Evaluating apoptosis-inducing effects of safranal and radiotherapy on human gastric adenocarcinoma cells

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

[a] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, 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

Abstract

Safranal is a cyclic monoterpene which occurs in saffron (*Crocus sativus*). Gastric cancer is a highly prevalent malignancy worldwide, which is also among the five most common cancers in Iran. Although surgery, chemotherapy and radiotherapy are routine approaches for gastric cancer, the survival rate of patients at advanced stages is low. The purpose of current research was to study apoptosis-inducing effects of safranal and radiotherapy on human gastric cancer cells for the first time. To do so, MKN-٤٥ cells were treated with ١,٦ mM safranal for ٩٦ h. Meanwhile, cells were treated with ١,٦ mM safranal for ٤٨ h, exposed to ٦ Gy ionizing radiation and recovered for ٤٨ h. Cells only exposed to ٦ Gy radiation and recovered for ٤٨ h were also considered in our study. To study changes induced on the cell cycle, treated cells were harvested and suspended in the staining solution containing propidium iodide (PI). For detection of apoptosis, cells were suspended in the binding buffer containing FITC-annexin V and PI and finally analyzed by flow cytometry. Our findings revealed that treatment of human gastric adenocarcinoma cells with safranal led to the accumulation of cells in the sub G١ phase of the cell cycle, and increased the number of early and late apoptotic cells. Likewise, treatment of cells with safranal + radiation also changed the distribution of cells in the cell cycle and increased the number of apoptotic cells. Radiation, however, did not induce considerable changes on MKN-٤٥ cells. More research is suggested to determine the mechanism of safranal action on gastric cancer cells.

Keywords: Safranal, Radiotherapy, Apoptosis, Gastric adenocarcinoma, Cell cycle.

Introduction

In recent years, natural products have been widely used for the prevention and treatment of various diseases. For instance, natural terpenes have attracted a lot of attention as anticancer agents with valuable effects *in vitro* and *in vivo* (Samarghandian and Shabestari, ٢٠١٣). Safranal (٢،٦،٦-trimethylcyclohexa-١،٣-diene-١-carbaldehyde) is a cyclic monoterpene which occurs in saffron (*Crocus sativus*). Safranal produces the aroma of saffron via enzymatic and thermal degradation of the picked stigmas (Amin and Buratovich, ٢٠١٠).

Cancer is one of the most common causes of illness and death worldwide, making it the second highest cause of death globally (Zhou, Y et al.,٢٠١٦). In Iran, cancer is known as the second largest group of chronic diseases and the third most common cause of death after heart disease and accidents (Dolatkhah et al., ٢٠١٥). Gastric cancer is a highly prevalent malignancy worldwide (Farmanfarma et al., ٢٠١٩), which is also among the five most common cancers in Iran (Dehdari, Dehdari and Jazayeri, ٢٠١٦). Routine therapeutic approaches for gastric cancer include resection surgery, chemotherapy, radiotherapy and immunotherapy. Nevertheless, survival rate of this cancer still remains low, specifically when the disease is diagnosed at advanced stages.

Apoptosis is a common type of eukaryotic cell death, which could be induced by external stress like ionizing radiation (Vali F et al., ٢٠١٥). Flow cytometry is a powerful tool to study cell cycle and also detect apoptosis. The purpose of current research was to study apoptosis-inducing effects of safranal and radiotherapy on human gastric cancer cells for the first time.

Methods:

The human gastric adenocarcinoma cell line used in our study was MKN-٤٥ (purchased form Pasteur institute, Tehran, Iran). Cells were maintained in Dulbecco's modified Eagle's medium (Capricorn) supplemented with ١٠٪ fetal bovine serum (Gibco) and ١٪ penicillin-streptomycin (Betacell). MKN-٤٥ cells were incubated at ٣٧°C in a humidified atmosphere with ٥٪ $CO_{٢}$ (Memmert) and subcultured by trypsin ٠,٢٥٪-١mM EDTA (Betacell).

To study the effects of safranal and radiotherapy on the cell cycle and apoptosis induction, MKN-٤٥ cells were seeded in ٩٦-well plates (SPL) and treated with ١,٦ mM safranal (Sigma aldrich) for ٩٦ h. Meanwhile, cells were also treated with ١,٦ mM safranal for ٤٨ h, exposed to ٦ Gy ionizing radiation (Elekta Compact™ linear accelerator, Crawley) and recovered for ٤٨ h (overall treatment time was ٩٦ h). Cells only exposed to ٦ Gy radiation and recovered for ٤٨ h were also considered in our study.

To study changes induced on the cell cycle, cells were harvested using trypsin and centrifuged at ١٤٠٠ rpm for ٥ min. After washing with phosphate buffered saline, the cell pellet was dissolved

in the staining solution containing triton X-١٠٠, sodium citrate and propidium iodide (PI, Sigma). Then, cells were analyzed by BD FACSCalibur using FL-٢ filter. For the detection of apoptosis, cells were harvested and washed similarly, but suspended in biding buffer containing FITC-conjugated annexin V and PI (Zist Pajoohan) and finally analyzed by FL-١ and FL-٢ filters.

Results:

Findings of the present study revealed that safranal changed the distribution of MKN-٤٥ cells in the cell cycle. As shown in Figure ١, ٣٨,٧٪, ٤,٨٪ and ٥٢,٦٪ of cells were detected in $G_{١}$, S and $G_{٢}$/M phases of the cell cycle, respectively. On the other hand, upon ٩٦ h treatment of cells with ١,٦ mM safranal, ٦,٩٪, ٤٥,٦٪, ٧,٨٪ and ٣٨,٨٪ of cells were detected in sub $G_{١}$, $G_{١}$, S and $G_{٢}$/M phases, respectively. Exposure of cells to ٦ Gy radiation did not induce considerable changes in comparison with untreated cells, while after safranal + radiation treatment, ٨,٧٪, ٤١,٢٪, ١٠,٣٪ and ٣٨,٥٪ of cells were detected in sub $G_{١}$, $G_{١}$, S and $G_{٢}$/M phases, respectively.

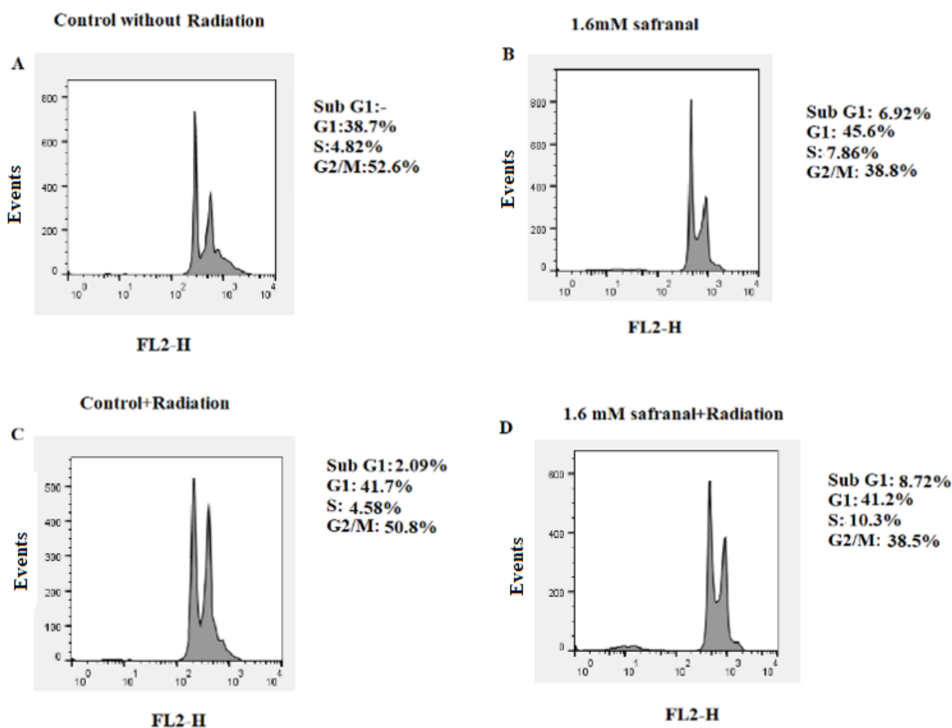

Figure ١: Cell cycle analysis after treatment of MKN-٤٥ cells with safranal, radiation and safranal + radiation. Distribution of cells in different phases of the cell cycle was detected by PI staining and flow cytometry.

To determine whether accumulation of cells in the sub $G_{١}$ phase was due to necrosis or safranal induced apoptosis, cells were examined after staining with FITC-annexin V and PI. Figure ٢ shows analyzed data represented by dot plots. As indicated, ٩٢,٧٪, ١,٢٪, ٥,٧٪ and ٠,٣٪ of untreated cells were alive, necrotic, late apoptotic and early apoptotic, respectively. However, ٧٢ h after treatment with safranal, ٧٦,٢٪, ١,١٪, ٢٠,٦٪ and ٢,١٪ of cells were alive, necrotic, late apoptotic and early apoptotic, respectively. Similar to the cell cycle histograms, no considerable change was observed after radiation exposure in comparison with untreated cells. Nevertheless, after treatment with safranal + radiation, ٦٨,٤٪, ٠,٨٪, ٢٧,٥٪ and ٣,١٪ of cells were detected as alive, necrotic, late apoptotic and early apoptotic, respectively.

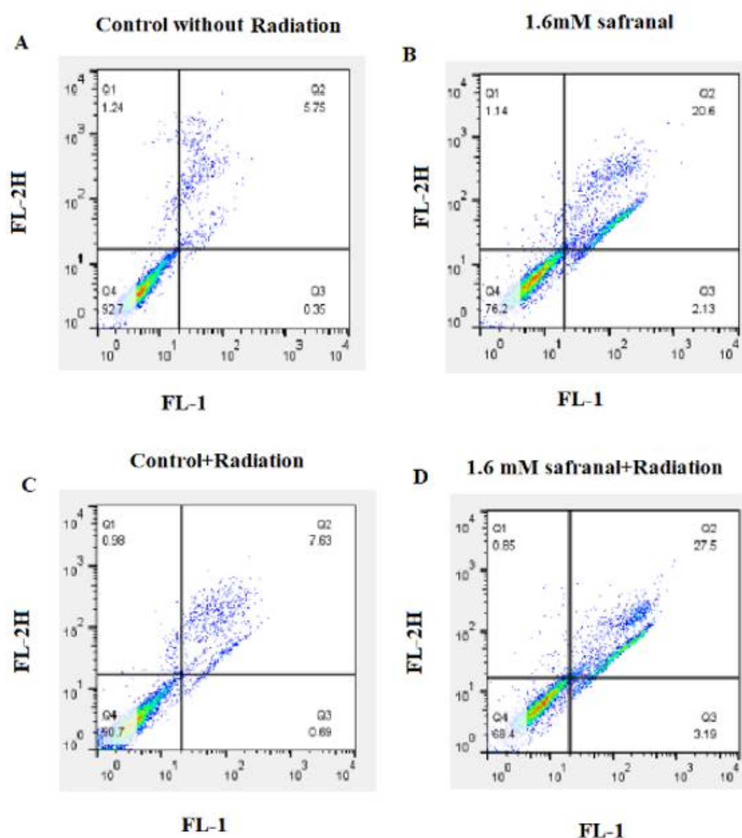

Figure ٢: Detection of apoptosis in MKN-٤٥ cells after treatment with safranal, radiation and safranal + radiation. Alive, necrotic, early apoptotic and late apoptotic cells were detected by FITC-annexin V and PI staining and flow cytometry.

Discussion:

Present study indicated, for the first time, that treatment of human gastric adenocarcinoma cells with safranal led to the accumulation of cells in the sub G١ phase, and increased the number of early and late apoptotic cells. Likewise, treatment of cells with safranal + radiation also changed the distribution of cells in the cell cycle and increased the number of apoptotic cells. Radiation, however, did not induce considerable changes on MKN-٤٥ cells. More research is suggested to determine the mechanism of safranal action on gastric cancer cells.

نازنین شادان پور[الف]، حمید غلامحسینیان[ب]، پرستوآزادبیگی[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

سافرانال یک مونوترپن حلقوی است که در زعفران (Crocus sativus) وجود دارد. سرطان معده یک بدخیمی بسیار شایع در سراسر جهان است که در بین پنج سرطان شایع در ایران نیز قرار دارد. اگرچه جراحی، شیمی درمانی و رادیوتراپی رویکردهای معمول برای درمان سرطان معده هستند، اما میزان بقای بیماران در مراحل پیشرفته پایین است. هدف از پژوهش حاضر بررسی اثرات القا کننده آپوپتوز سافرانال و رادیوتراپی بر روی سلول های سرطان معده انسان برای اولین بار بود. برای انجام این کار، سلول های MKN-۴۵ با ۱,۶ میلی مولار سافرانال به مدت ۹۶ ساعت تیمار شدند. در همین حال، سلول هایی که با ۱,۶ میلی مولار سافرانال به مدت ۴۸ ساعت تیمار شدند، در معرض تابش یونیزان ۶ گری قرار گرفتند و به مدت ۴۸ ساعت ریکاوری شدند. سلول هایی که فقط در معرض تابش ۶ گری بودند و به مدت ۴۸ ساعت ریکاوری یافتند نیز در مطالعه ما در نظر گرفته شدند. برای مطالعه تغییرات ناشی از چرخه سلولی، سلول‌های تیمار شده برداشت و در محلول رنگ‌آمیزی حاوی یدید پروپیدیوم (PI) معلق شدند. برای تشخیص آپوپتوز، سلول ها در بافر اتصال حاوی FITC-annexin V و PI شناور شدند و در نهایت با فلوسیتومتری آنالیز شدند. یافته‌های ما نشان داد که درمان سلول‌های آدنوکارسینوم معده انسان با سافرانال منجر به تجمع سلول‌ها در فاز قبل G۱ (sub G۱) چرخه سلولی و افزایش تعداد سلول‌های آپوپتوز اولیه و ثانویه می‌شود. به همین ترتیب، تیمار سلول ها با پرتو +سافرانال نیز توزیع سلول ها را در چرخه

سلولی تغییر داد و تعداد سلول های آپوپتوز را افزایش داد. پرتودهی، با این حال، تغییرات قابل توجهی در سلول های MKN-۴۵القاء نمی کند. تحقیقات بیشتری برای تعیین مکانیسم اثر سافرانال بر روی سلول های سرطانی معده پیشنهاد می شود.

واژه‌های کلیدی: سافرانال، رادیوتراپی، آپوپتوز، آدنوکارسینوم معده، چرخه سلولی.

References:

Amin, A. and Buratovich, M. (۲۰۱۰) ‘The Anti-Cancer Charm of Flavonoids: A Cup-of-Tea Will Do You Good!’, Frontiers in Anti-Cancer Drug Discovery, pp. ٥٥٢–٥٨٧. doi: ١٠,٢١٧٤/٩٧٨١٦٠٨٠٥١٦١٨١١٠٠١٠١٠٥٥٢.

Dehdari, T., Dehdari, L. and Jazayeri, S. (٢٠١٦) ‘Diet-related stomach cancer behavior among iranian college students: A text messaging intervention’, Asian Pacific Journal of Cancer Prevention, ١٧(١٢), pp. ٦٠٦٥–٦٠٧٢. doi: ١٠,٢٢٠٣٤/APJCP.٢٠١٦,١٧,١٢,٦٠٦٥.

Dolatkhah, R. et al. (٢٠١٥) ‘Increased colorectal cancer incidence in Iran: A systematic review and meta-analysis Chronic Disease epidemiology’, BMC Public Health, ١٥(١). doi: ١٠,١١٨٦/s١٢٨٨٩-٠١٥-٢٣٤٢-٩.

Farmanfarma, K. K. et al. (٢٠١٩) ‘Brain Cancer in the World: an Epidemiological Review’, World Cancer Research Journal, ٦(٤), pp. ١–٥. doi: ١٠,٣٢١١٣/wcrj_٢٠١٩٧_١٣٥٦.

Samarghandian, S. and Shabestari, M. M. (٢٠١٣) ‘DNA fragmentation and apoptosis induced by safranal in human prostate cancer cell line’, Indian Journal of Urology, ٢٩(٣), pp. ١٧٧–١٨٣. doi: ١٠,٤١٠٣/٠٩٧٠-١٥٩١,١١٧٢٧٨.

Vali, F., Changizi, V. and Safa, M. (٢٠١٥) ‘Synergistic Apoptotic Effect of Crocin and Paclitaxel or Crocin and Radiation on MCF-٧ Cells, a Type of Breast Cancer Cell Line’, International Journal of Breast Cancer, ٢٠١٥. doi: ١٠,١١٥٥/٢٠١٥/١٣٩٣٤٩.

Zhou, Y. et al. (٢٠١٦) ‘Natural polyphenols for prevention and treatment of cancer’, Nutrients, ٨(٨). doi: ١٠,٣٣٩٠/nu٨٠٨٠٥١٥.