

Original Article

Evaluation of apoptotic effect of crocin, cisplatin, and their combination in human oral squamous cell carcinoma cell line HN5

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ABSTRACT

Background: Squamous cell carcinoma (SCC) is the most common oral malignancy with high rate of mortality. Cisplatin, as the most effective chemotherapy drug, has side effects. Considering the studies on the use of crocin in saffron in the treatment of various malignancies, this study aimed at investigating the effects of crocin and cisplatin and their combination on SCC and fibroblast cell lines.

Materials and Methods: In this interventional study, HN5 and fibroblast cell lines were treated with different concentrations of crocin (12.5–50 µg/mL) and cisplatin (2, 4, 8, 16, and 32 µg/mL), and the cells were counted after 24, 48, and 72 h by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Data were analyzed with SPSS Version 17, and $P < 0.05$ was considered the level of significance. In the final stage, flow cytometry after 24 h in terms of the pattern of cell death was done.

Results: Both drugs had a toxic effect on malignant cells. One point was the high toxic effect of 8 µg/mL cisplatin not only on cancer cells ($P < 0.001$) but also on fibroblasts. However, combination with 12.5 µg/mL of crocin had the same effect on HN5 cell line, despite the less toxic effect in fibroblasts in comparison with cisplatin alone ($P = 0.012$). Apoptosis was the pattern of cell death showed by flow cytometry.

Conclusion: Crocin in high concentrations can have not only significant toxicity in cancer cells but also side effects in healthy tissue. It seems that lower doses of crocin, in combination with cisplatin, besides having anticancer effect, can reduce the toxicity of cisplatin in healthy tissue.

Key Words: Apoptosis, carcinoma, cell culture techniques, cisplatin, crocus, squamous cell

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts for >90% of oral cavity malignancies. Various factors contribute to the development of this malignancy. Several epidemiological studies suggest that high intake of fruits and vegetables reduces the risk of cancers, including oral cancer.^[1] In spite of significant advances in the treatment of such diseases in recent

years, the survival rate of patients with advanced carcinomas following surgery or radiotherapy is still disappointing. According to the reports, a combination therapy of surgery, radiotherapy, and chemotherapy increases the overall 5-year survival rate by 6.5%.^[2] It is also said that patients undergoing chemotherapy have a better overall survival rate than others.^[3]

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Chemotherapy seems to have many benefits to patients, especially in reducing metastasis, improving local control, improving longer survival, and maintaining organ function,^[4] but resistance to drugs can affect the success of chemotherapy.^[5] Today, the link between apoptosis and cancers has attracted a lot of attention. Since the mechanism of many chemotherapy drugs is to induce apoptosis in cancerous cells, the study of apoptosis can provide researchers with good clues about the effects of chemotherapy drugs.^[6] One of the important chemotherapy agents commonly used to induce apoptosis in OSCC is cisplatin, a first-generation anticancer agent.^[7-10] In different parts of the world, natural compounds and herbal extracts are used to induce apoptosis and control SCC.^[11] Due to the rapid progression of this malignancy and its inherited and acquired chemical resistance, treatment with cisplatin fails in some patients.^[12,13] In addition, there are reports of multiple side effects, including nephropathy and severe kidney damage, digestive toxicity, myelosuppression, autotoxicity, neuropathy, and vascular injury in patients treated with cisplatin.^[14,15]

Since the trend toward traditional and herbal medicines is nowadays rising alongside treatment with chemical drugs, research was performed on plants that appear to have potential anticancer effects.^[16,17]

Saffron, *Crocus sativus*, a native Iranian herb, is one of the most expensive spices in the world, which is added to food for its taste and color; it is also used in traditional medicine for its remedial effects. Many studies showed that saffron has an anticancer effect, which is attributed to its bioactive compounds such as crocin and crocetin. These compounds, which are abundant in saffron, induce apoptosis and inhibit proliferation in cells. In pharmacological studies, antiseizure, antidepressant, anti-inflammatory, antioxidant, and antitumor effects are considered for active compounds of saffron.^[18,19] Crocin is the most important anticancer compound of saffron; it seems that this compound exerts this effect by making alterations at the gene level and induction of apoptosis in the cancerous cells.^[20] The results of an experiment on healthy individuals showed that saffron is a relatively safe drug.^[21] Another study was done on the effect of crocin on OSCC cell line as a potent anticancer drug.^[22] Both authors recommended other researchers to do further experiment. Hence, the present study aimed at

evaluating the simultaneous effects of crocin and cisplatin on HN5 cell line and healthy fibroblasts.

MATERIALS AND METHODS

The current experimental and interventional study was conducted on the effect of cisplatin and crocin on the squamous carcinoma cell line (HN5) and fibroblasts as soon as its protocol was approved by the Ethics Committee of Babol University of Medical Sciences (ethical code: IR.MUBABOL.REC.1397.039).

Cell culture

The HN5 cell line (code 196) was purchased from the National Cell Bank of Iran affiliated to the Pasteur Institute of Tehran, Iran. HN5 cell line (RRID: CVCL_8128) had been isolated from SCC of the tongue of a 73-year-old male patient with tumor stage T2N0M0 and moderate level of differentiation^[23] and cultured in the RPMI1640 medium (Biowest) and 10% fetal bovine serum (FBS; Biosera, South America). When cells reach 80% confluence, for cell passaging, 1% Trypsin-EDTA (ATOCEL Company, Budapest) was added and incubated. Then, a complete cell culture medium (10% FBS + 1% PS) was added. Penstrep (ATOCEL Company, Budapest), a solution containing standard antibiotics, penicillin and streptomycin, was added to prevent growth of a variety of Gram-positive and Gram-negative bacteria.

For fibroblast preparation, the foreskins removed under sterile conditions in the operating room of the Amirkola Children and Babol Clinic Hospitals were added to cell culture medium containing DMEM 80% +10% FBS + 10% penicillin-streptomycin to obtain fresh cells.

Crocin and cisplatin preparation

The powdered crocin extract was purchased from Bu Ali Research Institute of Mashhad University of Medical Sciences, Mashhad, Iran. The extract was prepared by crystallization method, and 80% ethanol was used as solvent. The crystals had >97% purity.^[24]

The 50-mL vials of 1 mg/mL cisplatin were purchased from Mylan Company, France.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

Cell proliferation was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 24, 48, and 72 h. MTT powder was mixed in PBS medium, and the resulting

solution was added to the wells of 96-well microplate containing cell lines and different concentrations of drug (cisplatin 2–32 µg/mL, crocin 12.5 and 50 µg/mL, and combinations of the two drugs). After 4 h incubation at 37°C, dimethyl sulfoxide was added to the wells and the absorption rate was read at 570 nm.

Flow cytometry

After treatment with crocin and cisplatin alone or in combination at different concentrations, the cells were trypsinized and incubated with annexin V-conjugated fluorescein isothiocyanate (FITC) and propidium iodide (PI) (Invitrogen; Thermo Fisher Scientific Company), according to the manufacturer's protocol. Then, apoptosis rate was evaluated on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA), and the data were evaluated using Cell Quest (BD Biosciences) and FlowJo (Tree Star Inc., Ashland, OR, USA) software. The early apoptotic cells (FITC⁺/PI⁻), late apoptotic cells (FITC⁺/PI⁺), necrotic cells (FITC⁺/PI⁺), and intact cells (FITC⁻/PI⁻) were differentiated according to the staining profile.

Statistical analysis

Data were analyzed with SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA) using ANOVA, repeated-measures ANOVA, and LSD *post hoc* test. A $P < 0.05$ was considered the level of significance.

RESULTS

Cytotoxic effects of crocin and cisplatin on HN5 cell line and fibroblast

Most of these different concentrations of cisplatin (2, 4, 8, 16, and 32 µg/mL) and crocin (12.5 and 50 µg/mL) had inhibitory effects on the cell proliferation in HN5 cell line and fibroblast in a dose- and time-dependent manner.

Combined concentrations of the two drugs were also tested on both cell lines. Results of cell counting after 24, 48, and 72 h are shown in Graph 1 and Tables 1 and 2. It is noteworthy that 12.5 µg/mL of crocin alone did not have a significant toxicity on cancer cells after 72 h ($P = 0.323$), but in combination with 2 and 4 µg/mL of cisplatin, besides the additional effect of the drug in the HN5 cell line, it had inhibitory effect in fibroblasts.

Combination of 50 µg/mL crocin + 16 µg/mL cisplatin had an additional effect on both cell lines, but the combination of 12.5 µg/mL crocin + 8 µg/mL cisplatin had toxic effect on HN5 cell line and less toxicity on fibroblasts in comparison with 8 µg/mL cisplatin alone ($P = 0.012$). Therefore, it seems that crocin at certain concentrations can reduce the cytotoxicity of cisplatin in the healthy tissue.

Table 1: The effect of different concentrations of crocin and cisplatin (µg/mL) on viability rate of the squamous carcinoma cell line (HN5) after 24, 48, and 72 h

Drug concentration (µg/mL)	Mean±SE		
	24 h	48 h	72 h
Control	100±2.13	100±1.64	100±1.86
Crocin 12.5	78.40±1.07*	94.44±2.65	96.10±1.19
Crocin 50	68.58±1.07**	82.76±0.70**	89.10±1.36*
Cisplatin 2	88.22±3.06*	87.62±1.41***	65.96±0.81**
Cisplatin 4	74.80±2.44*	67.59±1.48**	45.12±0.69***
Cisplatin 8	63.34±1.30**	56.25±0.80**	25.62±0.87***
Cisplatin 16	58.10±1.61**	10.42±0.72***	8.25±0.16***
Cisplatin 32	29.46±1.50***	9.14±0.23***	4.79±0.27***
Crocin 12.5+cisplatin 2	85.92±2.25	90.05±2.92	64.36±1.46***
Crocin 12.5+cisplatin 4	80.36±2.80*	66.32±1.45**	41.76±0.77**
Crocin 12.5+cisplatin 8	65.63±1.73*	62.27±1.98***	23.14±1.16***
Crocin 12.5+cisplatin 16	50.57±1.72**	12.50±1.97***	7.54±0.54***
Crocin 12.5+cisplatin 32	33.06±0.66***	9.34±0.12***	5.67±0.09***
Crocin 50+cisplatin 2	85.76±2.63	61.11±1.20***	55.67±1.54**
Crocin 50+cisplatin 4	64.16±1.56***	54.05±1.51***	33.87±0.79***
Crocin 50+cisplatin 8	57.12±1.15**	43.06±1.40***	18.17±1.00***
Crocin 50+cisplatin 16	50.08±1.70***	14.24±0.92***	6.29±0.36***
Crocin 50+cisplatin 32	36.99±1.71***	7.18±0.64***	4.52±2.27***
P	<0.001	<0.001	<0.001

Cell viability rate was assessed by MTT assay. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control group in every column. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SE: Standard error

Table 2: The effect of different concentrations of crocin and cisplatin ($\mu\text{g/mL}$) on viability rate of the fibroblast after 24, 48, and 72 h

Drug concentration ($\mu\text{g/mL}$)	Mean \pm SE		
	24 h	48 h	72 h
Control	100 \pm 1.32	100 \pm 2.19	100 \pm 1.30
Crocini 12.5	99.79 \pm 1.48	74.27 \pm 1.64***	82.74 \pm 3.83*
Crocini 50	94.30 \pm 3.95	74.10 \pm 3.31**	81.08 \pm 1.96*
Cisplatin 2	96.62 \pm 1.52	80.10 \pm 2.60***	90.72 \pm 1.47*
Cisplatin 4	91.56 \pm 4.28	79.59 \pm 4.05*	76.07 \pm 3.54*
Cisplatin 8	87.76 \pm 3.29	66.55 \pm 1.53*	67.72 \pm 2.45*
Cisplatin 16	70.46 \pm 2.11**	51.97 \pm 2.06**	58.07 \pm 2.58**
Cisplatin 32	64.14 \pm 1.06**	40.31 \pm 0.96**	34.51 \pm 4.18**
Crocini 12.5+cisplatin 2	103.17 \pm 1.32	80.28 \pm 3.37	89.61 \pm 1.93
Crocini 12.5+cisplatin 4	93.67 \pm 2.19	78.90 \pm 0.34**	85.90 \pm 2.41
Crocini 12.5+cisplatin 8	86.92 \pm 3.07	66.73 \pm 1.64*	79.78 \pm 1.13**
Crocini 12.5+cisplatin 16	87.98 \pm 1.93	59.69 \pm 1.57**	52.88 \pm 3.17**
Crocini 12.5+cisplatin 32	79.96 \pm 1.65*	36.19 \pm 0.45***	28.01 \pm 1.13***
Crocini 50+cisplatin 2	93.25 \pm 0.92*	71.19 \pm 1.79***	85.71 \pm 3.06*
Crocini 50+cisplatin 4	85.23 \pm 1.12*	66.73 \pm 2.47**	76.62 \pm 1.83**
Crocini 50+cisplatin 8	81.22 \pm 1.80*	70.84 \pm 0.17**	74.03 \pm 0.85***
Crocini 50+cisplatin 16	83.54 \pm 1.67*	49.74 \pm 1.13***	47.68 \pm 0.93***
Crocini 50+cisplatin 32	80.59 \pm 1.84*	39.80 \pm 2.23**	34.32 \pm 0.49***
P	<0.001	<0.001	<0.001

Cell viability rate was assessed by MTT assay. * P <0.05, ** P <0.01, *** P <0.001 versus control group in every column. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SE: Standard error

Microscopic images of HN5 cell line and fibroblasts after treatment with 16 $\mu\text{g/mL}$ cisplatin + 50 $\mu\text{g/mL}$ crocin are shown in Figure 1.

Flow cytometry

For this purpose, both cell lines were treated with 50 $\mu\text{g/mL}$ of crocin, 8 and 16 $\mu\text{g/mL}$ of cisplatin, as well as the combination of the two-drug concentrations. The results of flow cytometry after 24 h in HN5 cell line and fibroblasts are shown in Graphs 2 and 3, respectively.

In all of the studied groups, the dominant pattern of death was apoptosis in cancer cell line, while it was necrosis and apoptosis in fibroblasts.

DISCUSSION

OSCC is a common malignancy of head and neck in various societies, and despite significant advances in the treatment of malignancies, the rate of mortality in such patients are still high. Therefore, many studies examined the addition of herbal medicines to the therapeutic protocol of this disease to reduce the adverse effects of chemotherapeutic drugs. After reviewing various articles about the effects of herbal drugs on cancerous cells, authors chose crocin; since, in addition to results of various experiments on its efficacy in different cancerous cell lines, this compound

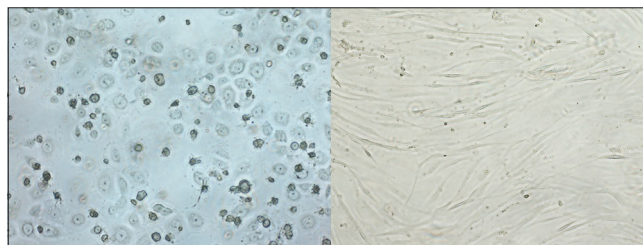
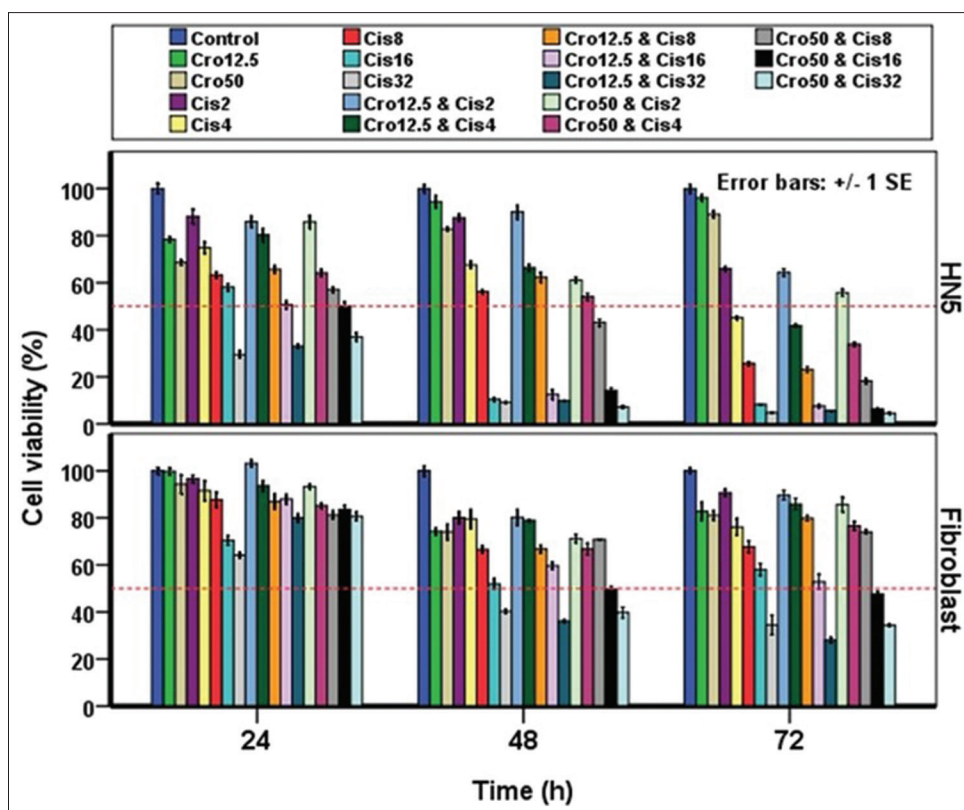


Figure 1: Microscopic view of fibroblasts (right) and HN5 cell line (left) after treatment with 16 $\mu\text{g/mL}$ cisplatin + 50 $\mu\text{g/mL}$ crocin, photographed at $\times 200$. Cytotoxicity is apparent in the HN5 cell line as roundness, vacuolation, and reduced cell density, while it is lower (i.e., reduced cell density, roundness) in fibroblast.

is derived from saffron, which is widely cultivated in different parts of Iran, and producing its extract and drugs seems feasible in domestic research institutes.

According to this fact that cisplatin is the first choice for treatment of OSCC,^[7-10] the current study finally aimed at assessing different concentrations of these two drugs. We also tried to achieve an appropriate dose of crocin in combination with cisplatin to suggest for patients suffering from side effects of chemotherapeutic drugs.

In our study, low concentrations of crocin alone did not have a significant effect on the HN5 cell line, but in combination with cisplatin, besides its additional



Graph 1: The effect of different concentrations of crocin and cisplatin ($\mu\text{g/mL}$) on viability rate of the squamous carcinoma cell line (HN5) and fibroblast after 24, 48, and 72 h. Cell viability rate was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Cro: Crocin, Cis: Cisplatinum).

effects on the cancer cell line, it reduced the cytotoxic effect of cisplatin on fibroblasts. The results showed that cisplatin at different concentrations could remove approximately 95% of cancer cells, but at higher doses, it also has a high cytotoxic effect on fibroblasts.

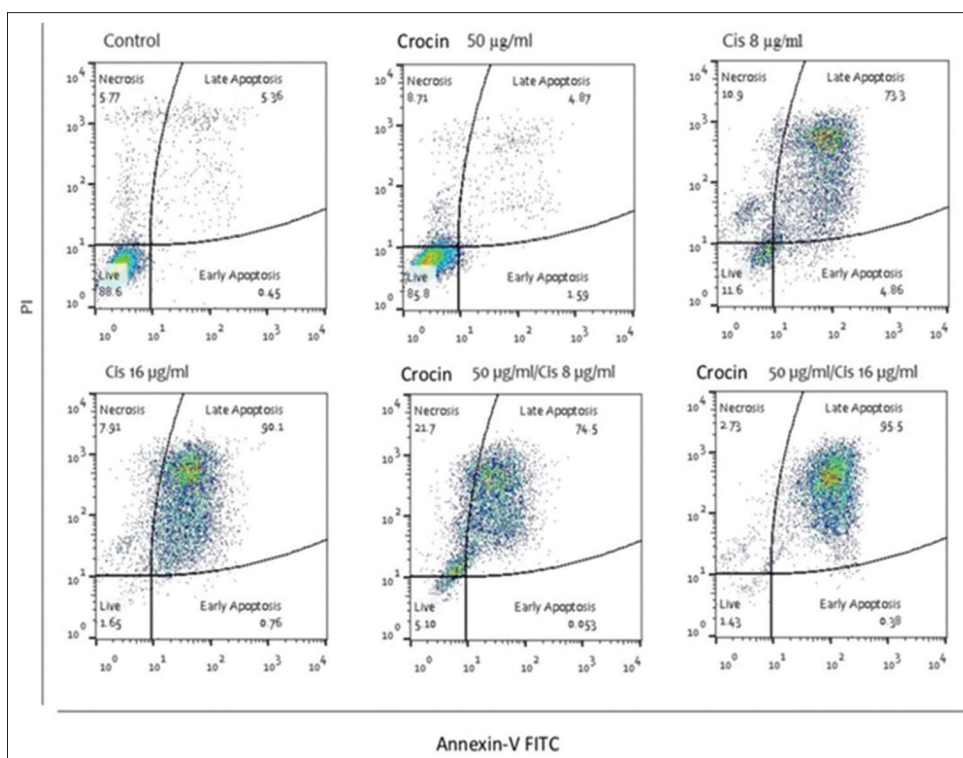
Both types of cells were treated with different concentrations of crocin, and the results indicated that the concentration of the drug that can reduce the viability of cancer cells to 25% of the primary population also kills almost half of the fibroblasts. In addition, with higher concentrations of crocin (800 $\mu\text{g/mL}$), cytotoxicity in fibroblasts was even higher than that of cancerous cells. Therefore, it is predicted that very high concentrations of crocin may lead to unwanted side effects in patients, and it is recommended to administer lower doses of crocin in combination forms.

Based on our finding, since crocin and cisplatin exhibited additional effects at different stages *in vitro*, it seems that reducing the concentration of each does not reduce the toxicity of drugs to cancer cells and may decrease their side effects in healthy tissue.

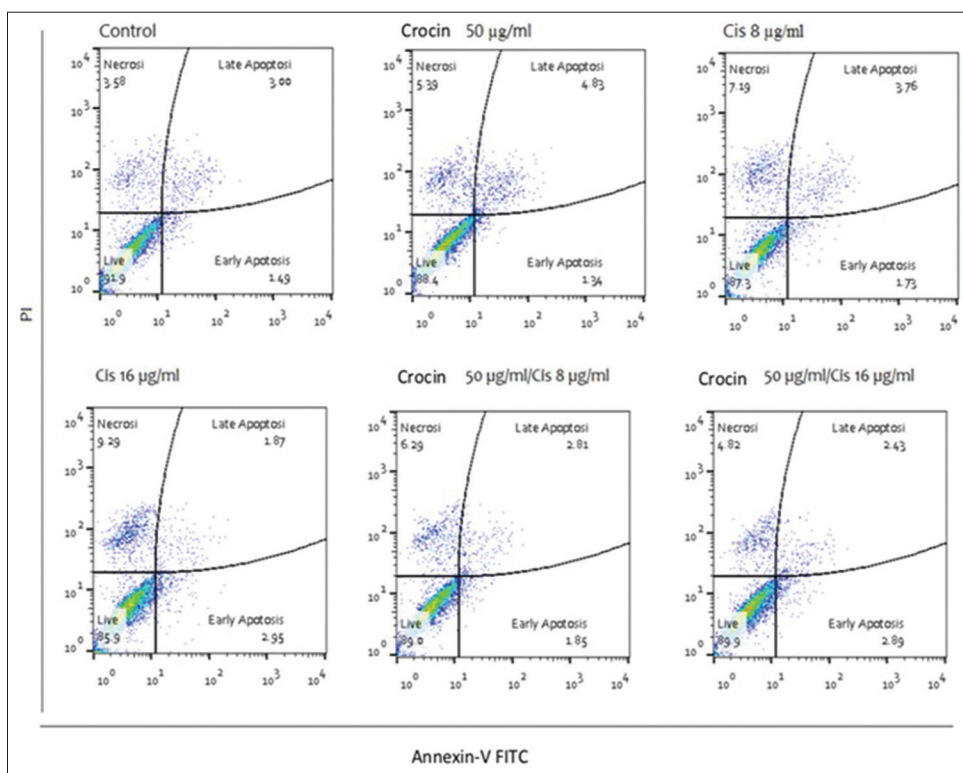
Similar to these findings, Garc-Olmo *et al.* found in their study that high concentrations of crocin that could stop the proliferation of colon cancer cells in the laboratory rats caused acute tubular necrosis in these animals.^[25] However, according to the findings of the study by Aung *et al.*, crocin had no toxicity on healthy cells, in contrast to its effects on malignant colonic cells.^[26] Interestingly, like our findings, about the role of crocin in inhibition of side effects of cisplatin, Khedr *et al.* showed in their study that crocin attenuates hepatotoxicity-induced by cisplatin.^[27]

There are also noticeable findings in other experiments. In the study by Milajerdi *et al.*, the toxic effects of saffron and its components on healthy cells were reported negligible via food intake;^[28] Melnyk *et al.* also reported that effective doses of crocin in lower amounts at micromolar levels have a selective cytotoxic effect on cancerous cells and do not affect healthy cells.^[29]

Along with the present study findings, it was also reported by el Daly that saffron could reduce the renal toxicity of cisplatin.^[30] The results of the study by Mehri *et al.* also showed that the administration



Graph 2: Evaluation of apoptosis by annexin V/propidium iodide in the squamous carcinoma cell line of the tongue (HN5). The cells were treated with different concentrations of crocin and cisplatin and after 24 h, apoptosis rate was measured by flow cytometry and annexin V-propidium iodide double-staining (Cis: Cisplatin).



Graph 3: Evaluation of apoptosis rate by annexin V/propidium iodide in the fibroblast cell line. The cells were treated with different concentrations of crocin and cisplatin and after 24 h, apoptosis rate was measured by flow cytometry and annexin V-propidium iodide double-staining (Cis: Cisplatin).

of crocin to the acrylamide-treated rats reduced the side effects of the drug such as motor disorders, in a dose-dependent manner.^[31]

Therefore, it can be concluded that if moderate doses of cisplatin are administered, its toxic effects in the healthy tissue can be neutralized by the addition of crocin. However, higher concentrations of cisplatin have irreparable toxic effects on fibroblasts that clinically manifest as side effects in patients.

According to the results of the present research and previous studies in this field, it can be concluded that crocin can neutralize the toxic effects of various drugs in the body. Therefore, lowering the dose of cisplatin and adding crocin to drugs are recommended for a more beneficial and less side effects.

The current study experiments indicated that cisplatin has a high toxic effect on the HN5 cell line, but in the clinic, commonly due to side effects and drug resistance, chemotherapy drugs are used in combination form to cumulate the optimum effects of drugs and minimize their side effects.

Similar to our research, Vazifedan *et al.* studied the effect of crocin on HN5 cell line, and the results confirmed that simultaneous crocin intake and radiotherapy could increase the sensitivity to radiation and cell death.^[22] Moreover, Luo *et al.* by studying on BGC-8223 cell line demonstrated for the first time that crocin plus cisplatin may be used as a new anticancer drug for the treatment of gastric cancer.^[32] Therefore, the present study aimed at examining the similar results in the combination of crocin with chemotherapy on HN5 cell; in addition to examining the possible effects of the treatment of fibroblasts on healthy tissue to determine the effective dose of the drug on cancerous cells with minimum side effects.

In a review study conducted by Badie Bostan *et al.*, in Mashhad University of Medical Sciences (2017) on toxic effects of saffron compounds, LD50 in animal samples was 1–5 g/kg of body weight,^[33] which, according to the current study findings, is about 50 mg/kg of body weight, apparently much less than the dosage suggested by Badie Bostan *et al.* Since the present study was performed *in vitro*, this difference can be justified because, in a living body, other organs such as liver are responsible for detoxification, which is impossible in the laboratory environment.

In general, the effects of both drugs, especially at higher concentrations, were more evident after 48 and

72 h, and after 24 h, they had a much lower effect, especially in the fibroblastic cell line. Very little effect after 24 h in the fibroblast cell line may indicate a delay in the manifestation of the side effects of drugs.

In the final stage, concentrations of the drugs with more significant effects were selected for flow cytometry. Flow cytometric assays were performed 24 h after the treatment, and in the fibroblast cell line, the highest amount of necrosis was observed for 16 µg/mL cisplatin, while using the combination of 16 µg/mL cisplatin + 50 µg/mL crocin, the amount of necrosis declined and the apoptosis rate elevated. The flow cytometric results showed that cytotoxicity increased with the addition of crocin to cisplatin, while apoptosis also increased. Of course, changes in the pattern of cell death may occur over time, which did not assess in the present study since flow cytometry was not performed after 48 and 72 h.

A positive point about crocin was that the pattern of cell death in fibroblasts was more likely to apoptosis than necrosis, which can predict the low side effects of this drug in clinical studies. Similarly, in the study by Sun *et al.*, 0.4 µM crocin could induce apoptosis in the squamous carcinoma cell line of the tongue.^[18] Saffron also reduced the viability rate of the cells by inducing apoptosis in the study by Samarghandian *et al.*, on lung cancer cell lines, which is consistent with the current study results.^[34] Moreover, Jabini *et al.* stated that apoptotic effect of crocin in the tumor cells were more than normal cells.^[35]

Colapietro *et al.* showed in their review that saffron and its carotenoids have chemopreventive activity through cancer cell apoptosis and inhabitation of cell proliferation in tongue carcinoma.^[36] In the other similar experiment, Li *et al.* declared that the cell proliferation on KYSE-150 cell line significantly decreased and cell apoptosis was induced with combined crocetin and cisplatin, in comparison with either of them only.^[37]

In a similar study by Mollaei *et al.*, the effects of crocin and cisplatin on another cancerous cell line (human cervical cancer) were studied. The results were the same as those found in the present study, including the fact that cell viability in different concentrations of crocin (0–4 mg/mL) in combination with cisplatin (0.003 mg/mL) significantly reduced after 24, 48, and 72 h, dose and time dependently. Further, in the performed flow cytometry, 1.5 mg/mL crocin and 0.003 mg/mL cisplatin increased initial

apoptosis in a time-dependent manner.^[38] It is also interesting to note that crocin has a synergistic effect with paclitaxel and gamma rays in the MCF-7 cell line (breast cancer). In their study, crocin concentration ranged 1500–6000 µg/mL, which had more significant effects, but the effect of drugs on the healthy cells was not investigated.^[39] According to the current study findings, higher concentrations, although seem to have very good anticancer effects, also showed a high toxicity to fibroblasts, and administration of these amounts in the living creature can lead to severe side effects and even death.

The study by Alizadeh and Bolhassani was conducted on the effect of cytotoxicity of saffron and its compounds on cancerous and healthy cell lines, and the cytotoxicity in cancerous cells was much higher than healthy cells.^[40]

The difference between the current study and most of the similar studies was the simultaneous effect of drugs on the cancer cell line and healthy fibroblasts.

In summary, to the best of authors' knowledge, both cisplatin and crocin had a toxic effect on the HN5 cell line, and this effect was even increased over time. It was also shown that the addition of low concentrations of crocin to cisplatin increases its toxicity in cancerous cells but has an inhibitory effect in the fibroblast cell line. Therefore, we think that crocin, in addition to its anticancer activity, may reduce the toxic effect of cisplatin in healthy cells, and in patients who suffer from the side effects of cisplatin, we can reduce the dose of cisplatin.

CONCLUSION

It can be concluded as a final result that crocin can be used as an auxiliary composition along with cisplatin in the treatment of various malignancies, but its side effects (albeit slight) on healthy tissue should be considered and appropriate doses of crocin should be used as auxiliary treatment.

Finally, it is noted that laboratory research on the effects of drugs on various cell lines is the first step in introducing new compounds and drugs, and similar research and clinical trials are needed to confirm the results.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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