Inhibitory and Cytotoxic Activities of Chrysin on Human Breast Adenocarcinoma Cells by Induction of Apoptosis

Saeed Samarghandian¹, Mohsen Azimi-Nezhad²,³, Abasalt Borji¹, Malihe Hasanzadeh⁴, Farahzad Jabbari⁴, Tahereh Farkhondeh⁵, Mohammad Samini³

¹Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, ²Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, ³Allergy Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, ⁴Department of Immunogenetic and Cell Culture, Immunology Research Center, School of Medicine, ⁵Department of Gynecology Oncology, Woman Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Submitted: 30-08-2015 Revised: 07-12-2015 Published: 30-09-2016

ABSTRACT

Objectives: Chrysin, an active natural bioflavonoid found in honey and many plant extracts, was first known for its antioxidant and anti-inflammatory effects. The fact that antioxidants have several inhibitory effects against different diseases, such as cancer, led to search for food rich in antioxidants. In this study, we investigated the antiproliferative and apoptotic effects of chrysin on the cultured human breast cancer cells (MCF-7).

Materials and Methods: Cells were cultured in Roswell Park Memorial Institute medium and treated with different chrysin concentrations for three consecutive days. Cell viability was quantitated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The percentage of apoptotic cells was determined by flow cytometry using Annexin V-fluorescein isothiocyanate. Results: The MTT assay showed that chrysin had an antiproliferative effect on MCF-7 cells in a dose- and time-dependent manner. The 50% cell growth inhibition values for chrysin against MCF-7 cells were 19.5 and 9.2 µM after 48 and 72 h, respectively. Chrysin induced apoptosis in MCF-7 cells as determined by flow cytometry.

Conclusion: This study shows that chrysin could also be considered as a promising chemotherapeutic agent and anticancer activity in treatment of the breast cancer cells in future.

Key words: Apoptosis, chrysin, honey, human breast cancer cells, lung cancer, viability

SUMMARY

• Chrysin had an antiproliferative effect on human breast cancer cells (MCF-7) cells in a dose- and time-dependent manner
• Chrysin induced apoptosis in MCF-7 cells, as determined by flow cytometry

INTRODUCTION

Breast cancer is the main health problem in women. It is the one of major causes of death of middle age (40–50-year-old) women.[1] About 23% of total cancer diagnoses and 14% of deaths in women are related to breast cancer; therefore, incident rate of breast cancer is 1 out of 35 people in Asia and one out of eight in the United States.[2,3] Recently, it looks that there is a critical need for amelioration in detection, diagnosis, and treatment of breast cancer. Unlucky, progression of resistance to chemotherapeutic materials is an important obstruction in the treatment of breast cancer.[4] On the other hand, the present treatments (surgery, chemotherapy, and/or radiotherapy) are involved by critical side effects mainly loss of appetite, hair loss, decreased resistance to infections, weakness and fatigue, weight gain and bleeding, premature menopause, and development of tumor resistant.[5] In addition, major cytotoxic treatments mainly target dividing cells both normal and malignant cells that makes meaningful morbidity and limited clinical benefits of the patients. Thus, discovering novel and effective treatments against breast cancer is now-a-days a scientific challenge. Enhanced focus has been currently attended to naturally compounds as novel strategies.[6]

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Samarghandian S, Azimi-Nezhad M, Borji A, Hasanzadeh M, Jabbari F, Farkhondeh T, Samini M. Inhibitory and cytotoxic activities of chrysin on human breast adenocarcinoma cells by induction of apoptosis. Phcog Mag 2016;12:436-40.
Utilization of antioxidants has been related to the several preventative effects against different diseases such as cancer, aging, inflammatory disorders, coronary diseases, and neurological degeneration10-14 and led to search for natural foods rich in antioxidants. One such food includes honey15-16 which has a long tradition of use in folk medicine for various purposes and has also been referred to extensively in the medical literature17-20. Its major ingredients are fructose and glucose and it also including amino acids, proteins, carbohydrates, minerals, vitamins, water, and enzymes. Honey indicates a broad spectrum of therapeutic aspects, containing antifungal, antibacterial, wound healing, and anti-inflammatory.21-25 Gribel and Pashinski showed that honey possessed antitumor and also antimitotic effects in the different strains of mouse and rat tumors.22 It was also currently elucidated that honey could make apoptosis in cancer cell lines.9,17 Some minor ingredients of honey are confirmed to have antioxidant aspects23 including polyphenol compounds.24,25 Thus, it is possible that the antitumor aspects of honey are related to its polyphenols. With the development of extraction methods for various polyphenols, it is possible to study and concentrate the polyphenolic ingredients extracted from honey rather than crude honey itself. Phenolic ingredients can be separated into several different types based on their structure: Consisting flavonoids and phenolic acids. Flavonoids (the most important polyphenolic class) are natural antioxidants that exhibit a wide range of biological effects, including antithrombotic, antibacterial, anti-inflammatory, antiallergic, and vasodilatory actions.26 As natural products, flavonoids are considered as healthy, safe, and easily obtained in normal diet, making them proper treatment for cancer chemoprevention or associated material in clinical treatment. Chrysin (5,7-dihydroxyflavone) is a natural phytochemical flavonoid and biologically active flavone extracted from honey, vegetables, propolis, and fruits [Figure 1].17,28 Chrysin, especially, has been detected as indicating a broad range of pharmacological aspects, such as anti-oxidation, anti-inflammatory properties, and promotes cell death by perturbing cell cycle progression.17,29 The present study was aimed to investigate the cytotoxic and apoptotic effects of chrysin against breast cancer cell line.

MATERIALS AND METHODS

Chemicals and reagents
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amersco (USA). Roswell Park Memorial Institute (RPMI) medium 1640 was purchased from Gibco BRL (Grand Island, NY, USA). Annexin V-fluorescein isothiocyanate (FITC) was obtained from Invitrogen Corporation (USA). Fetal bovine serum (FBS) was purchased from PAA Laboratories GmbH, Austria. Chrysin (5,7-dihydroxyflavone) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals were of the highest, commercially available quality.

Cell culture

The human breast cancer cell line (MCF-7) and normal fibroblast mouse (L929) cell (as control) were obtained from the Pasteur Institute of Iran and cultured in RPMI medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin. The MCF-7 cells were cultured in a CO2 incubator (MCO-17AI, Sanyo Electric Co., Ltd., Japan) at 37°C in a humidified atmosphere enriched by 5% CO2 and subcultured every 3–4 days. The malignant and nonmalignant cells were cultured in Dulbecco’s modified Eagle’s medium with 5% (v/v) FBS, 100 units/ml penicillin and 100 µg/ml streptomycin.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay

Cell viability was determined using the MTT assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases. Briefly, the cells were seeded at a density of 1 × 104 cells/ml in 96-well plates and allowed to attach for 24 h, resulting in log phase growth at the time of drug treatment. Chrysin at different concentrations (5, 10, 20, and 30 mM) was added to the wells for 24, 48, and 72 h. After removing the medium, cells were then labeled with MTT solution (5 mg/ml in phosphate-buffered saline [PBS]) for 4 h at 37°C and the resulting formazan was solubilized with dimethyl sulfoxide (100 µl). Absorbance was measured at 550 nm using an automated microplate reader (Bio-Rad 550). Cell viability was expressed as a percentage of the value for control culture value. The cytotoxic effects of chrysin on MCF-7 cells were expressed as 50% cell growth inhibition (IC50) values (the drug concentration that reduced the absorbance of treated cells by 50% compared to untreated cells). Experiments for each concentration were carried out in triplicate, including untreated cell control and a blank cell-free control.

Assessment of apoptosis by Annexin V-fluorescein isothiocyanate

Apoptotic cell death was measured using an FITC-conjugated Annexin V/propidium iodide (PI) assay kit by flow cytometry. Briefly, 5 × 105 cells were washed with ice-cold PBS, resuspended in 100 ml binding buffer, and stained with 5 ml of FITC-conjugated Annexin V (10 mg/ml) and 10 ml of PI (50 mg/ml). The cells were incubated for 15 min at room temperature in the dark, 400 ml of binding buffer was added, and the cells were analyzed (FACScan, Becton-Dickinson, USA). The MCF-7 cells were gated separately according to their granularity and size on forward scatter versus side scatter plots. Early and late apoptosis was evaluated on fluorescence 2 (FL2 for PI) versus FL1 (for Annexin) plots. Cells stained with only Annexin V were evaluated as being in early apoptosis; cells stained with both Annexin V and PI were evaluated as being in late apoptosis or a necrotic stage.

Statistical analysis

All results were expressed as mean ± standard error mean. Significance was evaluated using ANOVA and Bonferroni’s test. A probability level of P < 0.05 was considered statistically significant.
RESULTS

Effects of chrysin on cell viability

MCF-7 cancerous cells were incubated with different concentrations of chrysin for 24, 48, and 72 h. The cytotoxicity of chrysin on cell viability was quantitated by MTT assay. Exposure of the MCF-7 cells with chrysin showed significantly high growth inhibitory effects on the breast carcinoma cell line in a concentration- and time-dependent manner \((P < 0.001)\). The exposure of MCF-7 cells for 24 h showed no significant result at any concentration of chrysin. However, there were significant decreases in viability for the concentrations of 5, 10, 15, and 20 \(\mu M\) after 48 and 72 h \((P < 0.05, P < 0.01\) and \(P < 0.001\), Figure 1). The dose inducing \(IC_{50}\) against the malignant cell \(\text{(MCF-7)}\) was determined at being 19.5 ± 0.5 \(\mu M\) and 9.2 ± 0.7 \(\mu M\) at 48 and 72 h, respectively.

Quantification studies for apoptosis by chrysin

To study the roles of chrysin in apoptosis, chrysin was used to setup apoptosis system on the MCF-7 cell line. The MCF-7 cells were treated with concentrations of 5 and 20 \(\mu M\) of chrysin for 48 h. After treatment, the cells were harvested and apoptosis was examined by flow cytometry [Figure 2]. Quantitative analysis using Annexin V/PI assay further showed that the proportion of early stage apoptotic cells (Annexin V+/PI−) increased significantly from 20.13% to 49.76% while proportion of late apoptotic cell (Annexin V+/PI+) increased significantly from 13.82% to 38.47% when the cells were treated with the concentrations of 5 and 20 \(\mu M\) of chrysin, respectively [Figure 3]. Apoptosis induced from 5 and 20 \(\mu M\) of chrysin was statistically higher than control and the percentage of the early and late apoptotic cells significantly increased by increasing chrysin concentration \((P < 0.001)\), and also the number of the late apoptotic cells versus early apoptotic cells at concentration of 5 and 20 \(\mu M\) of chrysin treated cells were statically significant \((P < 0.01, P < 0.001)\) [Figure 3].

DISCUSSION

Cancer is a very complex disorder and the occurrence and progression of cancer cells are strongly connected to abnormal intracellular signal transduction system.

One of the basic strategies of new cancer treatment is chemotherapy. However, the common anticancer agents currently used for treating different types of cancer have severe side effects. Therefore, in the present time, the recent research has mainly concentrated on herbs and plants which have been studied for being nontoxic and for the treatment and prevention of breast cancer. Thus, it is substantial to screen natural products, either as isolated components or as crude extracts, for apoptotic abilities to detect potential anticancer compounds. Over 60% anticancer drugs recently applied come from natural sources, including marine organisms, plants, and microorganisms, and they offer an opportunity to investigate the molecular mechanisms of tumorigenesis. Significant interest is recently focused on the usage of foods for the protection of human health. Especially, there is severe interest in the function of dietary antioxidants, which can scavenge the free radicals and oxidants responsible for developing and initiating different diseases. The confirmed data that antioxidants have several preventative effects against various disorders, such as coronary diseases, cancer, neurologic degeneration, inflammatory disorders, and aging, have led to a investigate for foods containing antioxidants.

Research on the antioxidant aspects of various beverages, foods, spices, and herbs have been done and the number of articles addressing the health-protective and antioxidant characteristics of honey is increasing. Honey has for a long time been used as a natural source of sugars, as well as strong ingredient in traditional medicine, having antitumor, antimicrobial, and antiinflammatory properties. Honey contains a different ingredient including benzoic acid, and caffeic acid, esters, substitute's phenolic acids, flavonoid glycosides, and beeswax. Some of the illustrated biological activities of honey may be traced to its chemical ingredients.

The therapeutic and health-protective impacts of honey were previously explained by the existence of various antioxidant ingredients, such as organic acids, flavonoids (such as chrysin), phenolic acids, enzymes, and vitamins. In the current study, chrysin could reduce cell viability of the MCF-7 breast cancer cell line. Chrysin-induced cell toxicity is achieved through the inhibition of cell proliferation and induction of apoptosis. Our research showed that chrysin prevented the growth of the MCF-7 cells in a dose-dependent manner. The prevention of cell growth was in agree with that mentioned by Parajuli et al., who applied chrysin extracted from the roots, stem, and leaf of various Scutellaria plants. According to their research, chrysin at a concentration of 100 \(\mu M\) significantly prevented the growth of MDA-MB-231 cells after 4 days’ treatment (about 50.0%, \(P < 0.05\)). In our study, 20 \(\mu M\) chrysin was sufficient to prevent the growth of the MCF-7 cells during 3 days’ treatment (73.9%, \(P < 0.05\)). A 48 h treatment of chrysin was applied to discover the preliminary mechanisms of the chrysin on the MCF-7 cells because this was the condition under which the induction of early growth inhibition in the MCF-7 cells was
antioxidants with their ability to scavenge free radicals can protect the cells from different diseases such as cancer. [46,47]

CONCLUSION

Taken together, this study indicates that chrysin has a potential cytotoxicity and apoptotic properties on the MCF-7 cells. Further studies are needed to fully recognize the mechanism involved in cell death, and chrysin could be considered as promising chemotherapeutic agent in breast cancer treatment.

Acknowledgement

We would like to thank Research Affairs of Neyshabur University of Medical Sciences for financially supporting this work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
3. Akbari A, Razzaghi Z, Hornaes F, Khayamzadeh M, Movahedi M, Akbari ME. Parity and breastfeeding are preventive measures against breast cancer in Iranian women. Breast Cancer 2011;18:51-5.
4. Haghighat S, Akbari ME, Ghaffari S, Yavari P. Standardized breast cancer mortality rate compared to the general female population of Iran. Asian Pac J Cancer Prev. 2012;13:5525-8.
5. Asadzadeh Vostakolaei F, Broeders MJ, Mousavi SM, Kiemeneij LA, Verbeek AL. The effect of demographic and lifestyle changes on the burden of breast cancer in Iranian women: A projection to 2030. Breast 2013;22:277-81.
6. Samarghandian S, Hadjzadeh MA, Afshari JT, Hosseini M. Antiproliferative activity and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line. BMC Complement Altern Med 2014;14:192.
7. Hernandez-Aya LF, Gonzalez-Angulo AM. Adjuvant systemic therapies in breast cancer. Surg Clin North Am 2013;93:473-91.
8. Lee KW, Bode AW, Dong Z. Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer 2011;11:211-8.
9. Samarghandian S, Afshari JT, Davoodi S. Chrysin reduces proliferation and induces apoptosis by Annexin V/propidium iodide. ***P < 0.001, compared with control; ** P < 0.01; *P < 0.05, versus the other chrysin concentration.
10. The ability to establish tumor cell apoptosis is a critical property of a candidate anticancer drug, which differentiates between toxic compounds and anticancer drugs. [43,44] Our investigation identified that chrysin has a significant proliferation inhibitory activity against the MCF-7 cells in a dose-dependent manner [Figure 1]. Much effort has been managed toward the research of the effect of chrysin on apoptosis and understanding the mechanisms of action. The apoptosis evoked by chrysin was verified by the Annexin V-FITC [Figures 2 and 3]. In the present research, chrysin evoked apoptosis that was implicated by the Annexin V/propidium iodide. ***P < 0.001, compared with control; ** P < 0.01; *P < 0.05, versus the other chrysin concentration.
in the human prostate cancer cell line pc-3. Clinics (Sao Paulo) 2011;6:1073-9.

18. Samarghandian S, Aftshari JT, Davoodi S. Honey induces apoptosis in renal cell carcinoma. Pharmacogn Mag 2011;7:46-62.

19. Dustmann JH. Antibacterial effect of honey. Apiacta 1979;14:7-11.

20. Milan P. The antibacterial activity of honey. The nature of the antibacterial activity. Bee World 1992;1:5-28.

21. Eferm SE. Clinical observations on the wound healing properties of honey. Br J Surg 1988;75:679-81.

22. Gribel’ NV, Pashinskii VG. The antitumor properties of honey. Vopr Onkol 1990;36:704-9.

23. Antony SM, Han PY, Rieck JR, Dawson PL. Antioxidative effect of maillard reaction products formed from honey at different reaction times. J Agric Food Chem 2000;48:3985-9.

24. Cherchi A, Spadella L, Tuberoso C, Cabras P. Solidphase extraction and HPLC determination of organic acid in honey. J Chromatogr B Analyt Technol Biomed Life Sci 2002;770:77-82.

25. Davies AM, Harris RG. Free amino acid analysis of honeys from England and of the geographical origin of honeys. J Apicult Res 1982;21:168-73.

26. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 1996;7:66-76.

27. Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids: A review. Analyst 1997;122:11-34.

28. Pietta PG. Flavonoids as antioxidants. J Nat Prod 2000;63:1035-42.

29. Farkhondeh T, Samarghandian S, Azimin‑Nezhad M, Samini F. Effect of chrysin on nociception in formalin test and serum levels of noradrenalin and corticosterone in rats. Int J Clin Exp Med 2015;8:2465‑70.

30. Samarghandian S, Nezhad MA, Mohammadi G. Role of caspases, Bax and Bcl-2 in chrysin-induced apoptosis in the A549 human lung adenocarcinoma epithelial cells. Anticancer Agents Med Chem 2014;14:901‑9.

31. Wolf B, Brischwein M, Lob V, Ressler J, Wiest J. Cellular signaling: Aspects for tumor diagnosis and therapy. BiomedTech (Berlin) 2007;52:164‑8.

32. Cragg L, Fox A, Nation K, Reid C, Anderson M. Neural correlates of successful and partial inhibitions in children: An ERP study. Dev Psychobiol 2009;51:533‑43.

33. Adhikari AS, Iwakuma T. Mutant p53 gain of oncogenic function: In vivo evidence, mechanism of action and its clinical implications. Fukuoka Igaku Zasshi 2009;100:217‑28.

34. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996;20:933-66.

35. Butkovic V, Klasinc L, Bors W. Kinetic study of flavonoid reactions with stable radicals. J Agric Food Chem 2004;52:2816‑20.

36. Weissstein A, Luchter E, Bittrmann S. Medical honey and its role in paediatric patients. Br J Nurs 2014;23:S30, S32-4.

37. Greenaway W, Scaybrook T, Whately FR. The analysis of bud exudates and propolis by gas chromatographymass spectrometry. Biol Sci 1987;232:249-54.

38. Samarghandian S, Farkhondeh T, Samini F. Protective Effects of Carvacrol against Oxidative Stress Induced by Chronic Stress in Rat’s Brain, Liver, and Kidney. Biochem Res Int 2016;2016:2645237.

39. Specter AA, Gordon JA, Moore SA. Hydroxyeicosatetraenoic acids (HETEs). Prog Lipid Res 1988;27:373‑32.

40. Mijanur Rahman M, Gan SH, Khalil MI. Neurological effects of honey: Current and future prospects. Evid Based Complement Alternat Med 2014;2014:958721.

41. Samarghandian S, Farkhondeh T, Samini F. Available form: http://www.ncbi.nlm.nih.gov/pubmed/26863330. Chrysin treatment improves diabetes and its complications in liver, brain, and pancreas in streptozotocin-induced diabetic rats. Can J Physiol Pharmacol 2015;1-6.

42. Parajuli P, Joshee N, Rimando AM, Mittal S, Yadav AK. In vitro antitumor mechanisms of various Scutellaria extracts and constituent flavonoids. Planta Med 2009;75:418.

43. Jaganathan SK, Mandal M. Honey constituents and its apoptotic effect in colon cancer cells. J Api Api Med Sci 2009;1:29-36.

44. Frankfurt OS, Krishan A. Apoptosis-based drug screening and detection of selective toxicity to cancer cells. Anticancer Drugs 2003;14:555‑61.

45. Kerr JF, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239‑67.

46. Lu JJ, Dang YY, Huang M, Xu WS, Chen XR, Wang YT. Anti-cancer properties of terpenoids isolated from Rhizoma Curcumae – A review. J Ethnopharmacol 2012;143:406-11.

47. Niture SK, Rao US, Srivenugopal KS. Chemopreventive strategies targeting the MGMT repair protein: Augmented expression in human lymphocytes and tumor cells by ethanolic and aqueous extracts of several Indian medicinal plants. Int J Oncol 2006;29:1269-78.