous}$ Non-Radioactive Cell Proliferation Assay kit (Promega KK, Tokyo, Japan), as previously described (Hamano et al., 2021). In brief, the cells were cultured in the medium with or without 50 μM cisplatin for 24 h after pre-treatment with the ethical Kampo extract formulation or single crude drug extracts for 1 h. Cell death was assessed 1 h after addition of the MTS reagent.

*Mouse model of cisplatin-induced nephrotoxicity*

C57BL/6J male mice, 7–8-weeks-old, weighing 22–25 g, were purchased from Nippon CLEA (Tokyo, Japan), and were randomly divided into the following groups: vehicle-injected group, cisplatin-injected group, and cisplatin-injected and orally administered the ethical Kampo extract formulation group. The mice were injected cisplatin (20 mg/kg) or vehicle intraperitoneally. The ethical Kampo extract formulation dissolved in water or water as a vehicle was administered two days before, one hour before, and one day after cisplatin injection (a total of four times). Mice were administered two doses of the Kampo extract formulation (0.5 g/kg/day or 1.0 g/kg/day). Forty-eight hours after cisplatin injection, the mice were sacrificed and their blood and tissue samples were collected and used for analysis. The experimental protocol and drug

dose were based on previous studies on the ethical Kampo extract formulations (Kamei et al., 2017; Sreedhar et al., 2015) and cisplatin (Hamano et al., 2021). All experimental procedures were performed in accordance with the guidelines of the Animal Research Committee of the Tokushima University Graduate School, and the protocol was approved by the Institutional Review Board of the Tokushima University Graduate School (permit numbers: T30-74 (2018/10/1), T2021-75 (2021/10/13)).

*RNA extraction and mRNA expression*

The methods used for RNA extraction, cDNA synthesis, and quantitative RT-PCR were followed as described by a previous study (Hamano et al., 2021). The primer sets used in this study are listed in Table 1.

*Protein extraction and western blot analysis*

Protein preparation and western blotting were performed as described by a previous study (Hamano et al., 2021). The tissue or cell samples were homogenized or sonicated in a protein lysis buffer containing proteinase and phosphatase inhibitors, and the proteins were extracted. The extracted proteins were boiled for 5 min in Laemmli

sample buffer and used for western blotting. The detected immunoreactive bands were quantified by densitometric analysis using the Fiji software (https://imagej.net/software/fiji/).

*Measurement of plasma creatinine and blood urea nitrogen levels*

Plasma creatinine and blood urea nitrogen (BUN) levels were measured using an enzymatic method and the urease-GLDH method, respectively, as described by a previous study (Hamano et al., 2021).

*Histological analysis*

Renal tubular damage was evaluated as previously described (Hamano et al., 2021). Hematoxylin and eosin (HE)-stained sections were used for scoring tubular injury (tubular necrosis, brush-border loss, cast formation, tubule dilatation, and tubular degeneration) as follows: **0,** normal; **1**, < 25%; **2,** 25–50%; **3,** 50–75%; and **4,** > 75%.

*In situ superoxide detection*

Superoxide production in the kidney was detected using the dihydroethidium (DHE) staining method as described by a previous study (Hamano et al., 2021). Non-fixed frozen kidney sections were incubated with DHE in phosphate-buffered saline (10 μM) in a dark, humidified container at room temperature for 30 min, and then observed under a fluorescence microscope.

*TdT-mediated dUTP nick end labeling (TUNEL) staining*

Renal apoptosis was evaluated using the TUNEL staining (Apoptosis *in situ* Detection Kit; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), followed by counterstaining with methyl green. Semi-quantification of TUNEL-positive cells was performed in ten random fields (Hamano et al., 2021).

*Immunohistochemistry*

Frozen sections were used to detect Pt-(GpG) DNA adducts (Hamano et al., 2021). Briefly, the frozen sections were fixed in 4% paraformaldehyde. After blocking, the tissue sections were incubated with the primary antibodies (1:200) at 4 °C overnight. Antibody distribution was visualized using immunofluorescence (1:100; Alexa Fluor;

Life Technology, Tokyo, Japan). Pt-(GpG) DNA adduct-derived fluorescence signals were normalized to the corresponding DAPI fluorescence signals of the same nucleus, and expressed as arbitrary fluorescence unit (AFU).

**Results**

*Effect of the ethical Kampo extract formulations against cisplatin-induced cell death in renal proximal tubular HK-2 cells*

First, we investigated the effect of the ethical Kampo extract formulations (TJ-41, TJ-43, TJ-90, and TJ-114) against cisplatin-induced cell death *in vitro* using HK-2 cells. Both TJ-43 and TJ-90 suppressed cisplatin-induced cell death in the MTS assay. Next, we examined whether cisplatin-induced cleaved caspase-3 was inhibited by TJ-43 and TJ-90 treatment, and observed that only TJ-90 suppressed cisplatin-induced upregulated expression of cleaved caspase-3 (Figure S2). Collectively, TJ-90 exerted a preventive effect against cisplatin-induced cell death and cleaved caspase-3 upregulation (Figure 1). Further, to determine the preventive effect of TJ-90 against cisplatin-induced renal tubular cell death, we performed an *in vivo* experiment. TJ-90 did not affect cell viability or caspase-3 activation (Figure S3).

*Effects of TJ-90 in a mouse model of cisplatin-induced acute kidney injury*

We examined the preventive effect of TJ-90 against CIN *in vivo* using a mouse model. We assessed the effects of the two doses of TJ-90 on CIN. The mice treated with cisplatin exhibited reduced body weight with no change in kidney weight, regardless of TJ-90 treatment (Table 2). Histological analysis revealed that cisplatin-induced kidney injury was alleviated in mice after concomitant treatment with TJ-90 (Figure 2A and B). The mRNA expression of renal tubule damage markers such as KIM-1 and LCN-2 as well as renal function markers such as plasma BUN and creatinine levels worsened in mice after cisplatin administration, which was ameliorated in mice co-treated with both dose of TJ-90 (Figure 2C and Table 2). The cisplatin-induced mRNA upregulation of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, interleukin (IL)-6, and IL-1β, was also suppressed by TJ-90 treatment (Figure 2D). TJ-90 administration alone did not affect renal histology, function, and mRNA expression, except for MCP-1 and IL-6 genes (Figure S4). In contrast, TJ-43 treatment failed to prevent CIN (Figure S5 and Table S1).

*Effects of TJ-90 on oxidative stress, apoptosis, and MAPK pathway in mice with cisplatin-induced acute kidney injury*

Cisplatin administration increased superoxide production and lipid peroxidation, as indicated by DHE staining and 4-HNE expression, and these effects were inhibited by TJ-90 treatment (Figure 3A and C). In terms of apoptosis, cisplatin-induced TUNEL-positive cells and cleaved caspase-3 expression in the kidney were reduced in mice treated with TJ-90 (Figure 3B and C). Moreover, cisplatin-induced phosphorylation of the JNK, ERK1/2, and p38 MAPK pathways was inhibited by TJ-90 treatment (Figure 4A). No difference in cisplatin-induced DNA damage was observed between mice treated with and without TJ-90 (Figure 4B). Collectively, the preventive action of TJ-90 against cisplatin-induced acute kidney injury involves the inhibition of inflammatory response, oxidative stress, and apoptosis.

*Effect of TJ-90 on cancer cells treated with cisplatin*

The above findings suggest that TJ-90 inhibited cisplatin-induced kidney injury; therefore, we examined the effect of TJ-90 on anti-tumor activity of cisplatin in cancer cell lines. Cisplatin-induced cell death in both 3LL and colon-26 cancer cell lines

was not inhibited by concomitant treatment with TJ-90. Thus, TJ-90 is suggested to have no effect on anti-tumor activity of cisplatin *in vitro*（Figure 5）.

*Effect of single crude dug extracts present in TJ-90 on cancer cells treated with cisplatin*

We investigated which single crude drug present in Seihaito exerted a protective effect against CIN. In each single crude drug extract of the above four Kampo formulas, 9 crude extracts are uniquely blended in Seihaito (Platycodon Root (root of *Platycodon grandiflorum* A. De Candole (Kikyo)), Apricot Kernel (seed of *Prunus armeniaca* Linne (Kyonin)), Schisandra Fruit (fruit of Schisandra chinensis Baillon (Gomishi)), Gardenia Fruit (fruit of *Gardenia jasminoides* Ellis (Sanshishi)), Mulberry Bark (root bark of *Morus alba* Linne (Souhakuhi)), Bamboo Culm (inner layer of a woody ringed stem, culm, of *Bambusa tuldoides* Munro (Chikujyo)), Asparagus Root (root of *Asparagus cochinchinensis* Merrill (Temmondo), Fritillaria Bulb (bulb of *Fritillaria verticillata* var. thunbergii (Baimo)), and Ophiopogon Root (enlarged part of root of *Ophiopogon japonicus* Ker-Gawler (Bakumondo)). In the MTS assay, Gardenia Fruit, Bamboo Culm and Ophiopogon Root inhibited cisplatin-induced cell death, and only Bamboo Culm suppressed cisplatin-induced upregulated expression of cleaved caspase-3 (Figure 6).

## Discussion

We observed that TJ-90 Seihaito alleviated CIN through suppression of apoptosis, inflammation, and oxidative stress, and it did not interfere with anti-tumor effect of cisplatin. In single crude drug extracts from Seihaito, Bamboo Culm might be a potential component that can exert an inhibitory effect on CIN. Thus, our results indicate that Seihaito is a preventive medicine against CIN.

Many studies have shown that CIN is mediated through various mechanisms, including inflammation, apoptosis, and oxidative stress, and that the inhibition of these mechanisms is expected to alleviate CIN (Miller et al., 2010; Ozkok and Edelstein, 2014; Pabla and Dong, 2008). In the present study, we first examined the preventive action of the ethical Kampo extract formulations against CIN through *in vitro* experiments for screening purpose, and observed that only TJ-90 could inhibit both cell death and apoptosis induced by cisplatin. Cisplatin administration caused nephrotoxicity with renal dysfunction and increased inflammation, apoptosis, and oxidative stress, which were ameliorated by TJ-90 treatment in the mouse model, similar to *in vitro* experiment. The MAPK pathway, including ERK1/2, JNK, and p38MAPK, are involved in CIN and induce inflammation, apoptosis, and oxidative stress (Francescato et al., 2007; Jo et al.,

2005; Ramesh and Reeves, 2005). The activation of these pathways was alleviated by TJ-90 administration, resulting in the suppression of CIN. Thus, our study findings are the first, to the best of our knowledge, to reveal the protective action of TJ-90 against CIN.

TJ-90 is an ethical Kampo extract formulation for treating respiratory symptoms such as cough and sputum in clinical practice. Moreover, TJ-90 exhibited beneficial effects in aspiration pneumonia (Mantani et al., 2002) and chronic obstructive pulmonary disease (Kato et al., 2005). In aspiration pneumonia, fever and C-reactive protein level were reduced in patients receiving conventional therapy with TJ-90 compared to those receiving conventional therapy alone. A previous experimental study showed that TJ-90 suppressed oxidative stress and inflammation in the lung tissues of rabbits (Miyamoto et al., 1990). Moreover, TJ-90 ameliorated mortality in a mouse model of aspiration pneumonia by reducing oxidative stress *via* xanthine oxidase inactivation (Iwasaki et al., 1999). In addition, TJ-90 abolished caspase-3 activation and increased cisplatin-induced TUNEL-positive cells in the kidney, indicating the anti-apoptotic effect of TJ-90. Thus, TJ-90 inhibits inflammation, oxidative stress, and apoptosis, the molecular mechanisms of preventive effect of TJ-90 against CIN.

Cisplatin uptake into renal tubular cells of the kidney is mediated by organ cation transporter 2 (OCT2), which leads to its accumulation in the kidney, resulting in nephrotoxicity (Ciarimboli et al., 2005). Our previous study has demonstrated that the antihistamine drug diphenhydramine (DPH), which has been reported to inhibit OCT2 (Zolk et al., 2009), reduced cisplatin-induced DNA damage by inhibiting cisplatin accumulation in the kidney (Hamano et al., 2021). In the present study, no difference in cisplatin-induced DNA damage was observed between the vehicle-treated and TJ-90-treated groups, suggesting that the mechanism underlying the effect of TJ-90 against CIN does not involve cellular uptake of cisplatin into the kidney. Further experiments are required to clarify the molecular mechanisms of TJ-90 in CIN.

TJ-90 is composed of 16 crude drug extracts, and we examined which single crude extract in TJ-90 exert a preventive effect against CIN. Among the above 16 crude extracts, Scutellaria Root (root of *Scutellaria baicalensis* Georgi (Ougon)), Glycyrrhiza (root and stolon of *Glycyrrhiza uralensis* Fisher (Kanzou)), Ginger (rhizome of *Zingiber officinale* Roscoe (Shokyo)), Jujube (fruit of *Zizyphus jujuba* Miller (Taisou)), Poria Sclerotium (sclerotium of *Poria cocos* Wolf (Bukuryo)), Japanese Angelica Root (root of *Angelica acutiloba* kitagawa (Touki)), Citrus Unshiu Peel (pericarp of the ripe fruit of

*Citrus unshiu* Markovich (Chinpi)) were common in TJ-41, 43, 90, and 114. Therefore, we tested the remaining 10 crude extracts in TJ-90, and observed that only Bamboo Culm inhibited cisplatin-induced cell death and apoptosis. The major components of Bamboo Culm are N-p-coumaroyl serotonin and N-feruloyl serotonin (Tanaka et al., 2003). These serotonin derivatives as well as serotonin have recently been shown to exert a protective effect against CIN by inhibiting oxidative stress, inflammation, and apoptosis (Park et al., 2019). Therefore, these serotonin derivatives present in Bamboo Culm might have inhibited cisplatin-induced renal proximal cell death, contributing to the protective action of TJ-90 against CIN. However, it is suggested that the protective effect of each crude drug extract in TJ-90 against CIN can lead to additive or synergistic effect; however, further investigation is necessary to clarify this.

TJ-90 exerted a preventive effect on CIN, whereas it did not interfere with the anti-tumor action of cisplatin. Similar to our findings, some compounds have been shown to exert preventive effects against CIN without affecting the anti-tumor effects of cisplatin (Sadhukhan et al., 2018; Sanchez-Gonzalez et al., 2017). The cause of the differences in the nature and biological behavior between normal cells and tumor cells may be that renal

proximal tubular cells are quiescent, whereas tumor cells are proliferative. The disparity in cisplatin-induced cellular toxicity might be due to the different chromatin statuses (Faith A.A. Kwa, 2011), contributing to the differences in TJ-90 action in the kidney and cancer treated with cisplatin.

Currently, there are no available drugs to prevent CIN, although many studies have been performed to develop drugs for CIN. Recently, many researchers have focused on various natural products, including flavonoids, saponins, and alkaloids (Fang et al., 2021). For example, quercetin, a potent antioxidant flavonoid, inhibits CIN without affecting the anti-tumor activity of cisplatin in rats (Sanchez-Gonzalez et al., 2017), which is similar to the findings of the present study. Ethical Kampo extract formulations generally consist of several crude drug extracts, including the above natural products; therefore, Kampo medicine may be a more promising drug for preventing CIN. In addition, Kampo extract formulations are existing drugs widely used in clinical practice. It is advantageous in terms of development cost, duration, and period for clinical application compared to new drugs. Therefore, it may be comparatively easy to apply TJ-90 as a preventive drug against CIN in clinical practice.

In conclusion, the present study is the first, to the best of our knowledge, to reveal the novel preventive action of TJ-90 against CIN without interfering with the anti-tumor effect of cisplatin. This finding might contribute to the efficacy of the Kampo medicine as supportive therapy for cancer patients undergoing chemotherapy.

**Author contributions: Yasumasa Ikeda:** Conceptualization, Methodology, Validation, Investigation, Writing - Original draft preparation, Supervision. **Masafumi Funamoto:** Investigation, Writing - Reviewing and Editing. **Seiji Kishi:** Writing - Reviewing and Editing. **Masaki Imanishi:** Writing - Reviewing and Editing. **Ken-ichi Aihara:** Writing - Reviewing and Editing. **Yoshiki Kashiwada:** Writing - Reviewing and Editing. **Koichiro Tsuchiya:** Resources, Writing - Reviewing and Editing. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of the work, ensuring its integrity and accuracy.

## Study Approval

All experimental procedures involving mice were performed in accordance with the guidelines of the Animal Research Committee of Tokushima University Graduate School, and the protocol was approved by the Institutional Review Board of Tokushima University Graduate School for animal protection (Permit Number: T30-74, T2021-75).

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## Figure legends

**Figure 1**. Inhibitory effect of TJ-90 on cisplatin-induced renal proximal tubular cell death. (A) Cisplatin-induced cell death was significantly suppressed after TJ- 90 treatment in HK-2 cells. Values are expressed as the mean ± SEM (n = 8 in each group); **P < 0.01. (B) Upper panel: Representative protein bands of cleaved caspase-3, total caspase-3, and β-actin in HK-2 cells. Lower panel: Semi-quantitative densitometry analysis of cleaved Casp-3 corrected by total Casp-3. Values are expressed as the mean ± SEM (n = 4–6 in each group); **P < 0.01.

**Figure 2**. TJ-90 alleviates cisplatin-induced acute kidney injury in mice. (A) Representative images of hematoxylin and eosin staining of the kidney sections of mice from the vehicle-treated group, cisplatin-injected mice in the vehicle group, and TJ-90 (0.5 or 1.0 g/kg/day) treatment group. (B) Quantitative analysis of the renal tubular damage scores. Values are expressed as the mean ± SEM (n = 7–9 in each group); *P < 0.05, **P < 0.01. (C) mRNA expression levels of kidney injury markers (KIM-1 and lipocalin-2) in the kidneys of mice from all groups. Values are expressed as the mean ± SEM (n = 7–9 in each group); *P < 0.05, **P < 0.01. (D) TJ-90 prevents cisplatin-induced

renal inflammation. Quantitative analysis of mRNA expression of inflammatory cytokines in the kidneys of mice from all groups. Values are expressed as the mean ± SEM (n = 7–9 in each group); *P < 0.05, **P < 0.01.

**Figure 3.** Effect of TJ-90 on cisplatin-induced oxidative stress and apoptosis. (A) Left panel: Representative images of dihydroethidium (DHE) staining in the kidneys of mice from all groups. Right panel: Semi-quantitative DHE fluorescence intensity analysis. Values are expressed as the mean ± SEM (n = 5–7 in each group); *P < 0.05. (B) Left panel: Representative images of TdT-mediated dUTP nick end labeling (TUNEL) staining in the kidneys of mice from all groups. Right panel: Semi-quantitative analysis of TUNEL-positive cells. Values are expressed as the mean ± SEM (n = 8 in each group); *P < 0.05. (C) Left panel: Representative protein bands of 4-hydroxynonenal (HNE), cleaved caspase -3, total caspase-3, and β-actin in the kidneys of mice. Right panel: Semi-quantitative densitometry analysis of 4-HNE and cleaved caspase-3 corrected by total caspase-3. Values are expressed as the mean ± SEM (n = 4–5 in each group); *P < 0.05, **P < 0.01.

**Figure 4.** Effect of TJ-90 on cisplatin-induced activation of the mitogen-activated protein kinase pathway. (A) Left panel: Representative protein bands of phospho-c-Jun N-terminal kinase (JNK), total JNK, phospho-extracellular signal-regulated kinase (ERK) 1/2, total ERK 1/2, phospho-p38 protein, total p38 protein, and β-actin in the kidneys of mice. Right panel: Semi-quantitative densitometry analysis of JNK, ERK 1/2 phosphorylation, and p38 protein phosphorylation. Values are expressed as the mean ± SEM (n = 4–5 in each group); *P < 0.05, **P < 0.01. No effect of TJ-90 was observed on cisplatin-induced DNA damage in the kidney. (B) Left panel: Representative immunohistological images of Pt-DNA Adducts and DAPI in the kidney sections of cisplatin-injected mice with vehicle or TJ-90 treatment. Right panel: Semi-quantitative analysis of DNA adducts. Values are expressed as the mean ± SEM (n = 4 in each group).

**Figure 5.** Effect of TJ-90 on cisplatin-induced cell death in cancer cell lines. Cisplatin-induced cell death was not interfered by TJ-90 treatment in (A) 3LL mice lung carcinoma cells and (B) colon-26 mouse colon cancer cells. Values are expressed as the mean ± SEM (n = 8 in each group); *P < 0.05, **P < 0.01.

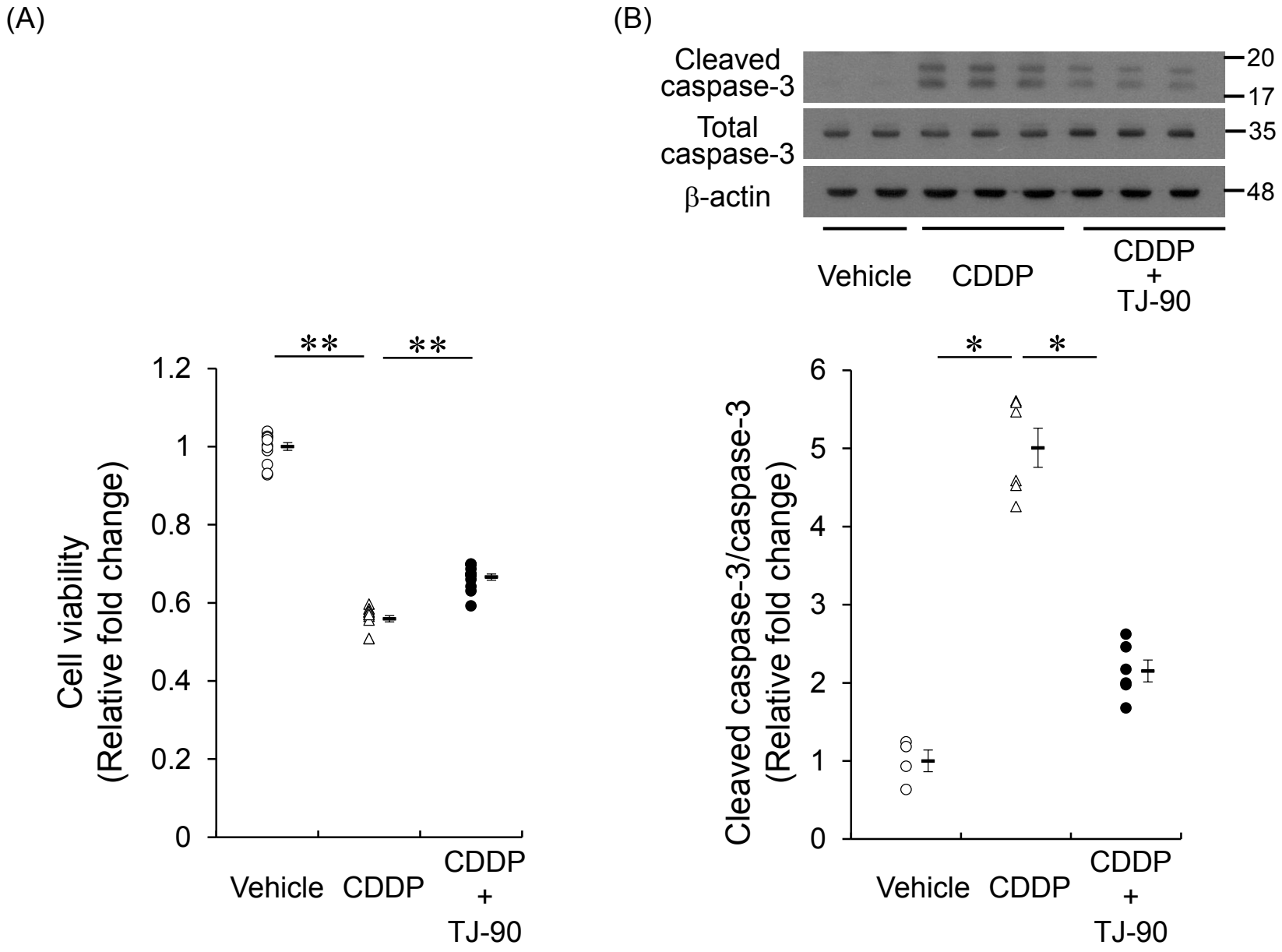
**Figure 6.** Inhibitory effect of single crude drug extract present in TJ-90 on renal proximal tubular cell death and cleaved caspase-3 expression induced by cisplatin. (A) Cisplatin-induced cell death was significantly suppressed by Sanshishi, Chikujyo, and Bakumondo treatment in HK-2 cells. Values are expressed as the mean ± SEM (n = 16 in each group); **P < 0.01. (B) Cisplatin-induced cell death was significantly suppressed by Chikujyo treatment in HK-2 cells. Upper panel: Representative protein bands of cleaved caspase-3, total caspase-3, and β-actin in HK-2 cells. Lower panel: Semi-quantitative densitometry analysis of cleaved caspase-3 corrected by total caspase-3. Values are expressed as the mean ± SEM (n = 8 in each group); **P < 0.01.

**CRediT Author statement**

Author contributions: Yasumasa Ikeda: Conceptualization, Methodology, Validation, Investigation, Writing - Original draft preparation, Supervision. Masafumi Funamoto: Investigation, Writing - Reviewing and Editing. Seiji Kishi: Writing - Reviewing and Editing. Masaki Imanishi: Writing - Reviewing and Editing. Ken-ichi Aihara: Writing - Reviewing and Editing. Yoshiki Kashiwada: Writing - Reviewing and Editing. Koichiro Tsuchiya: Resources, Writing - Reviewing and Editing. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of the work, ensuring its integrity and accuracy.

Figure 1 Ikeda, et al.





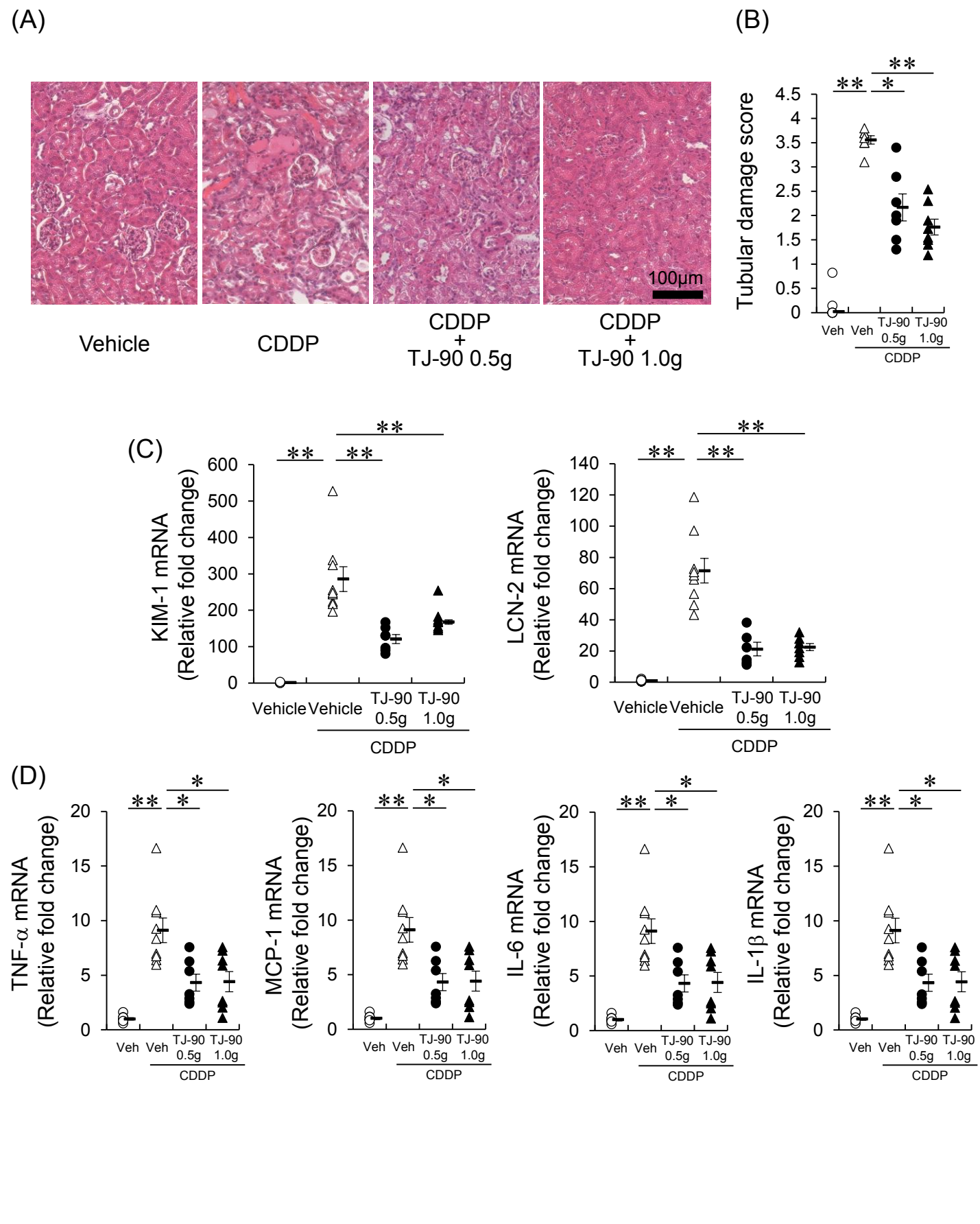


Figure 3 Ikeda, et al.



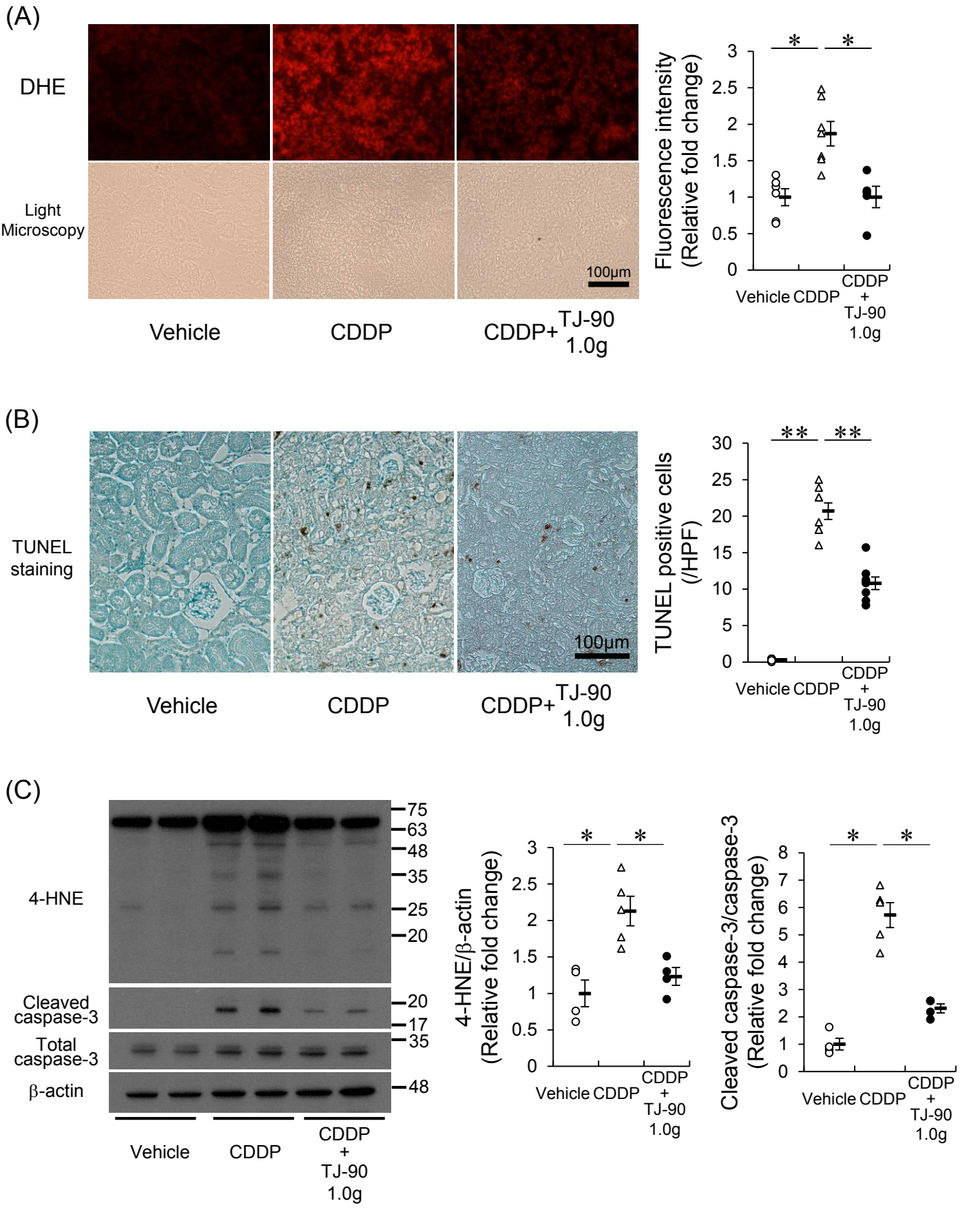
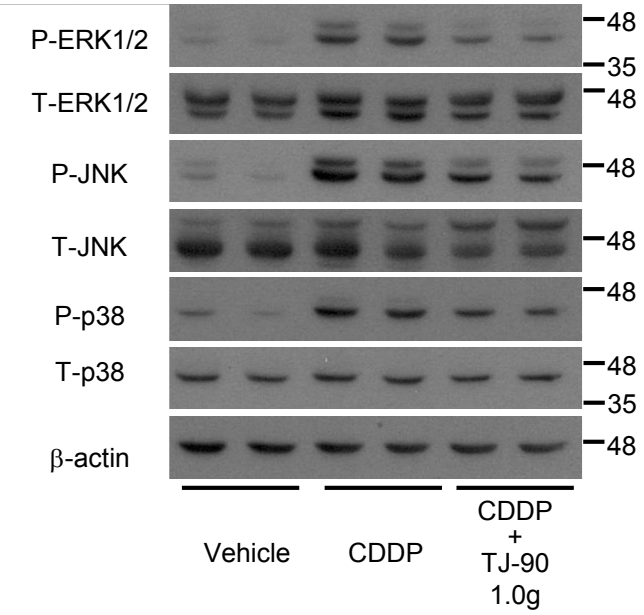
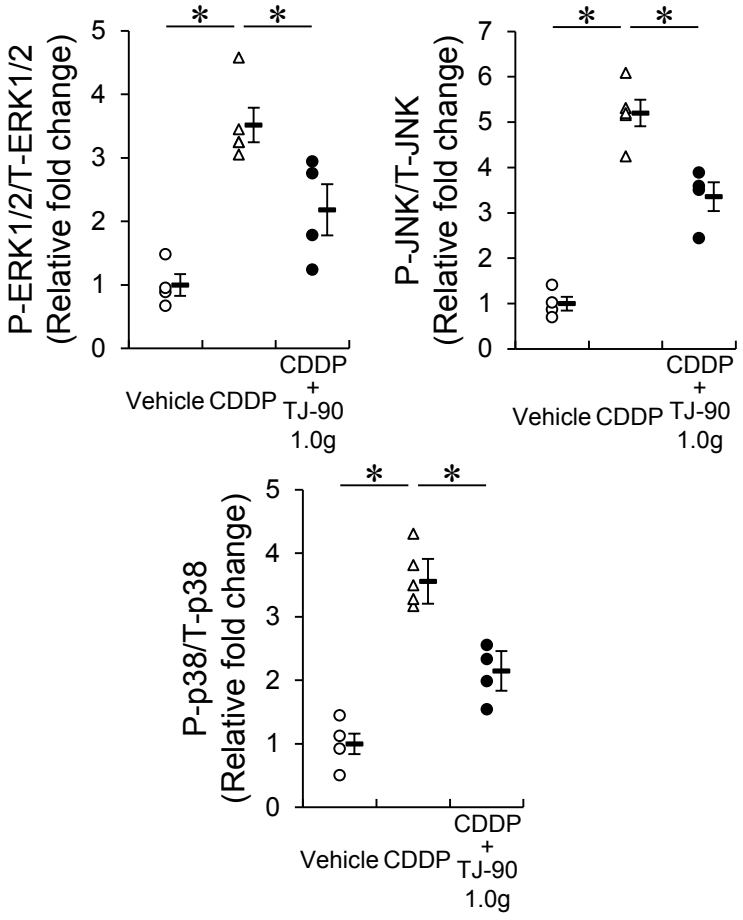


Figure 4 Ikeda, et al.



(A)

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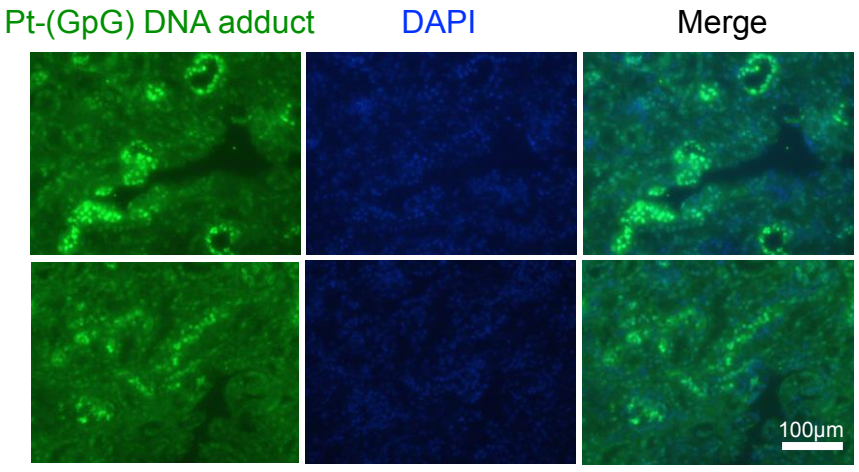


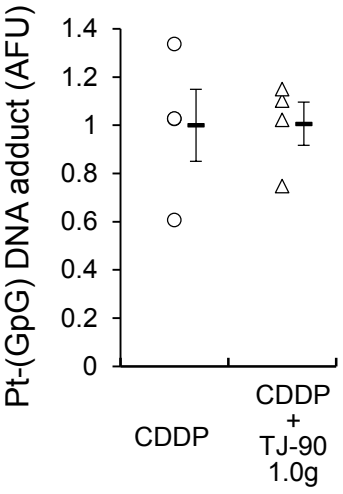
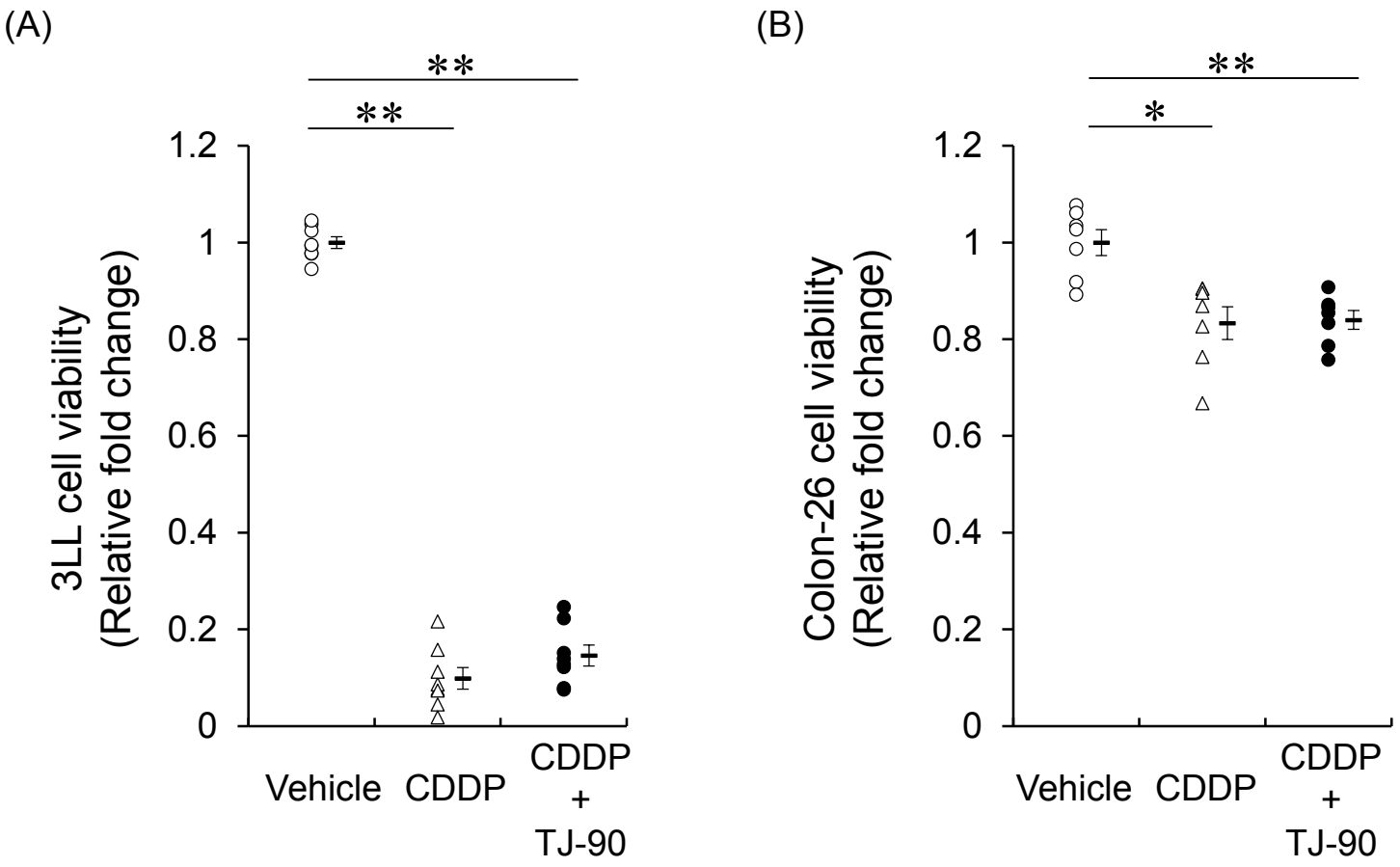



Figure 5 Ikeda, et al.





# Figure 6 Ikeda, et al.



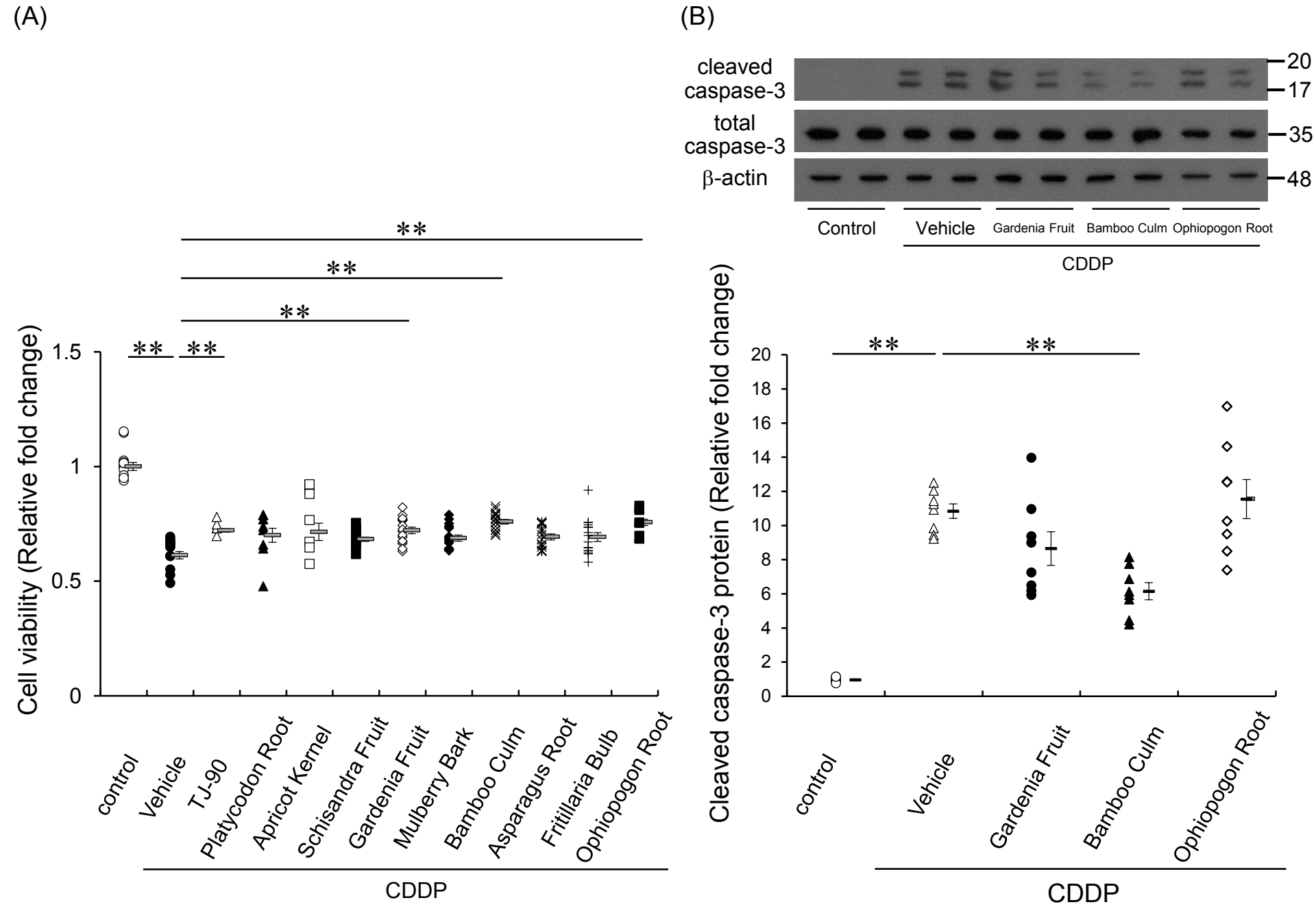


Table 1. Sets of primer sequences

| | Forward | Reverse |
|---|---|---|
| Mouse kidney injury molecule (KIM)-1 | AAACCAGAGATTCCCACACG | GTCGTGGGTCTTCCTGTAGC |
| Mouse lipocalin (LCN)-2 | TGGAAGAACCAAGGAGCTGT | GGTGGGGACAGAGAAGATGA |
| Mouse tumor necrosis factor (TNF)-α | ACGGCATGGATCTCAAAGAC | GTGGGTGAGGAGCACGTAGT |
| Mouse monocyte chemoattractant protein (MCP)-1 | GGAGCTCATGATGTGAGCAA | GACCAGGCAAGGGAATTACA |
| Mouse interleuikin-6 (IL-6) | CCGGAGAGGAGACTTCACAG | TCCACGATTTCCCAGAGAAC |
| Mouse interleuikin-1β (IL-1β) | CAGGCAGGCAGTATCACTCA | TGTCCTCATCCTGGAAGGTC |
| 36B4 | GCTCCAAGCAGATGCAGCA | CCGGATGTGAGGCAGCAG |

Table 2. Body weight, kidney weight, and renal function in vehicle-treated mice and cisplatin-treated mice with or without TJ-90

| | Vehicle | Cisplatin | Cisplatin+TJ-90 0.5g/kg/day | Cisplatin+TJ-90 1.0g/kg/day |
|---|---|---|---|---|
| Initial body weight (g) | 23.1 ± 0.5 | 23.0 ± 0.4 | 22.2 ± 0.3 | 22.8 ± 0.4 |
| Post body weight (g) | 23.7 ± 0.4 | 19.9 ± 0.4** | 19.0 ± 0.3** | 20.0 ± 0.5** |
| Right kidney weight (mg) | 137.0 ± 4.6 | 123.9 ± 3.2 | 128.0 ± 4.4 | 128.9 ± 1.7 |
| Left kidney weight (mg) | 130.4 ± 4,7 | 121.8 ± 2.6 | 115.5 ± 5.9 | 123.5 ± 2.8 |
| BUN (mg/dl) | 24.2 ± 1.6 | 63.7 ± 4.7** | 32.7 ± 1.4*$^{\#}$ | 25.0 ± 1.4$^{\#\#}$ |
| Creatinine (mg/dl) | 0.10 ± 0.00 | 0.40 ± 0.04** | 0.21 ± 0.04*$^{\#}$ | 0.17 ± 0.01**$^{\#\#}$ |

Data represent mean ± SEM; $n$ = 7-9; *$P < 0.05$, **$P < 0.01$ vs. vehicle mice, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. cisplatin mice.

## Highlights

- Seihaito inhibits CIN through reducing inflammation, apoptosis, and oxidative stress.
- Seihaito does not interfere anti-tumor effect of cisplatin.
- Bamboo Culm including Seihaito is a potential component to alleviate CIN.

**Supplemental Figure S1**. The 3D-HPLC-based profile of four Japanese ethical Kampo extract formulations (TJ-41 Hochuekkito, TJ-43 Rikkunshito, TJ-90 Seihaito, TJ-114 Saireito).

**Supplemental Figure S2**. Inhibitory effect of Kampo (TJ-41, 43, 90, and 114) on cisplatin-induced renal proximal tubular cell death. (A) Cisplatin-induced cell death was significantly suppressed by treatment with TJ-43 or 90 in HK-2 cells. Values are expressed as the mean ± SEM (n = 8 in each group); **P* < 0.05. (B) Upper panel: Representative protein bands of cleaved caspase-3, total caspase-3, and β-actin in HK-2 cells. Lower panel: Semi-quantitative densitometry analysis of cleaved caspase-3 corrected by total caspase-3. Values are expressed as the mean ± SEM (n = 8–10 in each group); *P < 0.05.

**Supplemental Figure S3**. No affect of TJ-90 on cell viability and caspase-3 activation in HK-2 cells. (A) Cisplatin-induced cell death was significantly suppressed by treatment with TJ- 90 in HK-2 cells. Values are expressed as the mean ± SEM (n = 8 in each group); ***P* < 0.01. (B) Upper panel: Representative protein bands of cleaved caspase-3, total caspase-3, and β-actin in HK-2 cells. Lower panel: Semi-quantitative densitometry

analysis of cleaved caspase-3 corrected by total caspase-3. Values are expressed as the mean ± SEM (n = 7–9 in each group); *P < 0.05.

**Supplemental Figure S4**. No affect of TJ-90 on kidney of mice. (A) Representative images of hematoxylin and eosin staining of the kidney sections of mice from the vehicle group, and TJ-90 treatment group. (B) Quantitative analysis of the renal tubular damage scores. Values are expressed as the mean ± SEM (n = 6–7 in each group) (C) The levels of blood urea nitrogen and plasma creatinine in mice from two groups. Values are expressed as the mean ± SEM (n = 6–7 in each group). (D) mRNA expression levels of kidney in the kidneys of mice from two groups. Values are expressed as the mean ± SEM (n = 6–7 in each group); **P < 0.01..

**Supplemental Figure S5**. TJ-43 exhibits no preventive action against cisplatin-induced acute kidney injury in mice. (A) Representative hematoxylin and eosin staining of the kidney sections of the mice from the control group, cisplatin-injected mice with vehicle group, or TJ-43 treatment group. (B) Quantitative analysis of the renal tubular damage scores. Values are expressed as the mean ± SEM (n = 7–9 in each group); **P < 0.01. (C) The levels of blood urea nitrogen and plasma creatinine in mice Values are expressed as

the mean ± SEM (n = 7–9 in each group); **P < 0.01. (D) mRNA expression levels of kidney injury marker (KIM-1) and inflammatory cytokine (TNF-α) in the kidneys of mice from all groups. Values are expressed as the mean ± SEM (n = 8–9 in each group); **P < 0.01.