Assessment of MKN-٤٥ cell viability after treatment with crocin, alone and in combination with radiation

Parastoo Azadbeigie[a], Hamid Gholamhosseinian[b], Nazanin Shadanpour[a], Fatemeh B. Rassouli[c]*, Farhang Haddad[a]*

[a] PhD in Cytogenetics,Associate Professor,Dept of Biology,Faculty of Sciences,Ferdowsi University of Mashhad

(FUM)Iran.

[b] Medical Physics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

[c] Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.
Corresponding authors' e-mail: behnam٣٢٠@um.ac.ir; haddad@um.ac.ir , bahadad@yahoo.com

Abstract

Crocin is the main biologically active carotenoid of saffron, which is generally derived from the dried stigma of *Crocus sativus* L. Crocin has considerable pharmaceutical activities including antioxidant, anti-inflammatory, hepatoprotective, hypotensive, antidiabetic and anticancer effects. Gastric cancer is the fifth most common malignancy and the fourth leading cause of cancer-associated death worldwide. The aim of current study was to investigate toxic effects of crocin, alone and in combination with radiation, on human gastric cancer cells for the first time. To do so, MKN-٤٥ cells were treated with ٢ mM crocin for ٧٢ h. Meanwhile, cells were also treated with ٢ mM of crocin for ٢٤ h and then exposed to ٦٠٠ cGy X-ray followed by ٤٨ h recovery. Cell viability was determined after both approaches by alamarBlue assay. Results revealed that ٢ mM crocin decreased cell density and ٢ mM crocin + ٦٠٠ cGy X-ray decreased cell density and changed the morphology of MKN-٤٥ cells. Calculating the viability of cells also indicated that ٦٤٪ of cells were alive after ٧ h treatment with ٢ mM crocin, while this amount was decreased down to ٤٣,٢٪ upon combination of ٢ mM crocin + ٦٠٠ cGy X-ray treatment. Further investigation on other human gastric adenocarcinoma cell lines is recommended to better evaluate combinatorial effects of crocin and radiation *in vitro*.

Key words: Crocin, Radiation, Gastric cancer, Viability assay, Combination.

Introduction

Secondary metabolites and synthesized chemical compounds derived from medicinal plants have attracted a lot of attention in the field of anticancer studies (Hatziagapiou, K et al., ۲۰۲۲). Crocin is the main biologically active carotenoid of saffron that is generally derived from the dried stigma of *Crocus sativus* L. (Vafaei, S et al.,۲۰۲۲). During the last decade, pharmacological studies have shown that crocin has multiple therapeutic effects including antioxidant, anti-inflammatory, hepatoprotective, hypotensive, antidiabetic, anticonvulsant, antidepressant effects to name a few (Mishra, Y. and Mishra, V., ۲۰۲۳). In addition, crocin is noticed for its considerable anticancer effects against liver, cervical, breast and colorectal carcinoma cells, which were manifested by the induction of apoptosis along with inhibition of cell proliferation, invasion and chemotherapy resistance. Gastric cancer is the fifth most common malignancy and the fourth leading cause of cancer-associated death worldwide. Approximately ۱،۱ million new cases and ۷۷۰،۰۰۰ deaths of gastric cancer were estimated in ۲۰۲۰ (Morgan, E et al.,۲۰۲۲). Although surgery, chemical drugs and radiotherapy are available therapeutic modalities for this neoplasm, survival rate in patients with advanced disease in low (Guan, W.L et al.,۲۰۲۳). To introduce a novel and more effective approach, the aim of current study was to investigate toxic effects of crocin, alone and in combination with radiation, on human gastric cancer cells for the first time.

Methods:

MKN-٤٥ cells, a human gastric cancer cell line, were purchased form Pasteur institute (Tehran, Iran). Cells were cultured in Dulbecco's modified Eagle's medium (Capricorn) containing ۱۰٪ fetal bovine serum (Gibco) and ۱٪ penicillin-streptomycin (Betacell), and incubated at ۳۷°C in a humidified atmosphere with ۵٪ CO_2 (Memmert). To evaluate the effects of crocin, MKN-٤٥ cells were seeded in separate ۹٦-well plates (SPL) and incubated outright. Half of the cells were treated with ۲ mM crocin (Sigma) for ۷۲ h, while other cells were first treated with ۲ mM of crocin for ۲٤ h and then exposed to ٦۰۰ cGy X-ray using Elekta Compact™ linear accelerator (Crawley) followed by ٤۸ h recovery. To note, total treatment time point for both groups was ۷۲ h, and untreated cells were considered as control.

To determine cell viability, alamarBlue assay was used. In summary, ۱۸ µl alamarBlue reagent (۰،۱ mg/ml, Sigma) was added to each well and cells were further incubated at ۳۷°C for ۲ h in the absence of light.

Then, optical density (OD) of each well was measured at ٦٠٠ nm using BioTek Epoch microplate spectrophotometer and cell viability (%) was calculated by the following formula: ١٠٠-((OD_T-OD_U)/(OD_B-OD_U)×١٠٠), in which OD_T, OD_U and OD_B stand for OD of treated cells, untreated cells and blank control, respectively.

Results and Discussion:

The viability of MKN-٤٥ cells was investigated upon treatment with crocin alone and in combination with radiation. As presented in Figure ١-A-C, the morphology and density of attached cells changed after treatments; ٢ mM crocin decreased cell density and ٢ mM crocin + ٦٠٠ cGy X-ray decreased cell density and changed the morphology of MKN-٤٥ cells. Calculating the viability of cells also indicated that ٦٤٪ of cells were alive after ٧ h treatment with ٢ mM crocin, while this amount was decreased down to ٤٣,٢٪ upon ٢ mM crocin + ٦٠٠ cGy X-ray treatment (Figure ١-D).

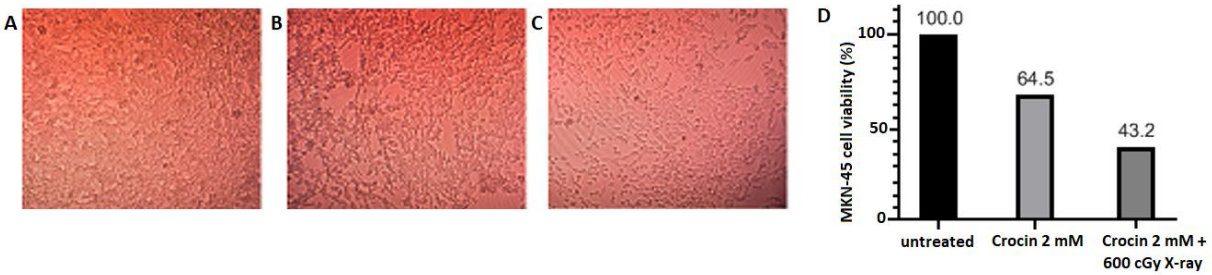

Figure ١: Morphological alterations and viability assessment of MKN-٤٥ cells. Phase contrast micrographs of untreated cells (A), cells treated with ٢ mM crocin (B) and cells treated with ٢ mM crocin + ٦٠٠ cGy X-ray (C) after ٧٢ h. Column graph presents quantitative analysis of cell viability (D).

In conclusion, findings of our study indicated that ٢ mM crocin induced toxic effects on MKN-٤٥ cells, and combination of crocin with X-ray reduced cells viability as well. Further investigation on other human gastric adenocarcinoma cell lines is recommended to better evaluate combinatorial effects of crocin and radiation *in vitro*.

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ارزیابی زنده ماندن سلول MKN-۴۵ پس از درمان با کروسین، به تنهایی و همراه با پرتو

پرستوآزادبیگی[الف] ، حمید غلامحسینیان[ب]، نازنین شادان پور[الف] ، فاطمه بهنام رسولی[ج*]، فرهنگ حداد[الف*]

الف) گروه زیست شناسی، دانشکده علوم، دانشگاه فردوسی مشهد، مشهد، ایران.
ب) مرکز تحقیقات فیزیک پزشکی، دانشگاه علوم پزشکی مشهد، مشهد، ایران.
ج) گروه تحقیقاتی نوین تشخیص و درمان، پژوهشکده بیوتکنولوژی، دانشگاه فردوسی مشهد، مشهد، ایران.

خلاصه:

کروسین کاروتنوئید فعال بیولوژیکی اصلی زعفران است، که عموماً از کلاله خشک Crocus sativus L بدست می‌آید. کروسین دارای فعالیت‌های دارویی قابل‌توجهی از جمله اثرات آنتی اکسیدانی، ضد التهابی، محافظ کبد، کاهش فشار خون، ضد دیابت و ضد سرطان است. سرطان معده پنجمین بدخیمی شایع و چهارمین علت مرگ و میر مرتبط با سرطان در سراسر جهان است. هدف از مطالعه حاضر بررسی اثرات سمی کروسین به تنهایی و همراه با پرتو بر سلول های سرطانی معده انسان برای اولین بار بود. برای انجام این کار، سلول های MKN-۴۵ با کروسین ۲ میلی مولار به مدت ۷۲ ساعت تیمار شدند. در همین حال، سلول ها نیز با ۲ میلی مولار کروسین به مدت ۲۴ ساعت تحت درمان قرار گرفتند و سپس در معرض اشعه ایکس (۶۰۰ سانتی گری) قرار گرفتند و سپس ۴۸ ساعت ریکاوری شدند. زنده ماندن سلول ها پس از هر دو روش با روش آلاماربلو تعیین شد. نتایج نشان داد که کروسین ۲ میلی مولار تراکم سلولی را کاهش داد و کروسین ۲ میلی مولار +اشعه ایکس(۶۰۰ سانتی گری) ، تراکم سلولی را کاهش داد و مورفولوژی سلول‌های MKN-۴۵ را تغییر داد. محاسبه میزان زنده ماندن سلول‌ها نیز نشان داد که ۶۴ درصد سلول‌ها پس از ۷ ساعت تیمار با کروسین ۲ میلی‌مولار زنده بودند، در حالی که این میزان با ترکیب ۲ میلی‌مولار کروسین + پرتو ایکس (۶۰۰ سانتی گری) به ۴۳,۲ درصد کاهش یافت. برای ارزیابی بهتر اثرات ترکیبی کروسین و تابش در شرایط آزمایشگاهی، تحقیقات بیشتر، بر روی سایر رده‌های سلولی آدنوکارسینوم معده انسان توصیه می‌شود.

کلمات کلیدی: کروسین، پرتو، سرطان معده، سنجش زنده ماندن، ترکیبی.

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