Original Article

Safflower seed extract synergizes the therapeutic effect of cisplatin and reduces cisplatin-induced nephrotoxicity in human colorectal carcinoma RKO cells and RKO-transplanted mice

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Summary   **Safflower seed is effective against oxidative stress, mediating the activation of the apoptotic signaling pathway in the renal tissues of cisplatin-treated mice. The anticancer activity of safflower in various cancer cell lines has also been reported. The present study was conducted to evaluate the potential synergistic anticancer effects of the co-treatment of safflower seed extracts and cisplatin in RKO cells and in BALB/c mice bearing RKO cell-derived human colorectal tumors. In the cellular system, RKO cells were treated with safflower seed extract in the presence or absence of cisplatin for 48 h and the cytotoxicity was evaluated by using microscopy. In the *in vivo* system, mice were injected with RKO cells and subsequently orally administered 100 or 200 mg/kg body weight safflower seed extract plus cisplatin-treated or untreated mice for 3 days to examine the inhibitory effect on the tumor. Treatment with safflower seed extract or cisplain to RKO cells resulted in a greater cell death than in with untreated cells. In the RKO cells co-treated with both safflower seed extract and cisplatin, greater cell damage was observed. In addition, mice co-administered safflower seed extract and cisplatin had lower concentrations of serum creatinine, which were indicative of less damage to the kidney, and had a lower solid tumor mass and higher expression of the caspase-3 protein. The results showed that safflower seed extract was highly toxic to RKO cells and inhibited tumor growth in cisplatin-treated mice through renoprotective effects.**

Keywords: Safflower seed, cisplatin, nephrotoxicity, anticancer activity

1. Introduction

Colorectal cancer has become one of the most common cancers in Asian countries and is now considered a serious health problem (*1*). Several factors have been suggested to increase the risk of colon cancer (*2*). Although many anticancer drugs have been developed,

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resistance to these drugs is ubiquitous. Moreover, most anticancer drugs are not selective toward cancer cells and attack rapidly dividing normal cells, which is a known harmful side effect of topoisomerase inhibitors [*e.g.* irinotecan (neutropenia and diarrhea), doxorubicin (cardiotoxicity), and alkylating agents, such as oxaliplatin, melphalan, carboplatin, cyclophosphamide, and cisplatin (gastrointestinal toxicity, cardiovascular toxicity, pulmonary, hematologic toxicity, and nephrotoxicity)]. Thus, the development of an adjuvant anticancer agent that protects against anticancer drug-induced toxicity, but does not impair the efficacy of chemotherapy, is an important clinical aim (*3*).

In the Asia-Pacific region, many plants have traditional uses as foods and medicines. Many candidate compounds have been identified for use in the prevention of human cancers (*4*). Among various oriental herbs, the

flowers and leaves of safflower (*Carthamus tinctorius* L.), a member of the chrysanthemum family, have a long history of use in traditional Chinese medicine in treatments for stroke, gynecological diseases, coronary heart disease, angina pectoris, and hypertension (*5-8*). Furthermore, safflower has been used for centuries to treat urological diseases in China (*9*), and the consumption of safflower seed oil is popular worldwide. Safflower seed is used as an herbal medicine in Korea to promote bone formation and to treat osteoporosis and rheumatism. Moreover, our previous study suggested that safflower seed was effective against oxidative stress, mediated the activation of the mitogen-activated protein kinase (MAPK)-related apoptotic signaling pathway in renal tissue, and exerted renoprotective effects in cisplatin-treated mice (*10*). Anticancer activity, through the induction of cyclin D1 proteasomal degradation, has also been reported in various human colorectal cancer cell lines (*11*). Thus, safflower seed may be a promising candidate adjuvant anticancer agent.

Therefore, the present study examined whether safflower seed extract acted synergistically as an anticancer agent with cisplatin chemotherapy, and evaluated its protective effect against cisplatin-induced nephrotoxicity, in RKO cells and mice bearing RKO cell-derived tumors.

2. Materials and Methods

2.1. *Materials*

Human colorectal carcinoma cell line, RKO, was obtained from American Type Culture Collection (Manassas, Virginia, USA). Minimum essential medium (MEM) was purchased from Gibco Laboratories (Paisley, UK). Fetal bovine serum (FBS) and [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) were purchased from Sigma-Aldrich Chemical Company (Dorset, UK). Six-well flat-bottom tissue culture plates were purchased from Nunc Inc. (Hereford, UK). Phenylmethylsulfonyl fluoride (PMSF) was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA) and protease inhibitor mixture solution was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Enhanced chemiluminescence (ECL) western blotting detection reagents and pure nitrocellulose membranes were purchased from GE Healthcare (Buckinghamshire, UK). Primary antibodies for the detection of caspase 3 and β-actin and goat anti-rabbit and goat anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich Co., Ltd. unless otherwise specified.

2.2. *Preparation of safflower seed extract*

Safflower seeds were purchased from Geunyang Oriental Medicine Co. (Chungju, Korea). A voucher for the herbarium specimen, identified by a plant systematist from the Herbal Crop Research Division, was deposited at the Department of Medicinal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration (voucher specimen No. MC20150528). The dried safflower seeds were pulverized and ultrasonically extracted twice in 70% ethanol (60 Hz, room temperature, 3 h). Subsequently, the solvent was evaporated *in vacuo* to obtain an extract with a yield of 4.65% by weight of the original safflower seed.

2.3. *Analysis of safflower seed extract*

An aliquot of 10 mg of the 70% ethanol extract of safflower seed was dissolved in 10 mL of 50% methanol by repeated vortexing, and passed through a Dismic-25 JP membrane filter (Advantec Toyo, Tokyo, Japan; pore diameter: 5 μm). Next, 5 μL of the sample was injected into a reverse-phase HPLC system with an INNO C18 column (4.6 × 250 mm, 5 μm pore size, YoungJin Biochrom, Korea) at 35°C. The mobile phase comprised (A) a 0.1% aqueous solution of formic acid and (B) acetonitrile, and the following gradient conditions were used: 0 min, 0% B; 5 min, 6% B; 10 min, 22% B; 50 min, 28% B; 66 min, 50% B; and 70 min, 100% B. The flow rate was 0.8 mL/min. The UV absorbance at 254 nm was monitored by using an Agilent 1200 series diode array detector (Agilent Technologies, Waldbronn, Germany). All peaks were assigned through co-injection tests with authentic samples and comparison of the UV spectral data; through this process, compounds including serotonin and its derivatives were detected from safflower seed extract. All measurements were repeated in triplicate. Representative HPLC results are illustrated in Figure 1. The quantification of serotonin, *N*-(*p*-coumaroyl)serotonin, *N*-feruloyl serotonin, luteolin, and luteolin-7-*O*-glucoside was achieved by peak area measurement. The quantity of each identified compound is shown in Table 1.

2.4. *Cell culture*

The human colon cancer cell line RKO was maintained and cultured in MEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. For all experiments, cells in the exponential growth phase were used.

2.5. *Cell cytotoxicity*

RKO cells were seeded at a density of 4×10^4 cells/ well in 6-well plates. After incubation for 24 h, the cells were treated with safflower seed extract (100 μg/mL),
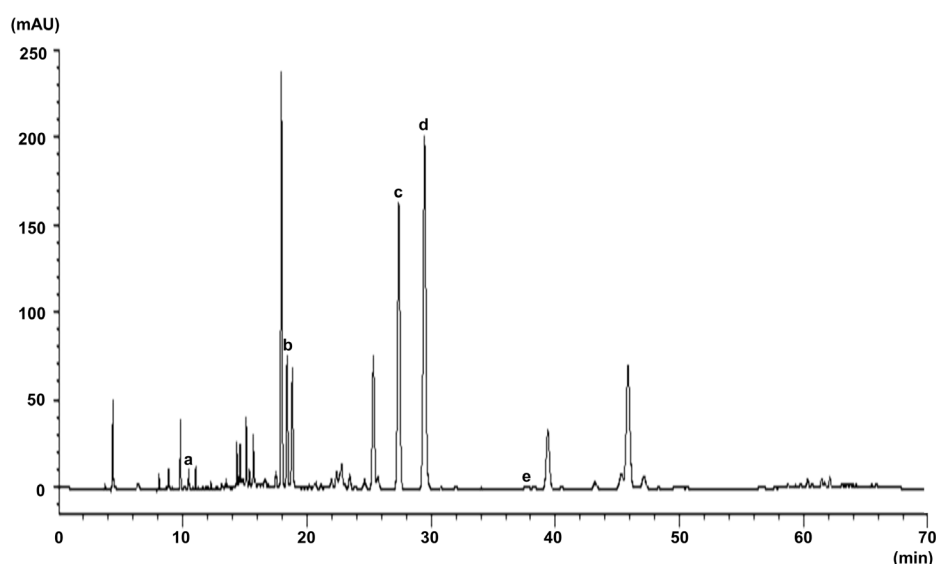
Figure 1. HPLC profile of safflower seed. a, serotonin; **b**, luteolin-7-*O*-glucoside; **c**, *N*-(*p*-coumaroyl)serotonin; **d**, *N*-feruloyl serotonin; **e**, luteolin.

Table 1. Contents of compound

Compound	Content (mg/g)	RSD (%)
Serotonin	1.47 ± 0.06	4.19
N-(*p*-Coumaroyl)serotonin	28.70 ± 1.82	6.35
N-Feruloyl serotonin	37.06 ± 2.25	6.07
Luteolin	0.89 ± 0.04	4.07
Luteolin-7-*O*-glucoside	12.85 ± 0.77	5.98

Values are the mean ± SEM.

cisplatin (1.5 μg/mL), or safflower seed extract (100 μg/mL) and cisplatin (1.5 μg/mL) for 48 h. Treatment doses were determined based on a previous study (*11*). The images of the cell cytotoxicity were obtained by using a Leica CTR 5000 microscope (×200) and a DM 5000B microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.6. *Xenograft mouse model experiments*

All experimental procedures were permitted using the guidelines established by the Pusan National University Institutional Animal Care and Use Committee. BALB/c nude mice (female, 5 weeks old; Joong-ang Animal Experiment Company, Republic of Korea) were used to construct the xenograft animal model. Mice were housed individually under a 12-h light/dark cycle at a controlled temperature (25 ± 2°C) and humidity (approximately 60%), and given free access to food and water. After adaptation (approximately 1 week), the animals were randomly divided into seven equal groups containing five mice: (1) mice were orally administered drinking water; (2) RKO cell-transplanted mice were orally administered drinking water; (3) RKO cell-transplanted mice were orally administered safflower seed extract (100 mg/kg body weight/day); (4) RKO cell-transplanted mice were

orally administered safflower seed extract (200 mg/kg body weight/day); (5) RKO cell-transplanted mice were administered an intraperitoneal injection of cisplatin (5 mg/kg body weight); (6) RKO cell-transplanted mice were orally administered safflower seed extract (100 mg/kg body weight/day) followed by an intraperitoneal injection of cisplatin (5 mg/kg body weight); (7) RKO cell-transplanted mice were orally administered safflower seed extract (200 mg/kg body weight/day) followed by an intraperitoneal injection of cisplatin (5 mg/kg body weight). To produce tumors, each mouse was implanted with RKO cells (5×10^6 cells per mouse) in the back next to the right hind leg. After 2 weeks, the safflower seed extract (100 or 200 mg/kg body weight in water) was administered *via* oral gavage. The treatment dose of safflower seed extract was determined from our previous report (*10*). Subsequently, cisplatin (5 mg/kg body weight) was injected intraperitoneally. The treatment compounds were administered a total of three times, with each administration 3 days apart. Sixteen hours after the test compound administration, the mice were euthanized by CO_2 gas (70%) and the tumor was removed and weighed. The blood samples were collected by cardiac puncture from anesthetized mice and the serum was immediately separated by centrifugation.

2.7. *Protein extraction and immunoblotting analyses*

Protein was extracted according to the method of Komatsu (*12*). Briefly, the solid tumor was homogenized with ice-cold lysis buffer, pH 7.5 [137 mM NaCl, 20 mM Tris-HCl, 1% Tween 20, 10% glycerol, 1 mM PMSF, and protease inhibitor mixture] and the homogenates were centrifuged at 3,000 × g for 10 min at 4°C. The protein concentration of each sample was determined by using a Pierce BCA protein assay kit
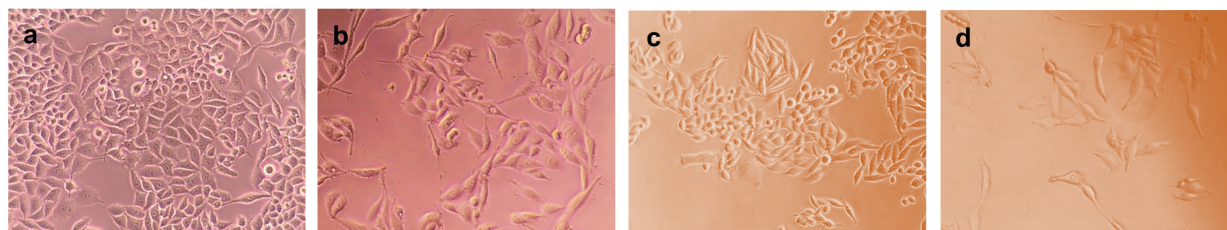
Figure 2. Cell cytotoxicity in human colorectal carcinoma RKO cells. a, untreated RKO cells; **b**, safflower seed extract-treated RKO cells; **c**, cisplatin-treated RKO cells; **d**, co-treated RKO cells.

(Thermo Scientific, Rockford, IL, USA) and equal amounts of protein were subjected to immunoblotting analyses. To determine caspase 3 and β-actin, each sample (10 μg of protein) was electrophoresed on an 8-15% sodium dodecylsulfate polyacrylamide gel. The separated proteins were electrophoretically transferred to a nitrocellulose membrane, which was blocked by incubation in 5% (w/v) skim milk solution for 1 h, and then incubated with the appropriate primary antibody overnight at 4°C. The blots were washed and then incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP-conjugated secondary antibodies for 90 min at room temperature. Each antigen-antibody complex was visualized by the application of ECL western blotting detection reagents and chemiluminescence was detected by using a Sensi-Q2000 Chemidoc (Lugen Sci Co., Ltd., Seoul, Korea). The band densities were measured by using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as a ratio to β-actin. Protein abundance was expressed relative to non-transplanted normal mice, which were considered to have an expression of 1.

2.8. *Measurement of renal functional parameter*

Serum creatinine level was measured using a commercial kit (CREA-Lq; Asan Pharm Co., Seoul, Republic of Korea).

2.9. *Statistical analysis*

The data are expressed as the mean ± SEM. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (SPSS 11.5.1 for Windows, 2002, SPSS Inc., USA), with values of $p < 0.05$ considered to indicate statistical significance.

3. Results

3.1. *Cytotoxicity*

The effect of safflower seed extract and cisplatin on the viability of human colorectal carcinoma RKO cells is shown in Figure 2. The cells were treated with the extract of safflower seed (100 μg/mL), cisplatin (1.5 μg/mL), or a combination of safflower seed extract (100 μg/
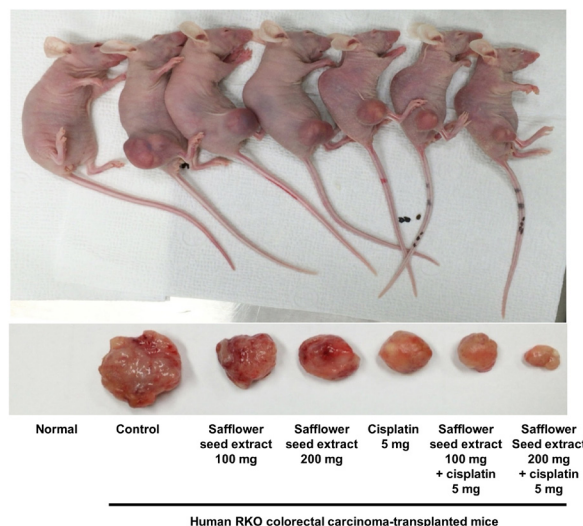

Figure 3. Xenograft mouse bearing RKO cell-derived human colorectal cancer.

mL) and cisplatin (1.5 μg/mL). Treatment with safflower seed extract (100 μg/mL) led to lower cell viability than that of untreated cells (Figure 2b). The changes in cell morphology induced by cisplatin are illustrated in Figure 2c. The combination treatment of safflower seed extract and cisplatin resulted in greater cell damage than the treatment of safflower seed extract or cisplatin alone (Figure 2d).

3.2. *In vivo evaluation*

Representative images of mice bearing RKO cell-derived human colorectal cancer xenografts are shown in Figure 3. In untreated mice bearing the xenografts (the control), the tumors weighed 1.57 ± 0.22 g, whereas the tumors in mice orally administered 100 and 200 mg/kg safflower seed weighed significantly less than the control tumors (0.94 ± 0.09 g and 0.91 ± 0.02 g, respectively). The tumor weight in cisplatin-treated mice was 51% lower than that in untreated and human RKO colorectal carcinoma-transplanted mice (0.70 ± 0.09 g), with greater reductions observed in mice co-administered safflower seed extract (0.52 ± 0.02 g and 0.43 ± 0.12 g, respectively), as shown in Figure 4. In addition, a significant increase in caspase 3 protein expression was observed in safflower seed-, cisplatin-,
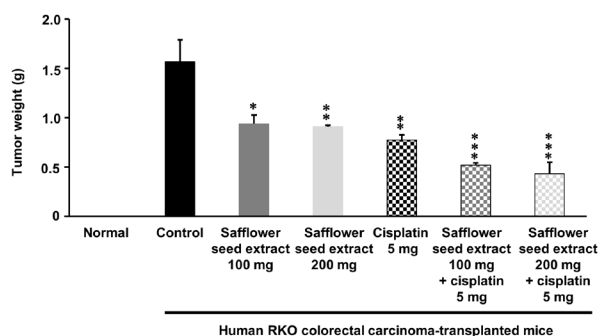
Figure 4. RKO cell-derived tumor weight. Data are the mean ± SEM. Significance: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 *vs.* human RKO colorectal carcinoma-transplanted control mouse values.
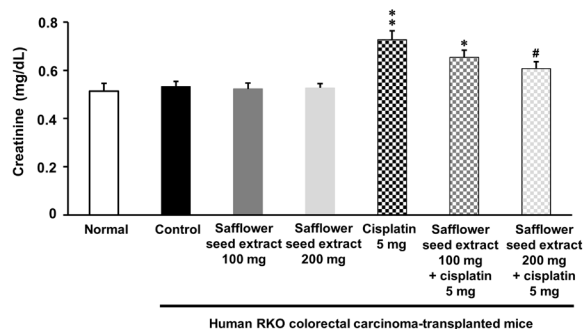

Figure 6. Serum creatinine level. Data are the mean ± SEM. Significance: * *p* < 0.05, ** *p* < 0.01 *vs.* human RKO colorectal carcinoma-transplanted control mouse values; # *p* < 0.05 *vs.* human RKO colorectal carcinoma transplantation and cisplatin-treated mouse values.
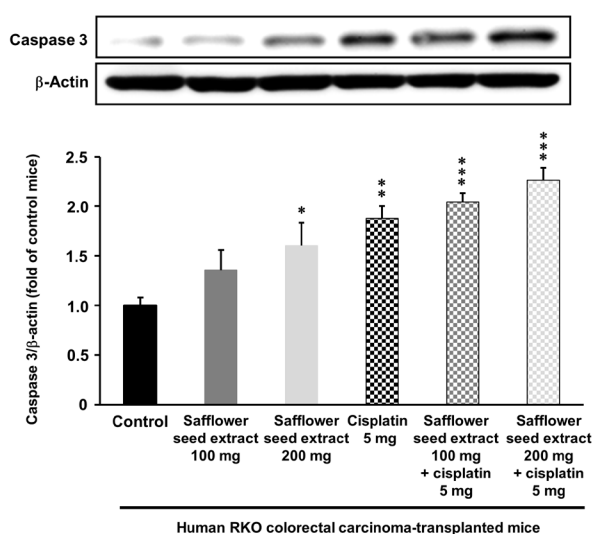

Figure 5. Caspase 3 protein expression in solid tumor. Data are the mean ± SEM. Significance: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001 *vs.* human RKO colorectal carcinoma-transplanted control mouse values.

and safflower seed plus cisplatin-treated mice compared with normal mice (Figure 5). Mice co-administered 200 mg/kg body weight safflower seed and cisplatin exhibited a more significant increase in caspase 3 protein expression. With regard to renal function, the mean serum creatinine concentration in cisplatin-treated mice (0.73 mg/dL) was markedly higher than the mean concentration in untreated mice (normal, human RKO colorectal carcinoma-transplanted control, or human RKO colorectal carcinoma-transplanted safflower seed-treated mice). However, the serum creatinine concentration of cisplatin-treated mice administered safflower seed extract decreased in a dose-dependent manner, as shown in Figure 6.

4. Discussion

Many therapeutic drugs have been developed and explored in clinical trials (*13,14*). Cisplatin is a potent anticancer agent, with clinical activity against a wide spectrum of solid neoplasms and has been in use for many years (*15*). However, the therapeutic efficacy of cisplatin is limited by its toxicity to normal tissues, notably the kidneys, where it induces cell injury and death in renal tubular cells and leads to acute renal failure (*16*). Hayes *et al.* (*17*) reported that high concentrations of cisplatin, between 3 and 5 mg/kg, induced renal toxicity; in particular, dose of 5 mg/kg resulted in dose-limiting toxicities to the kidney, bone marrow, and the ear, and led to death. Hence, we selected 5 mg/kg cisplatin for the induction of renal toxicity in the present study.

Traditional herbs have an important role in the prevention or the reduction of the symptoms of side effects from drugs. Therefore, we explored the potential renoprotective pharmacological properties of oriental herbs. We investigated the efficacy of safflower seed extract in a previous study by using the swine kidney-derived cultured epithelial cell line, LLC-PK$_1$, which retains proximal tubule characteristics. The oral administration of safflower seed extract to cisplatin-treated mice exerted a pleiotropic effect on several oxidative stress- and apoptosis-related parameters and was also renoprotective (*10*). Safflower has been used in drugs with pharmacological activities for decades worldwide (*18*). Recent studies have reported that the safflower seeds exert potential anti-inflammatory, anti-oxidant, and anti-cancer activities (*19-21*). Bae *et al.* (*21*) showed that the methanolic extract of safflower seeds exhibited inhibitory effects on three cancer cell lines (HepG2, MCF-7, and Hela cells), indicating that safflower seed may be moderately cytotoxic to cancer cells. Therefore, it was suggested that safflower seed might have the potential to reduce nephrotoxicity and enhance the anticancer activity of cisplatin. This study was designed to evaluate the anticancer activity of safflower seed in addition to its effects on the therapeutic efficacy of cisplatin and cisplatin-induced nephrotoxicity in RKO cells and RKO-transplanted mice.

Safflower seed was reported to reduce the proliferation of four human colorectal cell lines (HCT116, SW480, LoVo, and HT-29) (*11*). In addition, the treatment of safflower seed was shown to result in a high survival cell rate of human colon carcinoma SW 620 cell line, which indicated the protective effect of safflower seed treatment on intestinal cancer (*22*). As described in the Methods section, the cells were cultured in the presence of safflower seed extract, cisplatin, or co-cultured with safflower seed extract and cisplatin. Culture in the presence of safflower seed resulted in a decrease of RKO cell viability. We also observed the anticancer effect of safflower seed extract in a mouse with tumor xenografts. As He *et al.* (*23*) had shown that the tumor volume increased after 2 weeks in a RKO cell xenograft mouse model, the present study evaluated whether safflower seed exerted a beneficial effect on the growth of RKO-transplanted mice at this time, and found that the administration of the extract significantly inhibited the growth of RKO cell-derived tumors. In addition, mice orally administered safflower seed extract prior to intraperitoneal cisplatin administration showed greater reductions in tumor weight.

Serum creatinine concentration is commonly used for the measurement of renal function (*24*). Although cisplatin is used widely for the treatment of cancer, the toxicity to the kidney cannot be ignored. In the present study, the mean serum creatinine concentration in cisplatin-treated mice was significantly higher than the mean concentration in untreated control mice, and was significantly decreased by the administration of 200 mg/kg safflower seed extract. Safflower seed extract therefore represents a promising herbal medicine for the prevention or inhibition of cisplatin-induced renal damage.

Although multiple signaling pathways are activated by cisplatin in renal tubular cells (*25*), the mechanism of renal cell death during cisplatin nephrotoxicity is unclear. Effective renoprotective intervention during cisplatin chemotherapy is currently lacking. Programmed cell death (apoptosis) is an essential physiological process that plays a critical role in cell development and tissue homeostasis. The progress of apoptosis is regulated by a series of signal cascades (*26*). Caspases are crucial mediators of apoptosis; in particular, caspase 3 is required for some typical hallmarks of apoptosis (*26,27*). The activation and function of caspases are regulated by various types of molecules, such as the inhibitor of apoptosis protein and the Bcl-2 family of proteins. Asselin *et al.* (*28*) indicated that cisplatin treatment in human ovarian epithelial cancer cells induced cleavage of caspase 3. Moreover, we have previously shown the ameliorative effect of safflower seed on cisplatin-induced renal damage in a mouse model *via* caspase 3 protein expression, which may be used as a basis for the present study (*10*). Therefore, we examined the expression of caspase 3 protein by using western blotting
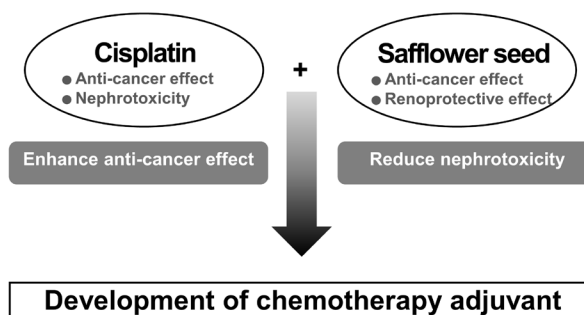

Figure 7. The potential of safflower seed as an adjuvant anticancer agent.

to clarify the responsible apoptotic-related proteins. We observed an increase in the expression of caspase 3 in the mouse xenograft model treated with safflower seed extract. These results contrasted with the inhibition of human colorectal tumor growth observed in mice bearing xenograft tumors.

As mentioned in the Introduction, traditional herbal medicines have emerged as one of the most important alternative therapies for cancer. We therefore believe that safflower seed extract may represent an effective nutritional supplement for cancer therapy. In this study, safflower seed extract was demonstrated as a potential adjuvant anticancer agent conferring protection against anticancer drug-induced toxicity without impairing the efficacy of chemotherapy (Figure 7).

5. Conclusion

Our results suggested that the reduction in tumor growth induced by safflower seed extract in RKO cells and mice bearing RKO xenografts was most likely associated with the chemical components, particularly the indolic phenols, *N*-(*p*-coumaroyl)serotonin and *N*-feruloyl serotonin, and the flavonoid luteolin-7-glucoside. Therefore, we will perform further characterization of the anticancer effects of the active compounds present in this extract and conduct a detailed investigation of the responsible molecular mechanisms.

Acknowledgements

References

1. John A, Al Kaabi S, Dweik N, *et al.* Emerging role for colorectal cancer screening in Asian countries. Trop Gastroenterol. 2014; 35:21-24.
2. Reddy BS, Kawamori T, Lubet R, Steele V, Kelloff G,

Rao CV. Chemopreventive effect of *S*-methylmethane thiosulfonate and sulindac administered together during the promotion/progression stages of colon carcinogenesis. Carcinogenesis. 1999; 20:1645-1648.

3. Ijaz S, Akhtar N, Khan MS, Hameed A, Irfan M, Arshad MA, Ali S, Asrar M. Plant derived anticancer agents: a green approach towards skin cancers. Biomed Pharmacother. 2018; 103:1643-1651.

4. Nishino H, Tokuda H, Satomi Y, Masuda M, Onozuka M, Yamaguchi S, Takayasu J, Tsuruta J, Takemura M, Ii T, Ichiishi E, Kuchide S, Okuda M, Murakoshi M. Cancer chemoprevention by phytochemicals and their related compounds. Asian Pacific J Cancer Prev. 2000; 1:49-55.

5. Han SY, Li HX, Ma X, Zhang K, Z. Z. Ma ZZ, Tu PF. Protective effects of purified safflower extract on myocardial ischemia *in vivo* and *in vitro*. Phytomedicine. 2009; 16:694-702.

6. Li HX, Han SY, Wang XW, Ma X, Zhang K, Wang L, Ma ZZ, Tu PF. Effect of the carthamins yellow from *Carthamus tinctorius* L. on hemorheological disorders of blood stasis in rats. Food Chem Toxicol. 2009; 47:1797-1802.

7. Lin WC, Lai MT, Chen HY, Ho CY, Man KM, Shen JL, Lee YJ, Tsai FJ, Chen YH, Chen WC. Protective effect of *Flos carthami* extract against ethylene glycol-induced urolithiasis in rats. Urol Res. 2012; 40:655-661.

8. Mandade R, Sreenivas SA, Wanare R. Antiulcer screening of *Carthamus tinctorius* on volume and acidity of stimulated gastric secretion in rats. J Pharmacol Pharmacother. 2012; 3:185-188.

9. Miyaoka R, Monga M. Use of traditional Chinese medicine in the management of urinary stone disease. Int Braz J Urol. 2009; 35:396-405.

10. Park CH, Lee AY, Kim JH, Seong SH, Jang GY, Cho EJ, Choi JS, Kwon J, Kim YO, Lee SW, Yokozawa T, Shin YS. Protective effect of safflower seed on cisplatin-induced renal damage in mice *via* oxidative stress and apoptosis-mediated pathways. Am J Chin Med. 2018; 46:157-174.

11. Park GH, Hong SC, Jeong JB. Anticancer activity of the safflower seeds (*Carthamus tinctorius* L.) through inducing cyclin D1 proteasomal degradation in human colorectal cancer cells. Korean J Plant Res. 2016; 29:297-304.

12. Komatsu S. Extraction of nuclear proteins. Methods Mol Biol. 2007; 355:73-77.

13. Lipson EJ, Forde PM, Hammers HJ, Emens LA, Taube JM, Topalian SL. Antagonists of PD-1 and PD-L1 in cancer treatment. Semin Oncol. 2015; 42:587-600.

14. Salazar R, Ciardiello F. Optimizing anti-EGFR therapy in colorectal cancer. Clin Cancer Res. 2015; 21:5415-5416.

15. Boulikas T, Vougiouka M. Cisplatin and platinum drugs at the molecular level. Oncol Rep. 2003; 10:1663-1682.

16. Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol. 2003; 23:460-464.

17. Hayes DM, Cvitkovic E, Golbey RB, Scheiner E, Helson L, Krakoff IH. High dose cis-platinum diammine dichloride. Amelioration of renal toxicity by mannitol diuresis. Cancer. 1977; 39:1372-1381.

18. Asgarpanah J, Kazemivash N. Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius* L. Chin J Integr Med. 2013; 19:153-159.

19. Bae SJ, Shim SM, Park YJ, Lee JY, Chang EJ, Choi SW. Cytotoxicity of phenolic compounds isolated from seeds of safflower (*Carthamus tinctorius* L.) on cancer cell lines. Food Sci Biotechnol. 2002; 11:140-146.

20. Koyama N, Kuribayashi K, Seki T, Kobayashi K, Furuhata Y, Suzuki K, Arisaka H, Nakano T, Amino Y, Ishii K. Serotonin derivatives, major safflower (*Carthamus tinctorius* L.) seed antioxidants, inhibit low-density lipoprotein (LDL) oxidation and atherosclerosis in apolipoprotein E-deficient mice. J Agric Food Chem. 2006; 54:4970-4976.

21. Kim DH, Moon YS, Park TS, Son JH. Serotonins of safflower seeds play a key role in anti-inflammatory effect in lipopolysaccharide-stimulated RAW 264.7 macrophages. J Plant Biotechnol. 2015; 42:364-369.

22. Arpornsuwan T, Petvises S, Thim-uam A, Boondech A, Roytrakul S. Effects of *Carthamus tinctorius* L. solvent extracts on anti-proliferation of human colon cancer (SW 620 cell line) *via* apoptosis and the growth promotion of lymphocytes. Songklanakarin J Sci Technol. 2012; 34:45-51.

23. He XW, Feng T, Yin QL, Jian YW, Liu T. NOB1 is essential for the survival of RKO colorectal cancer cells. World J Gastroenterol. 2015; 21:868-877.

24. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem. 1992; 38:1933-1953.

25. Zhuang S, Schnellmann RG. A death-promoting role for extracellular signal-regulated kinase. J Pharmacol Exp Ther. 2006; 319:991-997.

26. Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. Acta Biochim Biophys Sin. 2005; 37:719-727.

27. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999; 6:99-104.

28. Asselin E, Mills GB, Tsang BK. XIAP regulates Akt activity and caspase-3-dependent cleavage during cisplatin-induced apoptosis in human ovarian epithelial cancer cells. Cancer Res. 2001; 61:1862-1868.