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Flavonoids in safflower extract reduce cisplatin-induced damage to human follicle dermal papilla cells by inhibiting DNA damage and *Rad17/Chk1/Cdc25C* signaling

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Key words: Safflower extract, Cisplatin, Human hair follicle dermal papilla cells, Rad17, Hair loss, Cell cycle

Abstract: Background: Cisplatin is a chemotherapeutic agent commonly used clinically for the treatment of various human cancers. Patients often reduce the use of cisplatin due to its side effects, which in turn affects its treatment. This study explored the mechanism of action of safflower extract as an adjuvant traditional Chinese medicine for chemotherapy. **Methods:** Primary human follicle dermal papilla cells (HFDPCs) were used as target cells for cisplatin-induced damage to hair cells. Western blotting was used to investigate the molecular targets of cisplatin and safflower extract in causing HFDPCs damage. Cell survival and cell cycle were analyzed by mitochondrial staining reagent WST-1 and propidium iodide. **Results:** Cisplatin could reduce the viability of HFDPCs without causing cell death. Cisplatin increased the level of phospho-Rad17 in HFDPCs and activated the Chk1/Cdc25C signaling to reduce the expression of Cdc2 protein, thereby arresting the cells in the G2/M phase. The combination of safflower extract and the flavonoids could effectively inhibit the signal transduction of Rad17/Chk1/Cdc25 in cisplatin-treated cells and reduce the cell population in the G2/M phase. Finally, we also confirmed that safflower extract could effectively inhibit the damage to HFDPCs caused by cisplatin, mainly at the level of reducing the DNA damage caused by cisplatin. **Conclusions:** Safflower extract can be used as an adjuvant Chinese medicine for chemotherapy to reduce the damage caused by chemotherapy to normal hair follicle cells.

Introduction

Hair loss crises are a common occurrence that affects the scalp or the entire body. The symptoms may be transient or long-lasting. Additionally, hair loss symptoms may differ between men and women; various contributing factors may be family history of genetic disease, changes in hormonal regulation, infection, medication, medical treatment (e.g., radiation therapy), or stress, among others (York *et al.*, 2020). Chemotherapy-induced alopecia (CIA), continues to be a problem in clinical oncology (Palamaras *et al.*, 2011; Trueb, 2010). More than 50% of cancer chemotherapy patients perceive CIA as a painful side effect leading to poor treatment outcomes (Choi *et al.*, 2014; Wils *et al.*, 2019). The incidence of chemotherapy-induced hair loss varies with the type of chemotherapy drug given, the dosage used, and the route of administration.

Safflower is a traditional Chinese herbal medicine obtained from the dried corolla of *Carthamus tinctorius* L. In traditional Chinese medicine, the use of safflower promotes blood circulation and removes blood stasis (Adamska and Biernacka, 2021; Lu *et al.*, 2021; Yao *et al.*, 2016). Safflower extract was shown to promote the expression of vascular endothelial growth factor and keratinocyte growth factor in dermal papilla cells, thereby promoting the growth of living animal hair (Junlatat and Sripanidkulchai, 2014). Safflower oils on the market, such as

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HT26, are promoted as products for hair care, although there is no scientific evidence for these products to demonstrate their efficacy and targets.

Hair loss caused by chemotherapy is the direct consequence of the toxic effect of chemotherapy drugs on the rapidly dividing cells of hair follicles, in which dermal papilla cells (DPCs) play a key role in the hair growth cycle (Matsuzaki and Yoshizato, 1998; Higgins *et al*., 2013; Chen *et al*., 2020). These cells are also often used as target cells to study the cellular mechanism of hair follicle tissue growth (Madaan *et al*., 2018; Yang and Cotsarelis, 2010). Cisplatin, a chemotherapy drug, promotes DPC apoptosis through the activation of free radicals (Luanpitpong *et al*., 2011). The use of chemotherapy drugs induces the death of follicle cells in the dermal papilla and may lead to hair loss.

In this study, we found that cisplatin caused DNA damage in primary human follicle dermal papilla cells (HFDPCs) and further activated the Rad17 signal transduction pathway. The activation of the Rad17 pathway may lead to cell cycle arrest and decreased cell viability in HFDPCs. Safflower extract can reduce the DNA damage caused by cisplatin on HFDPCs and the activation of Rad17 pathway caused by cisplatin. This suggests that it can be used as an auxiliary traditional Chinese medicine for chemotherapy to reduce its side effects.

Materials and Methods

Preparation of safflower extract and materials

Ten grams of safflower powder (Shun Ten Pharmaceutical Co., Ltd., New Taipei City, Taiwan) was dissolved in 100 mL of distilled water. After incubation at 70°C for 30 min, the supernatant was collected by centrifugation at 14,000 × g and stored at 4°C after filtration through a 0.45 μm pore size filter. Hydroxysafflor yellow A, safflower yellow A, and luteolin were purchased from ChemFaces (Wuhan, Hubei, PRC) with a purity of 98%. Cis-diammineplatinum dichloride (cisplatin) was obtained from Sigma (St. Louis, MO, USA).

Cell culture

HFDPCs were purchased from PromoCell (Heidelberg, Germany). HFDPCs were subcultured with a medium purchased from the manufacturer (ready-to-use follicle dermal papilla cell growth medium). The HFDPCs selected for this study were from the 4^{th} to 10^{th} cell doubling generations. The alkaline phosphatase activity of the dermal papilla hair follicle cells was continuously maintained (Tsai *et al*., 2019).

Detection of cell viability

HFDPCs from the 4^{th} to 10^{th} cell doubling passages were seeded in 24-well culture dishes in triplicate at 3×10^4 cells per well, and the cells were cultured in follicle dermal papilla cell growth medium. The cells were treated with 10–100 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. The WST-1 reagent (100 μL) was added, and the cells were incubated at 37°C for 4–6 h. The supernatant was transferred to a 96-well dish, and the absorbance at 450 and 650 nm was read with an EIA reader (Infinite F200, Tecan, Durham, NC, USA) to estimate the cell viability of the cells.

Detection of cell death

HFDPCs from the 4^{th} to 10^{th} cell doubling passages were seeded in 24-well culture dishes in triplicate with a cell number of at 3×10^4 cells per well, and the cells were cultured in Dulbecco's modified Eagle medium containing 1% fetal calf serum. After treatment with 10–100 μg/mL safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h, the cell supernatant was collected. After removing the residual cell pellet by centrifugation at 1500 × g for 5 min, 20 μL of the cell culture supernatant was removed and analyzed for lactate dehydrogenase (LDH) activity with the Cytotoxicity Detection Kit (Sigma) to estimate cell death.

Western blotting

HFDPCs from the 4^{th} to 10^{th} cell doubling passages were plated in 10-cm culture dishes at a cell number of 1×10^6 and cultured for 24 h. After treatment with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h, RIPA buffer containing protease inhibitor cocktail (Roche Diagnostics, Germany) and phosphatase inhibitors (50 mM Tris [pH 8.0], 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium deoxycholate) was used to lyse the cells. Cell lysates were collected, and the protein concentration was measured. Proteins from 20–60 μg of cell extracts were separated on 10%–12% polyacrylamide gels, transferred to PVDF membranes, and allowed to react with various primary antibodies for 12 h at 4°C. Then, the corresponding horseradish peroxidase-conjugated secondary antibodies (Calbiochem, Darmstadt, Germany) was added at room temperature for 2 h, and the Amersham ECL kit (Amersham, Bucks, UK) was used for enzyme coloration, which was visualized by placing in a luminometer for image development and film scanning. The antibodies used in this study were phospho-Rad17 (Ser635), Rad17, phospho-Chk1 (Ser345), Chk1, phospho-cdc25C (Ser216), cdc25C, cyclin A, cyclin B, and phospho-Histone H2A.X (Ser139), obtained from Cell Signaling Technology (Beverly, MA, USA). Antibodies recognizing actin were purchased from Sigma–Aldrich. Antibodies recognizing Cdc2 were purchased from Proteintech (IL, USA).

Cell cycle analysis

HFDPCs from the 4^{th} to 10^{th} cell doubling passages were planted in 10-cm culture dishes at a cell number of 1×10^6 and cultured for 24 h. After treatment with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 48 h, cells were harvested and fixed in ice-cold 80% ethanol at −20°C for at least 12 h. Cells were stained with propidium iodide solution (20 μg/mL propidium iodide,

0.1% Triton-X 100 and 0.2 mg/mL RNase A) and analyzed by flow cytometry (Cytomics FC 500; Becton Dickinson, Franklin Lakes, NJ, USA). Finally, the percentage of cell cycle phase distribution was analyzed with Multicycle for Windows (Becton Dickinson) software.

Statistical Analysis

The data of each group were obtained in at least three replicates and expressed as the mean ± standard deviation. One-way ANOVA with Dunnett's *post hoc* test was used for statistical analysis, and p values < 0.05 were considered significantly different.

Results

Safflower extract reduces the cisplatin-induced decrease in dermal papilla cell viability

Like previous results (Luanpitpong *et al.*, 2011), cisplatin reduced the viability of HFDPCs. When HFDPCs were pretreated with safflower extract or treated with cisplatin simultaneously, increasing the dose of safflower extract reduced the effect of cisplatin on HFDPC viability. However, post-treatment with safflower extract did not alter the effect of cisplatin on HFDPC viability (Fig. 1A). To further clarify whether cisplatin reduces the cell viability of HFDPCs related to cell apoptosis, we analyzed the content of LDH released by cells. Cisplatin did not increase the amount of LDH released from cells (Fig. 1B), implying that cisplatin does not cause cell apoptosis. In addition, we also found that the cell morphology of HFDPCs did not change in cells treated with cisplatin or safflower extract (Fig. 1C). The above results imply that cisplatin reduces the viability of HFDPCs not due to cell death. Only high doses (50–100 μg/mL) of safflower extract significantly inhibited the effect of cisplatin on HFDPCs. For possible clinical use *in vivo* in the future, we adopted a concentration of 50 μg/mL in subsequent experiments to examine the effect of safflower extract on cisplatin-regulated intracellular signal transduction in HFDPCs.

Safflower extract reduces the level of phospho-Rad17 in primary human follicle dermal papilla cells induced by cisplatin

Cisplatin mainly affects intracellular DNA repair and correction systems, and previous literature studies have pointed out that the Rad17 signal transduction pathway plays an important role in cells affected by cisplatin (Belmonte-Fernandez *et al.*, 2023; Wang *et al.*, 2014; Zhou *et al.*, 2013). In order to further explore the target of cisplatin in inhibiting the cell viability of dermal papilla hair follicle cells, we analyzed the expression of Rad17 in the cells. The results in Fig. 2 show that cisplatin can increase the content of phospho-Rad17 in HFDPCs, but it will not affect the total protein expression of Rad17. Safflower extract reduced the level of cisplatin-induced phospho-Rad17 in cells. The levels of phospho-Rad17 and total Rad17 in HFDPCs treated with safflower extract alone were not affected (Fig. 2).

Safflower extract reduces cisplatin-activated Chk1/Cdc25C signaling in HFDPCs

Phosphorylation of Rad17 was found to be involved in the activation of Chk1 (Wang *et al.*, 2006), and Chk1/Cdc25C/Cdc2 was involved in the regulation of cell cycle G2→M (Aleem and Arceci, 2015; Donzelli and Draetta, 2003; Liu *et*

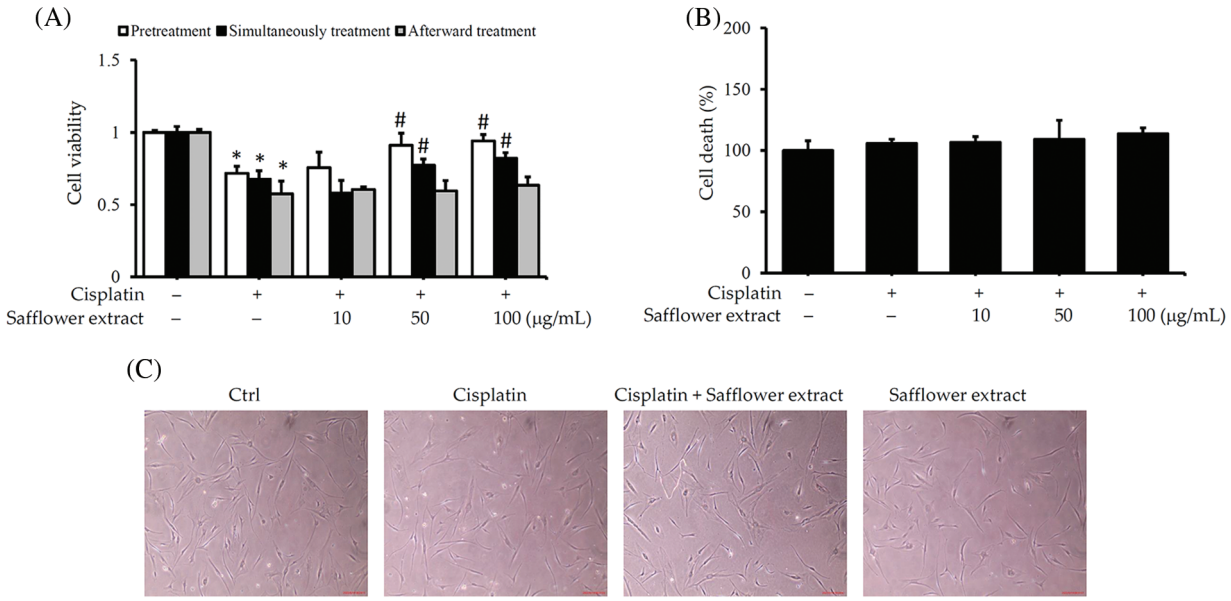

FIGURE 1. Safflower extract reduces the effect of cisplatin on the viability of primary human follicle dermal papilla cells (HFDPCs). HFDPCs were seeded in triplicate in 24-well dishes and cultured overnight. Cells were then treated with safflower extract at concentrations ranging from 10–100 μg/mL for 24 h and subsequently, treated with 100 μM cisplatin for 24 h in the pretreatment group, with both safflower extract and 100 μM cisplatin for 24 h in the simultaneous treatment group, or with 100 μM cisplatin for 24 h followed by safflower extract at concentrations ranging from 10–100 μg/mL for 24 h in the afterward treatment group. Cell viability (A) and cell death (B) were determined by the WST-1 assay and the release of lactate dehydrogenase. (C) Observation of cell type under an optical microscope at 40× magnification. * indicates p < 0.05 compared with the control group, # indicates p < 0.05 compared with the cisplatin-treated group.

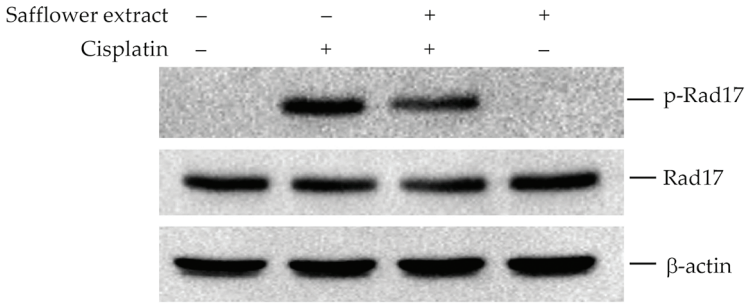

FIGURE 2. Effect of cisplatin on Rad17 protein expression in primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. Cell lysates were immunoblotted using phospho-specific and total Rad17 antibodies.

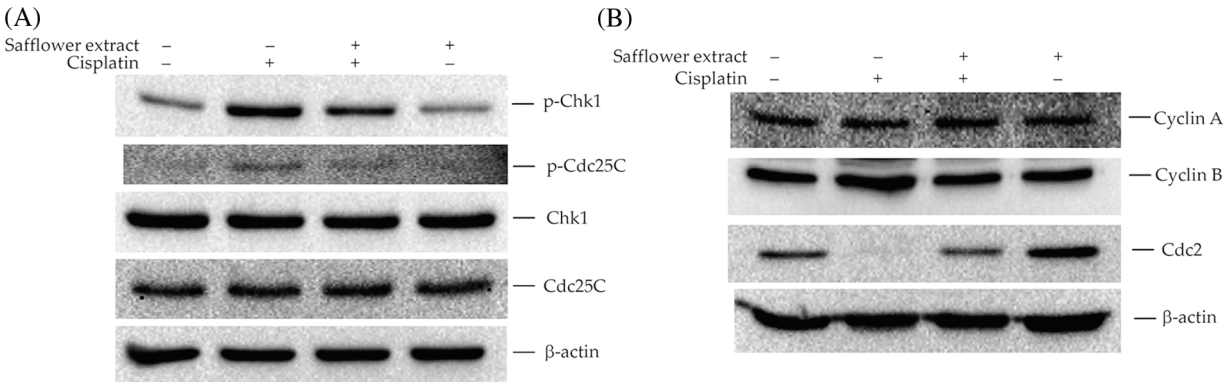

FIGURE 3. Effects of cisplatin and safflower extract on the expression levels of Chk1/Cdc25C signaling proteins in primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. The levels of Chk1 and Cdc25C (A) and cell cycle-related proteins (B) were analyzed by western blotting.

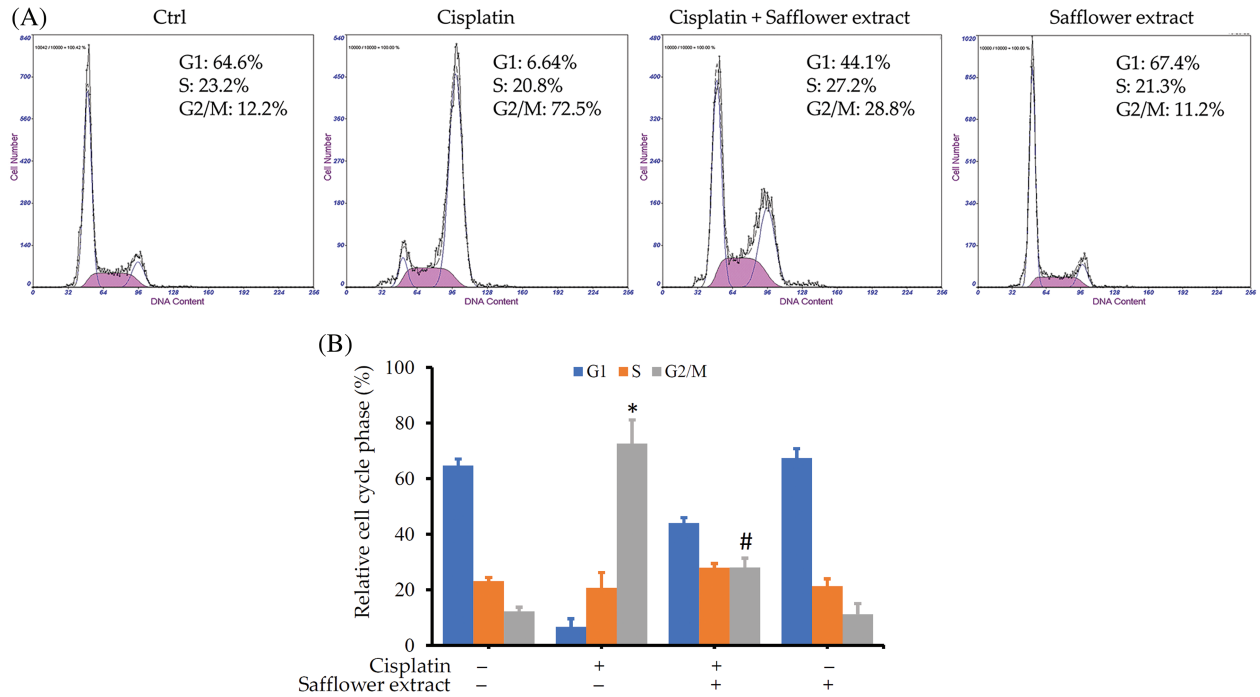

FIGURE 4. Effects of cisplatin and safflower extract on the cell cycle of primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract for 24 h and then treated with 100 μM cisplatin for 48 h. After fixation and staining with propidium iodide, the cell cycle was analyzed by flow cytometry. Shown is the representative chart (A). Experimental results are summarized as the mean (±SD) percentage (B). * indicates $p < 0.05$ compared with the control group, # indicates $p < 0.05$ compared with the cisplatin-treated group.

al., 2020). Therefore, we further analyzed the effect of cisplatin on the expression of cell cycle regulatory proteins in HFDPCs. As shown in Fig. 3A, cisplatin increased the expression levels of phospho-Chk1 (S345) and phospho-Cdc25C (S216) proteins in HFDPCs. Cisplatin also decreased the level of Cdc2 in cells but did not change the expression levels of cyclin A and cyclin B (Fig. 3B). Safflower extract reduced cisplatin-induced expression levels of phospho-Chk1 (S345)

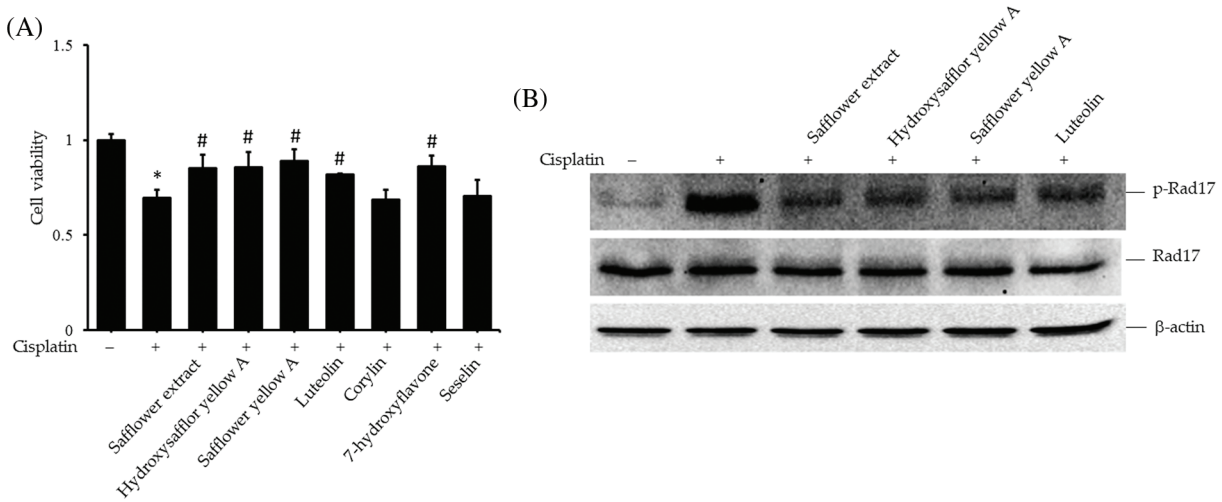

FIGURE 5. Effects of flavonoids found in safflower extract on cisplatin-induced reduction of primary human follicle dermal papilla cells (HFDPC) viability and induced Rad17 expression levels. Cells were then pretreated with 50 μg/mL safflower extract or 25 μg/mL flavonoids for 24 h and then treated with 100 μM cisplatin for 24 h. Cell viability was determined by WST-1 assay (A). HFDPCs were pretreated with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. Cell lysates were immunoblotted using phospho-specific and total Rad17 antibodies (B). * indicates $p < 0.05$ compared with the control group, # indicates $p < 0.05$ compared with the cisplatin-treated group.

and phospho-Cdc25C (S216) and partially restored the expression level of Cdc2 in cells (Fig. 3). Treatment with safflower extract alone did not affect the level of phosphorylated Chk1/Cdc25C in cells.

Safflower extract reduces cisplatin-induced primary human follicle dermal papilla cells cell cycle arrest in the G2/M phase

As shown above, cisplatin activates the Chk1/Cdc25C pathway and leads to a decrease in the expression of the Cdc2 protein. Cdc2 protein mainly regulates the G2/M phase of the cell cycle, so we further analyzed whether cisplatin can affect the cell cycle of HFDPCs. Cisplatin indeed increased the cell population of HFDPCs in the G2/M phase (Fig. 4). Cells treated with safflower extract significantly reduced the number of cells in the G2/M phase caused by cisplatin (Figs. 4A and 4B). However, treatment with safflower extract alone did not affect the cell cycle of HFDPCs.

Flavonoid compounds found in safflower extract reduce cisplatin-stimulated phospho-Rad17/Chk1/Cdc25C signals in primary human follicle dermal papilla cells

To clarify the active compounds of safflower extract in resisting the effect of cisplatin on HFDPCs, we purchased the commercially available and most potentially active flavonoid compounds in safflower extract (Mani *et al.*, 2020; Yu *et al.*, 2013) as well as other flavonoid compounds. We then analyzed whether these compounds have the same effect as safflower extract in inhibiting cisplatin from reducing the cell viability of HFDPCs. The results in Fig. 5A showed that the flavonoid compounds in safflower extract, such as hydroxysafflor yellow A, safflower yellow A, and luteolin, as well as the flavonoid compound 7-hydroxyflavone in the non-safflower extract, can inhibit the decline of HFDPCs cell viability caused by cisplatin (Fig. 5A). We observed that cisplatin treatment increased the level of phospho-Rad17 in

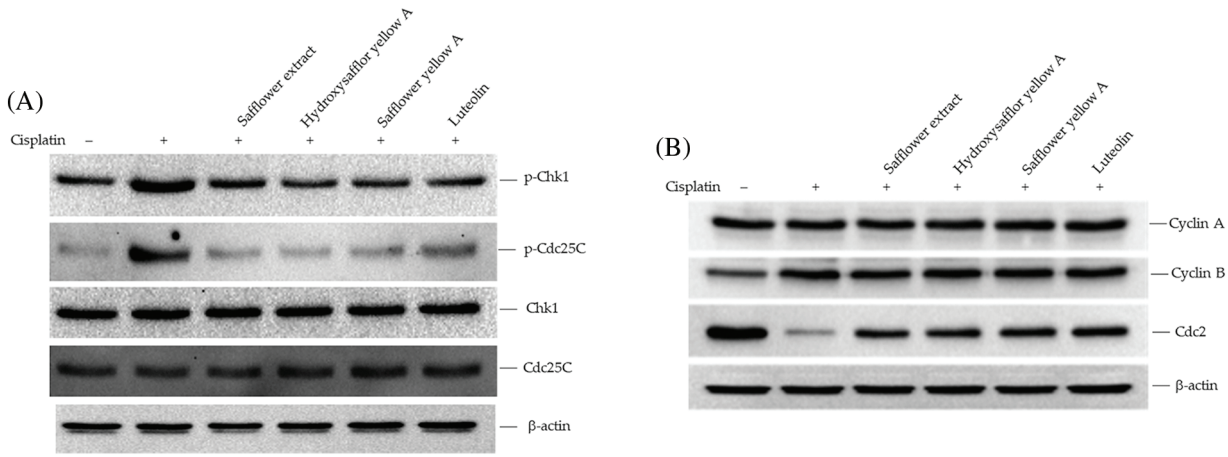

FIGURE 6. Effects of flavonoids found in safflower extract on the expression levels of Chk1/Cdc25C signaling proteins induced by cisplatin in primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. The levels of Chk1 and Cdc25C (A) and cell cycle-related proteins (B) were analyzed by western blotting.

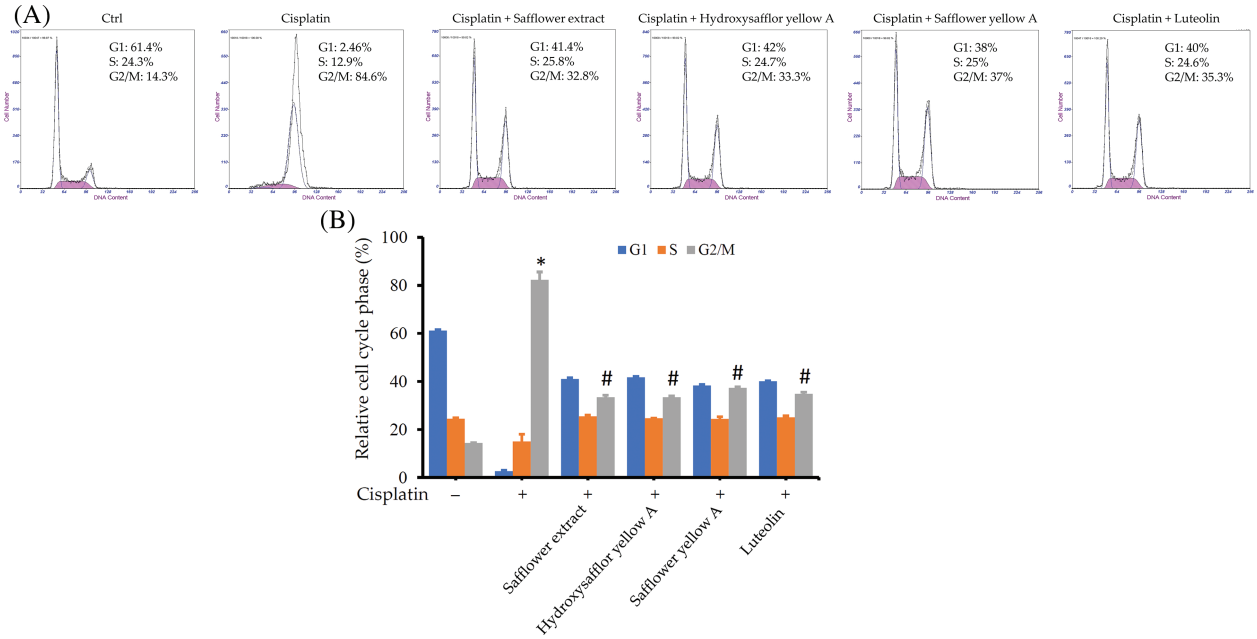

FIGURE 7. Effects of flavonoids found in safflower extract and cisplatin on the cell cycle of primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 48 h. After fixation and staining with propidium iodide, the cell cycle was analyzed by flow cytometry. Shown is the representative chart (A). Experimental results are summarized as the mean (±SD) percentage (B). * indicates $p < 0.05$ compared with the control group, # indicates $p < 0.05$ compared with the cisplatin-treated group.

HFDPCs, while the flavonoid compounds found in safflower extract were able to reduce the expression of phospho-Rad17 induced by cisplatin (Fig. 5B). We also further analyzed the effects of these flavonoid compounds on Chk1/Cdc25C signal transduction and downstream cyclin A/cyclin B/Cdc2 expression. These flavonoid compounds all inhibited increases in phospho-Chk1 and phospho-Cdc25C (Fig. 6A) and the decreased expression of Cdc2 (Fig. 6B) induced by cisplatin.

Flavonoid compounds found in safflower extract reduce cisplatin-induced primary human follicle dermal papilla cell cycle arrest in the G2/M phase

In addition to the protein expression in the Rad17 signaling pathway, we also further explored whether these flavonoid compounds found in safflower extract can also alter the cell cycle of HFDPCs affected by cisplatin. The addition of flavonoid compounds, such as safflower extract, reduced the increase in the number of cells in the G2/M phase of HFDPCs caused by cisplatin (Figs. 7A and 7B). These results suggested that flavonoid compounds may be related to the inhibition of cisplatin by safflower extract on the growth of HFDPCs.

Safflower extract reduces the cisplatin-induced DNA damage of primary human follicle dermal papilla cells

The main targets of cisplatin on cells are the formation of DNA bridges, interference with DNA repair, and the subsequent formation of intracellular free radicals leading to apoptosis of cancer cells (Dasari and Tchounwou, 2014; Go and Adjei, 1999; Tchounwou *et al.*, 2021). We, therefore, investigated whether safflower extract alleviates the effects of cisplatin on cells at the level of DNA damage. The expression of γ-H2AX was used to reflect DNA damage (Collins *et al.*, 2020; Mah *et al.*, 2010; Podhorecka *et al.*, 2010). As shown in Fig. 8, the addition of cisplatin increased the level of γ-H2AX in the cells, reflecting that cisplatin can indeed damage DNA in HFDPCs. The

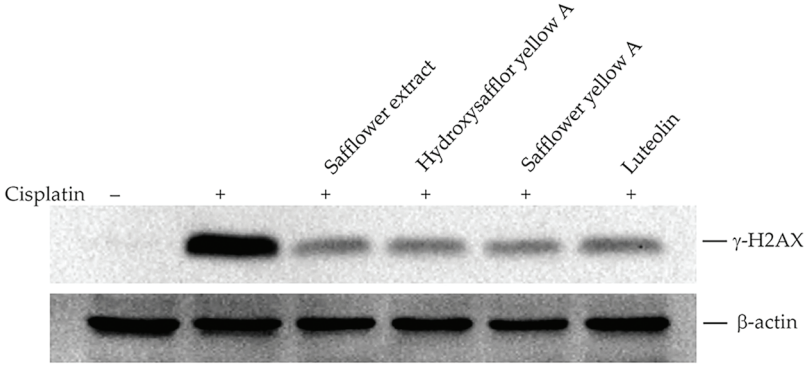

FIGURE 8. Effects of cisplatin and safflower extract on the expression level of γ-H2AX in primary human follicle dermal papilla cells (HFDPCs). HFDPCs were pretreated with 50 μg/mL safflower extract or 25 μg/mL flavonoids found in safflower extract for 24 h and then treated with 100 μM cisplatin for 24 h. The level of γ-H2AX was analyzed by western blotting.

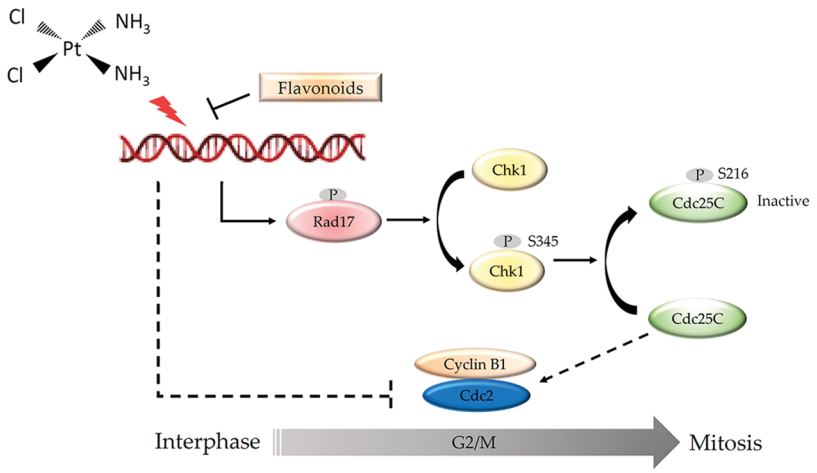

FIGURE 9. Schematic diagram of the mechanism by which flavonoids found in safflower extract inhibits cisplatin-induced G2/M cell cycle arrest in primary human follicle dermal papilla cells (HFDPCs). Cisplatin damages the DNA of HFDPCs in the dermal papilla, thereby increasing the phosphorylation of Rad, Chk1, and Cdc25C proteins and reducing the expression of Cdc2, thus arresting the cell cycle at the G2/M phase. Flavonoids found in safflower extract can inhibit the effect of cisplatin on DNA, thereby attenuating the effect of cisplatin on the cell cycle. The DNA structure was obtained by BioRender software.

addition of safflower extract and the flavonoid compounds found in the safflower extract significantly reduced the expression of cisplatin-induced γ-H2AX. These results indicated that the effect of safflower extract against cisplatin on cells may be at the level of DNA damage.

Discussion

Cisplatin is a commonly used chemotherapeutic agent in clinical practice. Its side effects mainly include ototoxicity and hair loss, and these side effects cause patients to reduce their willingness to use cisplatin, thereby affecting the curative effect of the drug. At present, there are many treatments of a combination of Chinese and Western medicines. For example, by integrating chemotherapy drugs with traditional Chinese medicines such as *Scutellaria barbata* and *Solanum nigrum* to increase the medical treatment willingness and compliance with chemotherapy patients. These traditional Chinese medicines have the ability to kill cancer cells (Fong *et al.*, 2008; Perez *et al.*, 2010; Wang *et al.*, 2015; Uen *et al.*, 2018; Liu *et al.*, 2019), but their effects on hair growth and ear nerve protection have not been reported in the relevant literature, suggesting that the use of these traditional Chinese medicines is mainly to reduce the dose of chemotherapy agents to alleviate the discomfort caused by chemotherapy.

Cisplatin was first used clinically to arrest the growth of gastric cancer cells in the clinic (Petrelli *et al.*, 2013; Sun *et al.*, 2021). Gastric cancer cell lines are cisplatin-sensitive cells (IC50 of 1–10 μM) (Mora-Lagos *et al.*, 2020), implying that the use of clinical drug doses should be less harmful to HFDPCs. Moreover, more than 60% of patients with hair loss due to chemotherapy drugs can usually recover completely within 6 months after the treatment (Bhoyrul *et al.*, 2021). These clinical data show that chemotherapy drugs do not cause irreversible damage to hair growth and support our findings that although cisplatin affects the growth of HFDPCs, it does not cause cell death. We found that cisplatin inhibited the growth of HFDPCs mainly through DNA damage, triggering the phosphorylation of the Rad17 protein and activating the Chk1/Cdc25C signaling pathway, resulting in a decrease in the expression of the Cdc2 protein and arresting the cells in the G2/M phase. The flavonoid compounds found in safflower extract can inhibit the DNA damage in HFDPCs caused by cisplatin so that the downstream Rad17/Chk1/Cdc25 signaling pathway will not be activated by cisplatin, allowing the cells to continue to proliferate (Fig. 9). In the past, studies have also shown that flavonoid compounds can indeed reduce DNA damage (Tiwari and Mishra, 2017, 2022), thereby reducing the generation of cancer cells.

Safflower extract can promote hair growth *in vivo* (Junlatat and Sripanidkulchai, 2014); however, in our study, the use of safflower extract alone did not increase the cell viability of dermal papilla cells, implying that the role of safflower extract *in vivo* may be to promote blood circulation and improve the local environment deep in the hair. The curative effect of promoting hair regeneration using safflower extract should be able to improve the phenomenon of hair loss caused by chemotherapy. However, since flavonoid compounds can reduce the DNA damage caused by cisplatin, whether they affect the ability of cisplatin to kill cancer cells still needs further investigation. A literature review presented that the flavonoids in many plant extracts can promote the apoptosis of gastric cancer cell lines (Lee *et al.*, 2012; Bhosale *et al.*, 2021; Abusaliya *et al.*, 2022). These studies demonstrated that flavonoids can stall the progression of the G2→M cell cycle in AGS gastric cancer cell lines and then cause apoptosis. Although their cellular targets are still not clear, flavonoids may have different activities in normal cells and cancer cells. However, whether these results can support flavonoids as adjuvant Chinese medicine for chemotherapy still needs to be confirmed through more research.

In addition to safflower extract, other plant extracts can also promote hair regeneration, such as *Dicerocaryum senecioides*, *Camellia sinensis* (L.) Kuntze, or *Coffea arabica* L., among others (Kwon *et al.*, 2007; Rambwawasvika *et al.*,

2019; Bussoletti *et al.*, 2020). Several potential active ingredients in hair growth-promoting plant extracts, including steroids, flavonoids, and triterpenoids, have been identified. Among these, flavonoids have been found to be the most effective in promoting hair regeneration in BalB/c mice (Rambwawasvika *et al.*, 2019). *In vivo* experiments have shown that flavonoids, such as the flavonoid-rich extract of *Pinus thunbergii* bark, can also promote hair regeneration, which promotes hair growth in C57BL/6 mice by regulating the expression of inflammatory substances and promoting growth factors (Her *et al.*, 2022). Similarly, the addition of flavonoid-rich extract of *Equisetum hyemale* L. was found to promote hair regrowth in Sprague Dawley rats with chemically-induced alopecia (David *et al.*, 2019).

In addition to flavonoids, other potential active substances, such as tocopherols, carotenoids, phytosterols, and saponins, are also found in safflower extract (Mani *et al.*, 2020). Tocopherol and carotenoids have been shown to reduce the toxicity of cisplatin on ear nerve cells (Leonetti *et al.*, 2003; Firdous and Kuttan, 2012; Kim *et al.*, 2016). Carotenoids and saponins can reduce acute kidney injury caused by cisplatin (Sindhu and Kuttan, 2013; Li *et al.*, 2021). Saponins can also mitigate intestinal damage caused by the drug (Hu *et al.*, 2021). Compared with other chemotherapeutic drugs, the main function of traditional Chinese medicine is to kill cancer cells and thus reduce the dose of chemotherapeutic drugs needed. The main function of safflower extract used clinically, is to directly reduce the damage caused by chemotherapeutic drugs to normal tissues or cells.

Although many studies currently use dermal papilla cells to simulate hair growth conditions, one of the many experimental limitations is that these cells cannot accurately reflect the *in vivo* environment. In a 2D culture environment, dermal papilla cells rapidly lose their hair inductive potential and, therefore cannot be cultured for a long time. However, now many 3D culture technologies are available for dermal papilla cells combined with special scaffolds that can simulate the *in vivo* environment and support long-term culture (Betriu *et al.*, 2020; Bejaoui *et al.*, 2022; Yoon *et al.*, 2022). This is beneficial for studying various drugs. Therefore, the use of a 3D culture environment to study the effects of safflower extract on cisplatin-induced damage to HFDPCs will help to explore more cellular mechanisms and better approximate the *in vivo* situation.

In summary, cisplatin inhibits hair growth, possibly by causing DNA damage in HFDPCs and causing cell cycle arrest through the Rad17/Chk1/Cdc25c signaling. Safflower extract can reduce the DNA damage caused by cisplatin in HFDPCs, thereby reducing the decrease in cell viability caused by cisplatin.

Conclusions

Our research demonstrated that the safflower extract and flavonoids contained in the extract can alleviate cisplatin-induced decrease in the viability of dermal papilla cells, and the main mechanism of action is to reduce the level of DNA damage caused by cisplatin. In conclusion, safflower extract or its flavonoid compounds can be developed as chemotherapy adjuvant drugs, which can slow down the side effects of hair loss caused by chemotherapy and increase the willingness of patients to take chemotherapy.

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