Original Article
Middle East Journal of Cancer; April 2021; 12(2): 261-268

Phycocyanin C a Natural Product with Impressive Therapeutic Efficacy for Inhibition of Breast Tumors' Growth and Metastasis in Vivo

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Please cite this article as: Kefayat A, Yuosefzadeh M, Goli P, Babayi A, Rezaei A, Esmaeil N. Phycocyanin C a natural product with impressive therapeutic efficacy for inhibition of breast tumors' growth and metastasis in vivo. Middle East J Cancer. 2021;12(2): 261-8. doi: 10.30476/mejc.2020.83064.1130.

Abstract

Background: Several studies have reported the anticancer effect of phycocyanin C, a natural extract isolated from the algae Arthrospira platensis. However, its therapeutic effects on the growth of breast cancer and its metastasis have not been determined yet.

Method: In this case-control study, we employed phycocyanin C for the treatment of 4T1 breast tumor as an applicable experimental animal model for human mammary cancer and metastasis. BALB/c mice were injected subcutaneously (s.c) into the 4th abdominal mammary fat pad with 1×10^6 4T1 cells. We randomly divided the mice into two groups; one group of mice were injected with PBS as the control, and the other group was intraperitoneally injected with phycocyanin C (80 mg/kg daily for 20 days). Tumor growth and metastasis were assessed in both groups.

Results: Phycocyanin C significantly inhibited 4T1 breast tumors growth (P<0.05). The mean tumors volumes at the control group were 2.73 times higher than those of the treatment group. In addition, phycocyanin C treatment could significantly inhibit the formation of metastasis colonies at vital organs like spleen, liver, and lung. Moreover, the survival rate of the tumor-bearing mice increased after about 22 days by phycocyanin C treatment in comparison with the control.

Conclusion: This is the first report demonstrating the anticancer effects of phycocyanin C on 4T1 breast tumor in vivo. Overall, our findings provided convincing evidence for the application of phycocyanin C as an anticancer therapeutic agent.

Keywords: Phycocyanin C, Breast neoplasm, Metastasis

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Received: August 25, 2019; Accepted: October 18, 2020

Introduction

Breast cancer is the most prevalent cancer among women worldwide.[1-3] Different therapeutic strategies, including chemotherapy, radiation therapy, and surgery are applied for the treatment of breast cancer. However, these therapies are not definitive treatments and are usually associated with several adverse effects.[4] On the other side, the incidence of breast cancer is on the increase worldwide, despite all the prevention strategies.[5] Regarding certain patients, breast cancer is defined as an aggressive malignancy accompanied by enhanced metastasis and poor prognosis.[6] Therefore, novel and alternative therapies, such as nutrition therapy, may be useful in these cases. In nutrition therapy, food supplements, ingredients, and a generally healthy diet provide various nutrients to the body so that it could fight cancer cells more effectively. In addition, nutrients exert chemopreventive and chemotherapeutic effects in these patients.[6] In fact, there is a balance between oxidants and antioxidants in an optimal physiological condition. Oxidative stress involves certain damages to different molecules, for instance lipids, proteins, and DNA, which could be considered as a risk factor for cancer induction.[7, 8] Studies have shown that phytochemical compounds exert antioxidant activity through scavenging free radicals, which leads to metabolic changes including gene regulation (oncogenes, as well as, tumor suppressor genes), cell differentiation, cell cycle arrest and apoptosis, modulation of enzyme activities in detoxification, oxidation and reduction, immune system stimulation, and regulation of hormone-related carcinogenesis.[9-11] To date, numerous researches have investigated ursolic acid, a phytochemical component in plants (fruits and vegetables), owing to its antiproliferative effects and its ability to induce apoptosis in breast cancer cells. Furthermore, its impacts on cell signaling and some metabolites have been demonstrated.[12,13] Another phytochemical compound is phycocyanin, which is isolated from the Arthrospira platensis. It is a natural, non-toxic component used as a food supplement in many

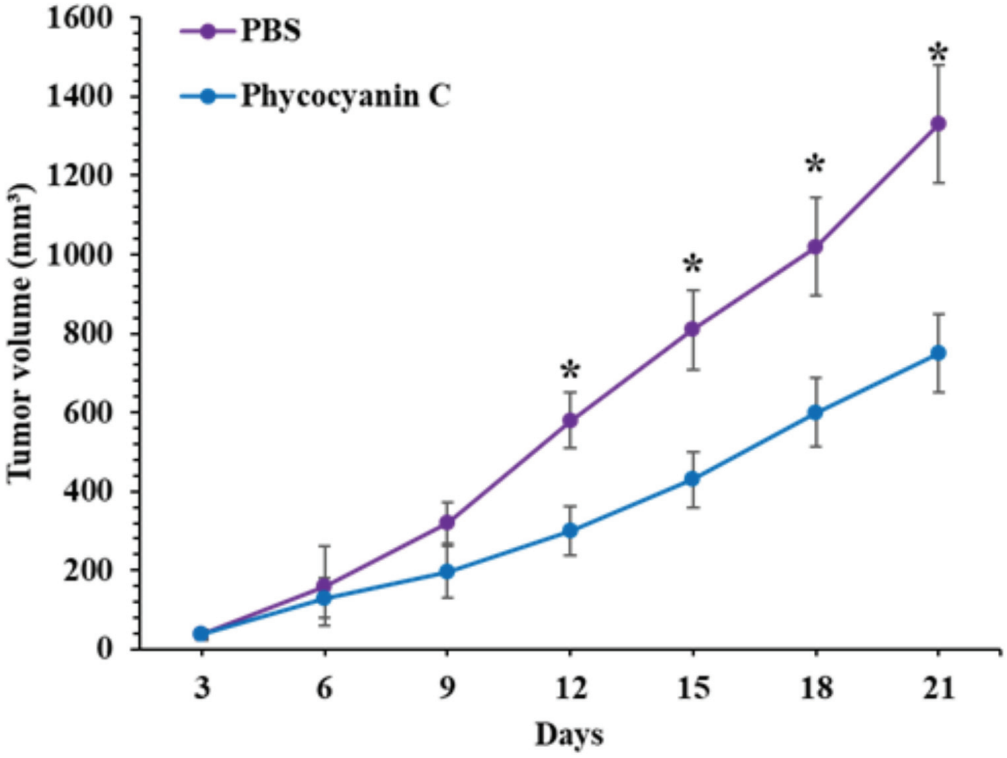

Figure 1. The tumors' volume progression in the PBS and phycocyanin C treated groups was monitored (n=10). Phycocyanin C treatment caused a significant inhibition in the 4T1 breast tumors' growth and volume progression (*: $P<0.05$).

countries. The antioxidant and anti-inflammatory characteristics of phycocyanin have been considered.[14,15] Reaserches have also reported its anticancer effects against certain types of cancers including colon, hepatocellular, service, and leukemic cell line.[16-19] Previous studies have indicated that phycocyanin activates proapoptotic and down-regulates antiapoptotic genes expression, which promotes apoptosis of cancer cells.[19, 20] The apoptotic effects of phycocyanin on K562 cell line results into the down-regulation of antiapoptotic proteins, such as Bcl-2, while it has no effects on proapoptotic proteins. These impacts of phycocyanin are probably mediated via the release of cytochrome c in the cytosol and poly (ADP) ribose polymerase (PARP) cleavage.[19, 20] Moreover, Cyclin E and CDK-2 levels, which are needed for G1-S transition, decrease in MDA-MB-231 cells in the presence of phycocyanin; and therefore, the cells push to enter apoptosis. The inhibition of COX-2 in triple negative breast cancers is another anticancer effect of phycocyanin.[21]

Most previous studies have reported the anticancer effects of phycocyanin on cancer cells in vitro. In the present study, we decided to evaluate the antineoplastic and antimetastatic effects of phycocyanin C on the 4T1 breast tumor-bearing BALB/c mice as an applicable experimental animal model for human breast cancer in vivo. To the best of our knowledge, this is the first time to investigate the therapeutic efficacy of phycocyanin C regarding the growth of breast tumor and metastasis in vivo.

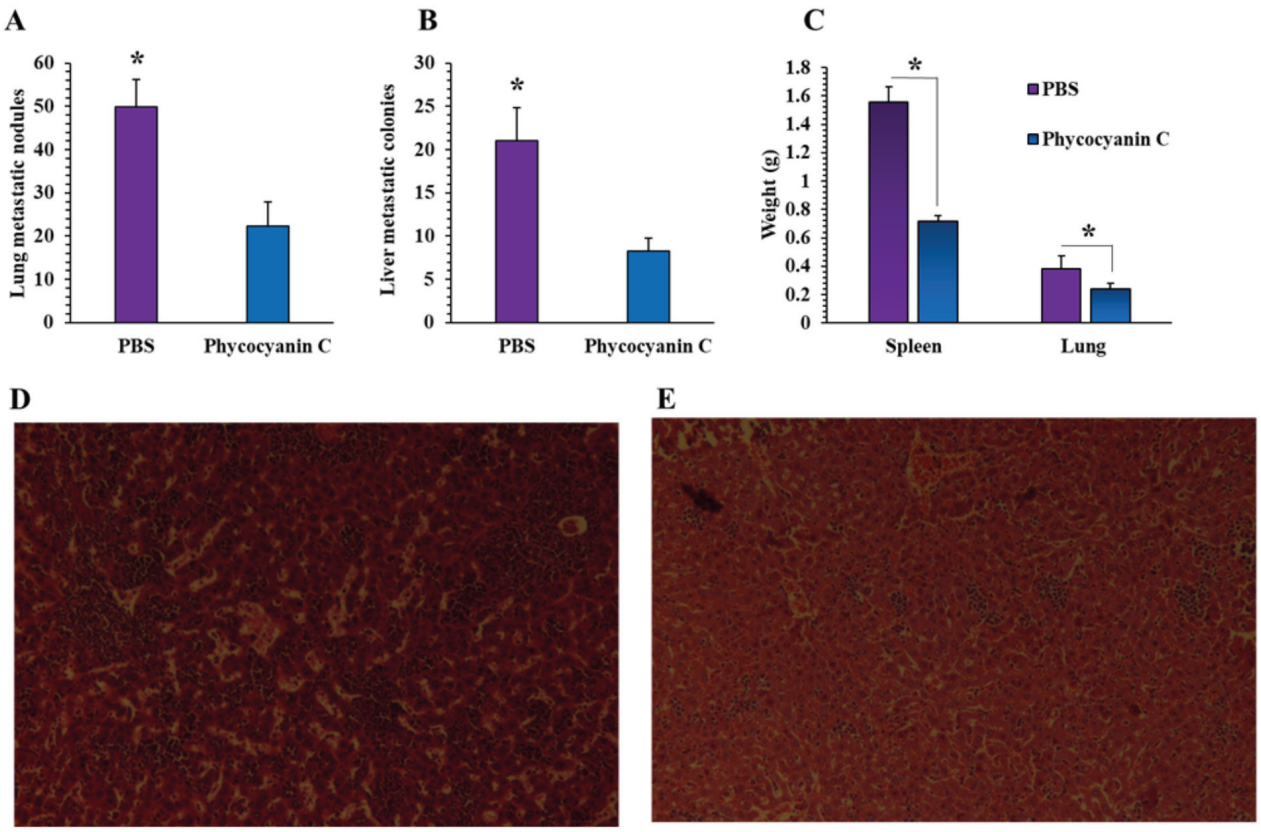

Figure 2. The metastatic burden at vital organs was evaluated at phycocyanin C treated and PBS groups (n=8), 65 days after cancer cell implantation. A) The average metastatic colonies per microscopic field of lungs and B) Liver sections were evaluated in phycocyanin C treated and PBS groups (n=8). C) Spleen and lung weights for different groups were evaluated and PBS group exhibited a significantly higher weight in comparison with the phycocyanin group (*:$P< 0.05$), which exhibited the higher metastatic burden. The microscopic photographs of liver sections at (D) control and (E) phycocyanin treated groups are presented to exhibit the difference of metastatic burden in this organ.

Materials and Methods

Cell culture

In this case-control study, mouse mammary carcinoma cell line (4T1) was purchased from Pasteur Institute of Tehran, Iran. Cells were cultured in RPMI 1640 medium (Sigma, USA) containing 10% fetal bovine serum (FBS) (Sigma, USA) and 1% antibiotics mixture containing penicillin (Sigma, USA) and streptomycin (Sigma, USA). The cells were incubated at 37 ºC in a humidified incubator at 5% CO2 atmosphere.

Animals husbandry and 4T1 breast tumor implantation

20 Female BALB/c mice (age, 6-8 weeks; mean weight, 25 ± 2 g) were purchased from the Pasteur Institute of Iran (Tehran, Iran) for this experiment. We verified all the procedures according to the guidelines of the Institutional Animal Care and Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1396.3.520). The mice were injected subcutaneously (s.c) into the 4th abdominal mammary fat pad with 1×10^6 cells suspended in 100 µl PBS (Sigma, USA). They were randomly divided into two groups (n=10 in each group). One group of mice were injected with PBS as the untreated group, and the other group was intraperitoneally (i.p) injected with 50 mg/kg phycocyanin C (Sigma-Aldrich, Germany) on a daily basis for 20 days. The injections started as tumors became palpable. We then measured the tumor sizes with a digital caliper every three days and calculated the tumors' volume using the below tumor volume equation:[22]

Tumor volume = Tumor length × Tumor width 2 × 0.5

Survival assay

16 female BALB/c mice were purchased from the Pasteur Institute of Iran (Tehran, Iran) for this experiment. The mice were injected with the 4T1 cancer cells and divided into two groups (n=8), including PBS and phycocyanin C (the same as previous section). For survival analysis, we

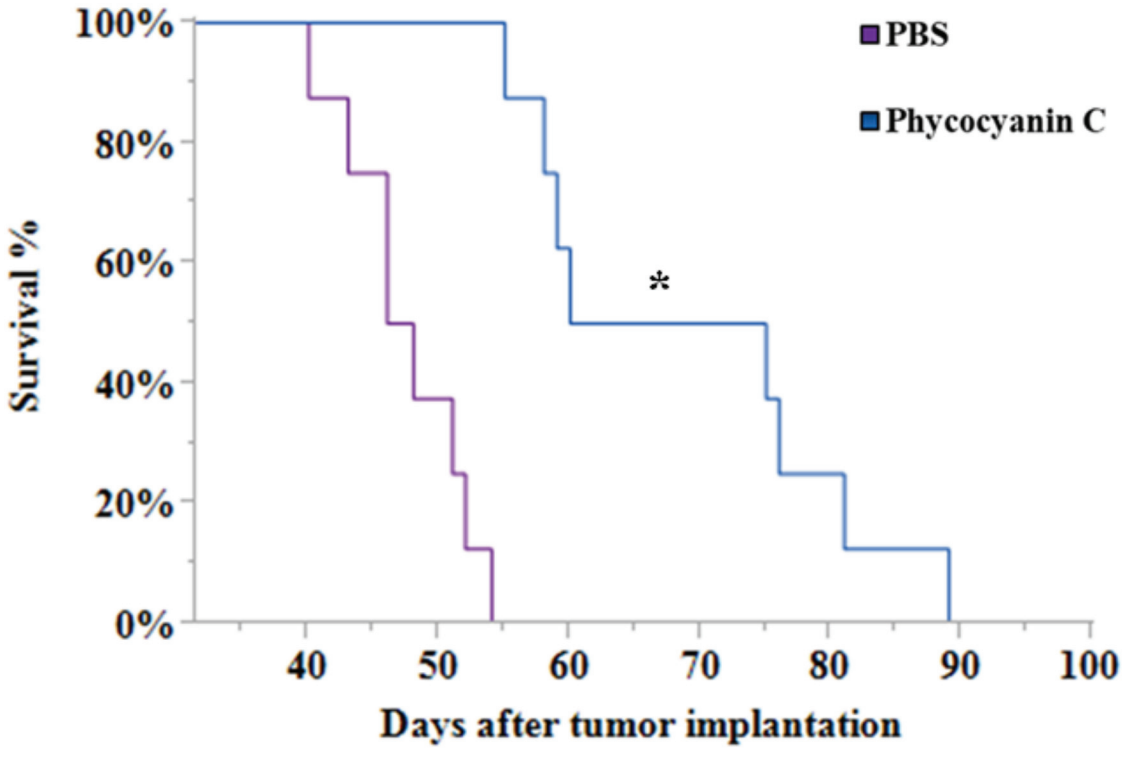

Figure 3. The survival time of the mice was monitored for 100 days after cancer cell implantation in different groups (n=8) and their Kaplan-Meier graph is illustrated in this figure. The horizontal axis exhibits the time progression and the vertical axis represents the survival percentage of the mice (*:$P< 0.05$).

observed the tumor-bearing mice for 100 days following the implantation of the cancer cells. The animals' death was recorded every day. The standardized humane endpoint employed to euthanize the animals was failure to eat and drink for over 3 days and without any limb movement.

Metastasis assay

16 Female BALB/c mice were purchased from the Pasteur Institute of Iran (Tehran, Iran) for this experiment. We initially injected them with the 4T1 cancer cells and then divided them into two groups (n=8), including PBS and phycocyanin C (the same as previous section). The mice were sacrificed 65 days after them implantation of the cancer cells. We harvested and weighted their spleen and lungs and compared the obtained weights between the PBS and treatment groups. Subsequently, their livers and lungs were fixed in 10% formalin neutral buffer solution and embedded in paraffin. Five µm sections were prepared from tissues blocks and stained with hematoxylin and eosin. Two sections were prepared for each liver. Afterwards, 10 random microscopic fields were evaluated for metastatic colonies at section, utilizing a digital light microscope (Olympus, Japan). We represented our results as the average number of metastatic colonies per group.[23]

Statistical analyses

We analyzed all the data utilizing One Way ANOVA with Tukey's post-hoc test with JMP 11.0 software (SAS Institute, Japan). The statistical significance was set at $P<0.05$. The results were expressed as mean ± SD (*: $P=0.05$, ns: not significant). All the experiments were replicated at least three times.

Results

Phycocyanin C inhibits 4T1 breast tumors' growth

4T1 tumor-bearing mice were injected with phycocyanin C so that we could evaluate its therapeutic effects as an anticancer agent. We started the injection as the tumors became palpable after 20 days. Phycocyanin C treatment (50 mg/kg, 20 days, i.p, daily) significantly inhibited the growth of 4T1 breast tumors 12 days following the treatment initiation (Figure 1). The tumors' volume at the 21st day of the treatment was about 2.73 times less than that in the untreated group, which was injected with PBS. We selected the phycocyanin dosage according to the study by Liao et al. on phycocyanin's therapeutic effects on pancreatic cancer in animal models. They observed a significant anticancer activity by inducing apoptotic and autophagic cell death at this dosage of phycocyanin C treatment.[24]

Phycocyanin C inhibits breast tumors' spontaneous metastasis and increases tumor-bearing mice survival

4T1 is a highly metastatic cell line and can spontaneously migrate to the vital organs and forms metastatic colonies. The liver, lung, and spleen of the tumor-bearing mice were harvested 40 days after the implantation of the cancer cells. Metastatic colonies at spleen and lung trigger an increase in their weights. We detected significant differences concernig the spleens ($P<0.05$) and lungs ($P<0.05$) weights between the treated tumor-bearing mice (20 days, 50 mg/kg i.p, daily) and the control group (100 µL PBS) (Figure 2C). The histopathological evaluations demonstrated a significant inhibition of cancer cells metastasis in the phycocyanin C treated mice, which exhibited lower metastatic colonies at the lung (Figure 2A) and liver (Figure 2B) in comparison with the untreated group. In addition, phycocyanin C significantly ($P<0.05$) increased the survival rate in the tumor-bearing mice. The treatment group exhibited about 22 days of more mean survival time than the control group (Figure 3).

Discussion

In the present study, we assessed antineoplastic effects of phycocyanin on 4T1 breast tumor-bearing BALB/c mice, and found that the treatment involving phycocyanin C, a marine drug, significantly decreased tumor volume. The efficacy of different therapeutic strategies commonly used for the treatment of breast cancer is not only dissatisfying, but also has several side effects. Thus, natural products have attracted a great deal of scientific attention concerning the

treatment of cancer in recent years. The 4T1 tumor is a proper model of human breast cancer since its metastasis ability is like human mammary cancer.[25] Previous studies have shown anticancer effects of phycocyanin C on different cancer cell types, such as breast cancer, liver cancer, and leukemia in vitro and in vivo,[19, 20, 26-28] However, its effects on 4T1 breast tumors have not been investigated so far.

Phycocyanin C selectively inhibits COX-2 and COX-2 and plays a vital role in tumor progression and metastasis. Therefore, phycocyanin C as a COX-2 inhibitor has an important role in cancer prevention.[29-32] In the present study, phycocyanin C treatment significantly inhibited 4T1 breast tumors growth 12 days after the initiation of the treatment. A previous study by Saini et al. indicated that phycocyanin C induces apoptosis in a rat model of colon cancer. They suggested that phycocyanin C and Piroxicam inhibit the production of inflammatory cytokines and promote the apoptosis of cancer cells.[32] Furthermore, they observed that in the treatment of triple-negative breast cancer MBA-MD-231 cells with phycocyanin, apoptosis of MBA-MD-231 cells increased with the increase in proapoptotic p21 protein and the decrease in antiapoptotic Bcl-2 protein levels.[21, 33] On the other side, the antineoplastic impacts of phycocyanin on the other breast cancer cell line (MCF-7 cells) have been related to increased FAS expression and the decrease of NF-κB, P53, and Bcl-2 expression.[20, 34] Other studies have found that phycocyanin C induces G0/G1 cell cycle arrest, diminishes cyclin D1 and CDK2, and also induces apoptosis in MBA-MD-231 cells and esophageal squamous cell carcinoma (ESCC) cell lines (EC9706 and EC1) in a dose-dependent manner.[35, 36] Recently, in a network analysis and phenotype experiment, Hao et al. illustrated that GRB2-ERK1/2 pathway is involved in the proliferation suppression of melanoma cells by phycocyanin C.[37] Accordingly, the significant decrease in the tumor size in our study is probably attributed to apoptotic impacts of phycocyanin C and also its role in cell-cycle arrest induction and proliferation suppression as mentioned above.

To determine the antineoplastic effects of phycocyanin C against liver and lungs metastasis, we performed histopathological evaluations of cancer cells' metastasis in the phycocyanin C treated mice compared to the untreated group. We found that liver and lung metastasis decreased in phycocyanin C treatment group, and the survival of phycocyanin C treated tumor-bearing mice increased in comparison with that in the untreated subjects. Previous studies have indicated that COX-2 mediates the metastasis of cancer cells to other tissues, and that this trend decreases in the presence of COX-2 inhibitors.[38, 39] Additionally, Kunte et al. reported the inhibitory effects of phycocyanin C on MMP-2 and MMP-9 (gelatinase) expression in a hepatocellular cancer cell line (HepG2).[40] Matrix metalloproteinases (MMPs), particularly gelatinase and collagenase classes, play leading roles in cancer metastasis.[41-43] Moreover, in another study, Hao et al. indicated that phycocyanin C suppresses proliferation and migration of non-small-cell lung cancer cells through downregulating RIPK1/NF-κB activity.[37] Accordingly, in our study, the decrease in liver and lung metastasis in the treatment of 4T1 breast tumor-bearing BALB/c mice with phycocyanin C may be due to the suppression of RIPK1/NF-κB signaling pathway, and also the inhibition of both COX-2 and MMP2/MMP9.

Conclusion

In the present study, we depicted that phycocyanin C significantly decreases tumor size and metastasis in 4T1 breast tumor animal models. This is the first report demonstrating the anticancer effects of phycocyanin C on 4T1 breast tumor. Overall, our findings provided convincing evidence for the application of phycocyanin C as an anticancer therapeutic agent.

Acknowledgments

This work was financially supported by Isfahan University of Medical Sciences (grant no. 396520).

Conflict of Interest

None declared.

References

1. Wang S, Liu Q, Zhang Y, Liu K, Yu P, Liu K, et al. Suppression of growth, migration and invasion of highly-metastatic human breast cancer cells by berbamine and its molecular mechanisms of action. *Mol Cancer.* 2009;8:81. doi: 10.1186/1476-4598-8-81.
2. Kamath R, Mahajan KS, Ashok L, Sanal TS. A study on risk factors of breast cancer among patients attending the tertiary care hospital, in Udupi district. *Indian J Community Med.* 2013;38(2):95-9. doi: 10.4103/0970-0218.112440.
3. DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. *CA Cancer J Clin.* 2016;66(1):31-42. doi: 10.3322/caac.21320.
4. Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. *N Engl J Med.* 2001;344(26):1997-2008.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics, 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018; 68(6):394-424.
6. Rahul V, Rui Hai L. Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid. *Food Science and Human Wellness.* 2012; 1(1): 1-13.
7. Liu RH, Hotchkiss JH. Potential genotoxicity of chronically elevated nitric oxide: a review. *Mutat Res.*1995;339(2):73-89.
8. Sakanaka S, TachibanaY, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry.* 2005; 89(4): 569-75.
9. Tor YS, Yazan LS, Foo JB, Armania N, Cheah YK, Abdullah R, et al. Induction of apoptosis through oxidative stress-related pathways in MCF-7, human breast cancer cells, by ethyl acetate extract of Dillenia suffruticosa. *BMC Complement Altern Med.* 2014;14:55. doi:10.1186/1472-6882-14-55.
10. St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet.* 1979;1(8124):1017-20.
11. Pellegrini N, Simonetti P, Brusamolino A, Bottasso B, Pareti FI. Composition of platelet phospholipids after moderate consumption of red wine in healthy volunteers. *Eur J Clin Nutr.* 1996; 50(8): 535-44.
12. Renaud S, Gueguen R. The French paradox and wine drinking. in Novartis Found. *Novartis Found Symp.* 1998; 216:208-17.
13. Sun L, Hui AM, Su Q, Vortmeyer A, Kotliarov Y, Pastorino S, et al. Neuronal and glioma-derived stem cell factor induces angiogenesis within the brain. *Cancer Cell.* 2006; 9(4): 287-300.
14. Romay Ch, González R, Ledón N, Remirez D, Rimbau V.C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci.* 2003;4(3):207-16.
15. Pardhasaradhi BV, Ali AM, Kumari AL, Reddanna P, Khar A. Phycocyanin-mediated apoptosis in AK-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS. *Mol Cancer Ther.* 2003;2(11): 1165-70.
16. Saini MK, Vaiphei K, Sanyal SN. Chemoprevention of DMH-induced rat colon carcinoma initiation by combination administration of piroxicam and C-phycocyanin. *Mol Cell Biochem.*2012; 361(1-2): 217-28.
17. Basha OM, Hafez RA, El-Ayouty YM, Mahrous KF, Bareedy MH, Salama AM.C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells. *Egypt J Immunol.* 2008;15(2): 161-7.
18. Li B, Gao MH, Zhang XC, Chu XM. Molecular immune mechanism of C-phycocyanin from *Spirulina platensis* induces apoptosis in HeLa cells in vitro. *Biotechnol Appl Biochem.*2006; 43(3): 155-64.
19. Subhashini J, Mahipal SV, Reddy MC, Mallikarjuna Reddy M, Rachamallu A, Reddanna P. Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. *Biochem Pharmacol.* 2004; 68(3):453-62. doi: 10.1016/j.bcp.2004.02.0.25.
20. Li B, Chu X, Gao M, Li W. Apoptotic mechanism of MCF-7 breast cells in vivo and in vitro induced by photodynamic therapy with C-phycocyanin. *Acta Biochim Biophys Sin (Shanghai).* 2009;42(1): 80-9.
21. Ravi M, Tentu S, Baskar G, Rohan Prasad S, Raghavan S, Jayaprakash P, et al. Molecular mechanism of anti-cancer activity of phycocyanin in triple-negative breast cancer cells. *BMC Cancer.* 2015;15(1):768.
22. Ghahremani F, Kefayat A, Shahbazi-Gahrouei D, Motaghi H, Mehrgardi MA, Haghjooy-Javanmard S. AS1411 aptamer-targeted gold nanoclusters effect on the enhancement of radiation therapy efficacy in breast tumor-bearing mice. *Nanomedicine (Lond).* 2018;13(20): 2563-78.
23. Huang Y, Pan L, Helou K, Xia Q, Parris TZ, Li H, et al. Mechanical ventilation promotes lung metastasis in experimental 4T1 breast cancer lung-metastasized models. *Cancer Manag Res.* 2018;10: 545.
24. Liao G, Gao B, Gao Y, Yang X, Cheng X, Ou Y. Phycocyanin inhibits tumorigenic potential of pancreatic cancer cells: role of apoptosis and autophagy. *Sci Rep.*2016;6:34564.
25. Pulaski BA, Ostrand-Rosenberg S. Mouse 4T1 breast

tumor model. *Curr Protoc Immunol.* 2001;Chapter 20:Unit 20.2. doi: 10.1002/0471142735.im2002s39.
26. Chen T, Wong YS. In vitro antioxidant and antiproliferative activities of selenium-containing phycocyanin from selenium-enriched *Spirulina platensis*. *J Agric Food Chem.* 2008;56(12): 4352-8.
27. Roy KR, Arunasree KM, Reddy NP, Dheeraj B, Reddy GV, Reddanna P. Alteration of mitochondrial membrane potential by *Spirulina platensis* C-phycocyanin induces apoptosis in the doxorubicinresistant human hepatocellular-carcinoma cell line HepG2. *Biotechnol Appl Biochem.* 2007;47(3): 159-67.
28. Li B, Gao MH, Chu XM, Teng L, Lv CY, Yang P, et al.The synergistic antitumor effects of all-trans retinoic acid and C-phycocyanin on the lung cancer A549 cells in vitro and in vivo. *Eur J Pharmacol.* 2015;749: 107-14.
29. Telliez A, Furman C, Pommery N, Hénichart JP. Mechanisms leading to COX-2 expression and COX-2 induced tumorigenesis: topical therapeutic strategies targeting COX-2 expression and activity. *Anticancer Agents Med Chem.* 2006; 6(3): 187-208.
30. Davies G, Martin LA, Sacks N, Dowsett M. Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. *Ann Oncol.* 2002; 13(5): 669-78.
31. Dohadwala M, Yang SC, Luo J, Sharma S, Batra RK, Huang M, et.al.Cyclooxygenase-2–dependent regulation of E-cadherin: prostaglandin E2 induces transcriptional repressors ZEB1 and Snail in non–small cell lung cancer. *Cancer Res.* 2006;66(10): 5338-45.
32. Saini MK, Vaish V, Sanyal SN. Role of cytokines and Jak3/Stat3 signaling in the 1,2-dimethylhydrazine dihydrochloride-induced rat model of colon carcinogenesis: early target in the anticancer strategy. *Eur J Cancer Prev.* 2013;22(3): 215-28.
33. Bharathiraja S, Seo H, Manivasagan P, Santha Moorthy M, Park S, Oh J. In vitro photodynamic effect of phycocyanin against breast cancer cells. *Molecules.* 2016; 21(11): 1470.
34. Rimbau V, Camins A, Pubill D, Sureda FX, Romay C, González R, et al. C-phycocyanin protects cerebellar granule cells from low potassium/serum deprivation-induced apoptosis. *Naunyn Schmiedebergs Arch Pharmacol.* 2001;364(2): 96-104.
35. Jiang L, Wang Y, Liu G, Liu H, Zhu F, Ji H, et al. C-Phycocyanin exerts anti-cancer effects via the MAPK signaling pathway in MDA-MB-231 cells. *Cancer Cell Int.* 2018;18(1): 12.
36. Zhang X, Fan T, Li S, Guan F, Zhang J, Liu H. C-phycocyanin elicited antitumor efficacy via cell-cycle arrest, apoptosis induction, and invasion inhibition in esophageal squamous cell carcinoma. *J Recept Signal Transduct Res.* 2019;39(2): 114-21.
37. Hao S, Li S, Wang J, Zhao L, Zhang C, Huang W, et al. Phycocyanin reduces proliferation of melanoma cells through downregulating GRB2/ERK signaling. *J Agric Food Chem.* 2018;66(41): 10921-9.
38. Singh B, Berry JA, Shoher A, Ayers GD, Wei C, Lucci A. COX-2 involvement in breast cancer metastasis to bone. *Oncogene.* 2007; 26(26): 3789.
39. Sorski L, Melamed R, Matzner P, Lavon H, Shaashua L, Rosenne E, et al. Reducing liver metastases of colon cancer in the context of extensive and minor surgeries through β-adrenoceptors blockade and COX2 inhibition. *Brain Behav Immun.* 2016;58: 91-8.
40. Kunte M, Desai K. The Inhibitory effect of c-phycocyanin containing protein extract (C-PC Extract) on human matrix metalloproteinases (MMP-2 and MMP-9) in hepatocellular cancer cell line (HepG2). *Protein J.* 2017;36(3): 186-95.
41. Sun S, Bay-Jensen AC, Karsdal MA, Siebuhr AS, Zheng Q, Maksymowych Wp, et al. The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. *BMC Musculoskelet Disord.* 2014;15(1): 93.
42. Halade GV, Jin YF, Lindsey ML. Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. *Pharmacol Ther.* 2013;139(1): 32-40.
43. Radisky ES , Radisky DC. Matrix metalloproteinases as breast cancer drivers and therapeutic targets. *Front Biosci.* 2015; 20:1144.