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Apoptotic and Anti-Metastatic Effect of Carvacrol in PANC-1 Human Pancreatic Cancer Cells

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Abstract

Pancreatic cancer is known to have one of the highest mortality rates among other cancer. Monoterpenes are natural compounds obtained from essential oils of plants. Carvacrol is a monoterpene phenolic derived from essential oils of various aromatic plants. In this study, the effects of carvacrol on proliferation, apoptosis and metastasis of PANC-1 human pancreatic cancer cells were investigated. XTT assay was used to determine cell proliferation and viability. mRNA level expression levels of BAX, BCL2, CASP3, CASP7, CASP8, CASP9, CYCS, FADD, FAS and P53 were evaluated for determining the effect of carvacrol on apoptosis by qRT-PCR analysis. In addition, gene expressions of CDH1, CDH2, TIMP1, TIMP2, TIMP3, ZEB1 and ZEB2 were analyzed to evaluate its effect on metastasis. Carvacrol inhibited the PANC-1 pancreatic cancer cell proliferation in dose and time dependent manner. Carvacrol treatment induced apoptosis by changing the expressions of genes important in apoptosis. Moreover, it suppressed the metastasis by effecting CDH1, CDH2, TIMP2, TIMP3 and ZEB2 expressions. In conclusion, carvacrol showed apoptotic and anti-metastatic effects in PANC-1 human pancreatic cancer cells. It is thereby concluded that carvacrol may have a therapeutic potential in pancreatic cancer.

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1. INTRODUCTION

Pancreatic cancer ranks seventh in cancer-related deaths in the worldwide (Sung *et al*., 2021). It is estimated that 62,210 new cases will be diagnosed and 49,830 patients are expected to die from the pancreatic cancer in the United States in 2022. The incidence of this disease has been increasing by approximately 1% per year. Mortality rates from pancreatic cancer have increased by 0.2% each year in recent years (American Cancer Society, 2022).

High toxicity in cancer treatment may affect the specificity of the treatment. For this reason, the search for non-toxic treatment methods for normal cells continues. Therefore, it has been observed that natural compounds can be used clinically and interest in the use of these compounds in medicine has increased. Monoterpenes are natural compounds obtained from essential oils of plants (Rejhová *et al*., 2018). One of these, carvacrol (5-isopropyl-2-methylphenol) is a natural bioactive monoterpenoid phenol derived from some aromatic plant essential oils of Lamiaceae family (Baj *et al*., 2020). Studies have demonstrated that this natural compound have anticancer (Li *et al*., 2021), gastroprotective (Souza *et al*., 2017), anti-inflammatory (Guimarães *et al*., 2012), antibacterial (Ben Arfa *et al*., 2006), antioxidant (Guimarães *et al*., 2010) and analgesic (Can Baser, 2008) effects. In addition, it was reported

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that carvacrol showed antiproliferative effect in many cancer cells including prostate (Luo *et al*., 2016), stomach (Günes-Bayir *et al*., 2018a), colon (Fan *et al*., 2015), liver (Yin *et al*., 2012) and lung (Koparal and Zeytinoglu, 2003) cancer cells. The anticancer effect of carvacrol however has not been fully elucidated.

Previously, it was shown that carvacrol suppressed cell viability of human prostate cancer PC-3 cell line in a dose and time dependent manner. Moreover, it induced apoptosis by modulating BAX, BCL2 and caspase (CASP) activity (Khan *et al*., 2019). Khan *et al*. (2018a) reported that carvacrol nanoemulsion caused apoptosis induction by elevating BAX, cytochrome c (CYCS), CASP3 and CASP9 by decreasing BCL2 in doxorubicin resistant human lung adenocarcinoma A549 cells. Previously, it was stated that carvacrol had an apoptotic effect by elevating BAX level and downregulating BCL2 expression in U2OS and 143B human osteosarcoma cells. Moreover, carvacrol suppressed MMP9 expression, invasion and migration in the same study (Zhang *et al*., 2021). Liang *et al*. (2022) showed that carvacrol significantly decreased leukemia cell viability. In a study, it was reported that carvacrol could contribute to human Jurkat T cell activity modulation by suppressing IL-2 and IFN-γ levels and also NFAT-2 and AP-1 transcription factors (Gholijani *et al*., 2015). In a previous study, MCF-7, MDA-MB-453, MDA-MB-231, BT-474 and BT-483 breast cancer cells were treated with 10-500 µM carvacrol for 24 h. CCK-8 cell viability assay results showed that carvacrol between 50 and 500 µM dose-dependently suppresses cell viability in all cells. In the same study, it was shown that 200 µM carvacrol treatment caused cell cycle arrest by regulating some cyclin proteins in MDA-MB-231 cells (Li *et al*., 2021). In this study, we aimed to investigate possible effect of carvacrol on cell proliferation and the expression of apoptosis and metastasis related genes in PANC-1 human pancreatic cancer cells.

2. MATERIAL and METHODS

2.1. Chemicals

Carvacrol was commercially purchased from Tokyo Chemical Industry (TCI). XTT kit and FBS were obtained from Biological Industries. DMEM, penicillin/streptomycin and PBS purchased from Gibco. QIAzol and cDNA synthesis kits were obtained from Qiagen and Bio-Rad, respectively.

2.2. Cell Culture

PANC-1 human pancreatic cancer cells (ATCC®CRL-1469™) were obtained from the American Type Culture Collection (USA). PANC-1 cells were cultured in DMEM medium containing 1% penicillin/streptomycin, 10% FBS and 2 mM L-glutamine, and proliferated in an incubator at 37ºC, 5% CO_2 and humidified 95% air.

2.3. Cell Viability Assay

PANC-1 cells were treated to prepared carvacrol solutions for 25, 50, 100, 200, 500 and 1000 µM for 24, 48 and 72 h. Carvacrol was dissolved in ethanol. The XTT method was used to evaluate the cytotoxic effect of carvacrol on PANC-1 cells. XTT test was performed according to the protocol in our previous study (Secme *et al*., 2018). IC_{50} value of carvacrol in PANC-1 cells was determined by calculating with the CompuSyn Version 1.0 software.

2.4. Total RNA Isolation, cDNA Synthesis and qRT-PCR Analysis

Expressions of important genes in apoptosis (BAX, BCL2, CASP3, CASP7, CASP8, CASP9, CYCS, FADD, FAS and P53) and metastasis (CDH1, CDH2, TIMP1, TIMP2, TIMP3, ZEB1 and ZEB2) pathways were evaluated after total RNA isolation and cDNA synthesis. ACTB was used as housekeeping gene and internal control. Expressions of these genes were

determined by qRT-PCR analysis using the primers and the protocol described in elsewhere (Eroğlu-Güneş *et al*., 2021).

2.5. Statistical Analysis

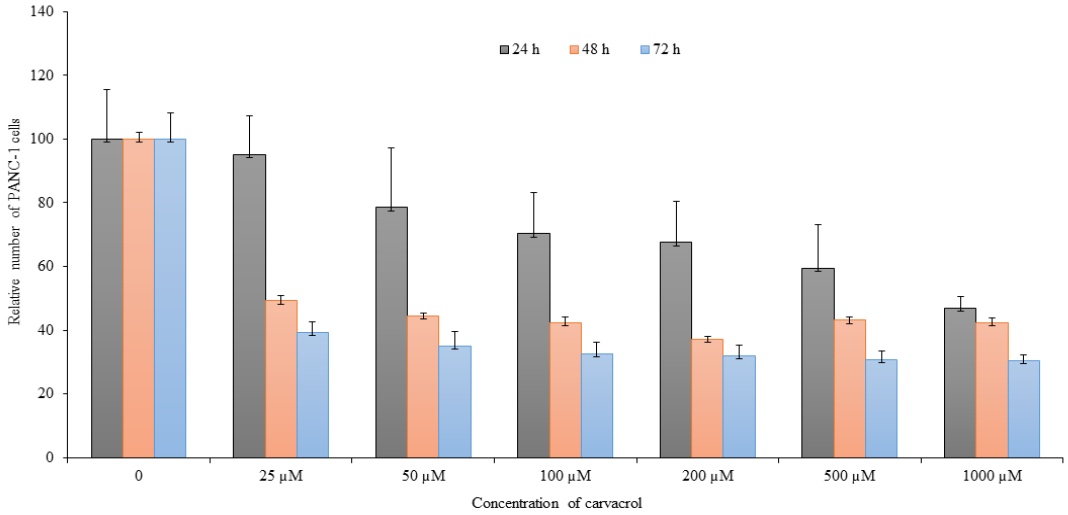
After three technical replicates, Ct values were obtained and normalized using the reference gene and the data were shown as mean ± SD. The qRT-PCR analysis was conducted with the $2^{(-\Delta Ct)}$ method. Experimental groups were compared using the independent samples *t-test* in SPSS 26.0 statistical analysis program. $p< 0.05$ was considered as statistically significant.

3. RESULTS and DISCUSSION

3.1. Cytotoxic Effect of Carvacrol in PANC-1 Cells

Cytotoxic effect of carvacrol in PANC-1 was determined by XTT assay. Carvacrol inhibited the cell proliferation of pancreatic cancer cells time and dose dependent manner. The half maximal inhibitory concentration (IC_{50}) dose of carvacrol in PANC-1 cells was found to be 664.02 µM for 24 h using CompuSyn version 1.0 software (Figure 1).

Figure 1. Effect of carvacrol on the PANC-1 cell viability. IC_{50} dose of carvacrol in PANC-1 cells was found to be 664.02 µM.



Apoptosis is an important cell death mechanism in cancer therapy. Anti-apoptotic BCL2, pro-apoptotic BAX, apoptotic proteins including CASP3, CASP7, CASP8, CASP9, CYCS, FADD and FAS play a critical role in regulation of programmed cell death. P53, known as a tumor suppressor protein and transcription factor, regulates the expression of several genes, including the cell cycle and apoptosis (Aubrey *et al*., 2018).

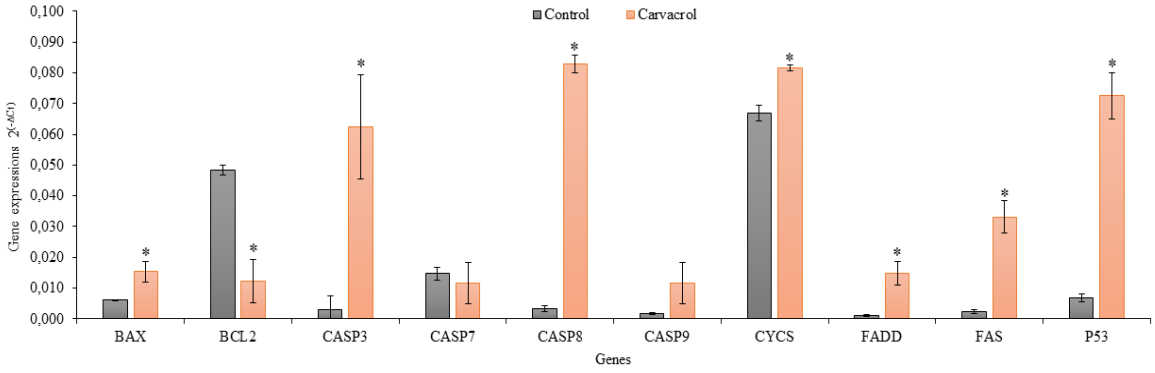
Essential oils obtained from aromatic plants are known to have strong anticancer effects. In a previous study, it was shown that carvacrol suppressed cell viability in dose and time dependent manner in human prostate cancer DU145 cells. In the same study, it was reported that the IC_{50} dose of carvacrol in these cells was 84.39 µM for 24 h and 42.39 µM for 48 h (Khan *et al*., 2017). Günes-Bayir *et al*. (2018b) showed that IC_{50} value of carvacrol was 82.57 µM for 24 h in gastric adenocarcinoma AGS cells. In addition, carvacrol has been reported to induce cytotoxicity, apoptosis and increased reactive oxygen species in these cells. In a previous study, effects of carvacrol in eight liver hepatocellular carcinoma cell lines were evaluated. Carvacrol suppressed cell viability of all these cells between 10-300 µM. Hep-G2 was the most

sensitive cell with inhibition rate 90% at 300 µM. SNU-182 and SNU-389 were least sensitive cells with 40% inhibition rate at 300 µM (Yin *et al*., 2022). In another previous study (Heidarian and Keloushadi, 2019), IC_{50} dose of carvacrol was 360 µM in PC3 human prostate cancer cells. Fan *et al*. (2015) reported that IC_{50} doses of carvacrol were 544.4 and 530.2 µM in HCT116 and LoVo human colon cancer cells, respectively. Also, the cell viability of HeLa human cervical cancer cells was suppressed with carvacrol treatment at IC_{50} dose of 556 µM (Potočnjak *et al*., 2018). In a study investigating the effect of carvacrol on ovarian cancer, it was reported that its IC_{50} value in SKOV-3 ovarian cancer cells was 322.50 µM at 24 h and 289.54 µM at 48 h (Elbe *et al*., 2020).

3.2. Expression of Apoptosis Related Genes in Carvacrol Treated PANC-1 Cells

Expression changes of genes in apoptosis pathway were evaluated by using qRT-PCR in carvacrol treated PANC-1 cells. Carvacrol caused a significant increase in BAX, CASP3, CASP8, CYCS, FADD, FAS and P53 but a decrease in BCL2 expression. Moreover, it was also observed that it insignificantly downregulated CASP7 expression but elevated CASP9 expression (Figure 2).

Figure 2. Effect of carvacrol on apoptosis genes in PANC-1 cells. (*, $p<0.05$)

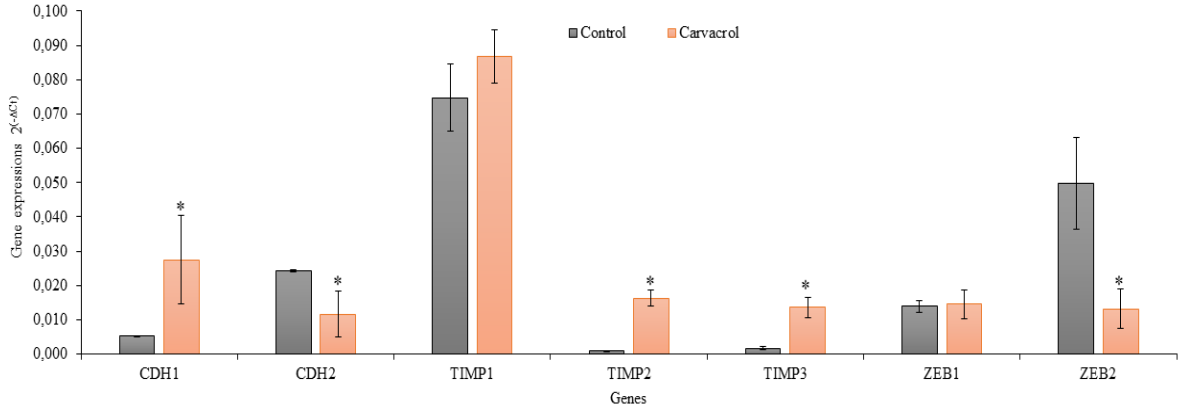


One of the most important points in the occurrence of cancer is the disruption of the balance between cell proliferation and apoptosis. Pancreatic cancer is treated with standard treatment options that cause cell death including chemotherapy and radiotherapy. However, the side effects experienced in the treatments limit the therapeutic applications. For pancreatic cancer therapy, it is important to discover novel agents that specifically target cancer cells and are less toxic to normal cells. Bhakkiyalakshmi *et al*. (2016) showed that carvacrol induced apoptosis in HL-60 human acute promyelocytic leukemia and Jurkat human acute T cell leukemia cells. The IC_{50} value of carvacrol was reported to be 100 µM for HL-60 cells and 50 µM for Jurkat cells. It was stated that BAX and CASP3 expressions were increased, however BCL2 expression was downregulated in carvacrol treated cells (Bhakkiyalakshmi *et al*., 2016). Khan *et al*. (2018b) reported that carvacrol nanoemulsion caused apoptosis by effecting proteins associated with apoptosis including BAX, BCL2, CYCS, CASP3 and CASP9 in A549 human lung cancer cells. Carvacrol also suppressed proliferation of cervical cancer HeLa cells via caspase-dependent apoptosis (Ahmad and Ansari., 2021). In a previous study, 300 µM of carvacrol significantly induced population of apoptosis in both JAR and JEG3 choriocarcinoma cells compared with nontreated cells by activating expression of BAX, BAK and CYCS (Lim *et al*., 2019).

3.3. Expression of Metastasis Related Genes in Carvacrol Treated PANC-1 Cells

In order to determine the effects of carvacrol on metastasis, changes in CDH1, CDH2, TIMP1, TIMP2, TIMP3, ZEB1 and ZEB2 gene expression were evaluated by qRT-PCR. The results showed that carvacrol significantly upregulated CDH1, TIMP2 and TIMP3 expressions. It also significantly reduced CDH2 and ZEB2 expressions. No significant changes were observed in ZEB1 expression (Figure 3).

Figure 3. Effect of carvacrol on metastasis genes in PANC-1 cells. (*, p<0.05)



Approximately 80% of pancreatic cancer patients are diagnosed after metastasis and disease progression due to the late onset of symptoms (Kuehn, 2020). EMT (Epithelial mesenchymal transition) is an important step in the formation of invasion and metastasis. In the EMT process, epithelial cells lose their epithelial properties by proteins altering the expression of cell-cell junction proteins. For example, E-cadherin was downregulated and initiate expression of mesenchymal-related proteins including N-cadherin (Hay and Zuk, 1995). In addition, ZEB1 and ZEB2 transcription factors are considered as the main transcriptional regulators of epithelial mesenchymal transition in this process (Peinado *et al*., 2007). Carvacrol application resulted in an elevated E-cadherin (CDH1) level and a downregulation in N-cadherin (CDH2) and vimentin expression on oral squamous cell carcinoma CAL-27 cells in a previous study (Liu *et al*., 2021). Heidarian and Keloushadi (2019) reported that carvacrol significantly reduced invasion in dose dependent manner in PC3 human prostate cancer cells. In a study, 500 µM doses of carvacrol significantly reduced expression of MMP2 protein in PC-3 and DU145 prostate cancer cells (Luo *et al*., 2016). Chen *et al*. (2015) stated that 561.3 µM IC_{50} dose for 24 h of carvacrol suppressed the proliferation, migration and invasion in U87 glioblastoma cells. In a previous study, carvacrol significantly suppressed invasion and migration in human oral squamous cell carcinoma Tca-8113 and SCC-25 cells by inhibiting MMP2, MMP9, ZEB1 and β-catenin expressions (Dai *et al*., 2016). It was shown that carvacrol suppressed cell invasion by decreasing MMP2 and MMP9 expression levels in HCT116 and LoVo human colon cancer cells (Fan *et al*., 2015). The results of the present study showed that increased E-cadherin (CDH1) and decreased N-cadherin (CDH2) and ZEB2 expression may cause suppression of EMT, an important process in metastasis, by carvacrol. Moreover, it showed antimetastatic effect by reducing TIMP2 and TIMP3 expressions, which play an important role in metastasis.

4. CONCLUSION

In this study, apoptotic and anti-metastatic effects of carvacrol were investigated in PANC-1 human pancreatic cancer cells. Carvacrol stimulated apoptosis by effecting expression levels of BAX, BCL2, CASP3, CASP8, CYCS, FADD, FAS and P53 genes. Moreover, it showed anti-metastatic effect by changing gene expressions of CDH1, CDH2, TIMP2, TIMP3 and ZEB2. In conclusion, further studies are needed to determine whether carvacrol can be used as an anti-cancer agent in the treatment of pancreatic cancer because of its apoptotic and anti-metastatic effect on PANC-1 cells.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in NatProBiotech belongs to the author(s).

Author Contribution Statement

Canan Eroglu Gunes: Study design, conducting experiments, statistical analyzes, evaluation of the results, preparation of the manuscript. **Mucahit Secme:** Study design, conducting experiments, statistical analyzes, preparation of the manuscript. **Ercan Kurar:** Statistical analyzes, preparation of the manuscript. **Huseyin Donmez:** Study design, statistical analyzes, preparation of the manuscript.

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5. REFERENCES

Ahmad, A., & Ansari, I. A. (2021). Carvacrol exhibits chemopreventive potential against cervical cancer cells via caspase-dependent apoptosis and abrogation of cell cycle progression. *Anticancer Agents Med. Chem*., *21*(16), 2224-2235. https://doi.org/10.2174/1871520621999201230201258

American Cancer Society. (2022). *Cancer Facts & Figures 2022*, Atlanta: American Cancer Society.

Aubrey, B. J., Kelly, G. L., Janic, A., Herold, M. J., & Strasser, A. (2018). How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ., 25*(1), 104-113. https://doi.org/10.1038/cdd.2017.169

Baj, T., Biernasiuk, A., Wróbel, R., & Malm, A. (2020). Chemical composition and in vitro activity of *Origanum vulgare* L., *Satureja hortensis* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. essential oils towards oral isolates of candida albicans and candida glabrata. *Open Chem., 18*(1), 108–118. https://doi.org/10.1515/chem-2020-0011

Ben Arfa, A., Combes, S., Preziosi-Belloy, L., Gontard, N., & Chalier, P. (2006). Antimicrobial activity of carvacrol related to its chemical structure. *Lett. Appl. Microbiol., 43,* 149–154. https://doi.org/10.1111/j.1472-765X.2006.01938.x

Bhakkiyalakshmi, E., Suganya, N., Sireesh, D., Krishnamurthi, K., Saravana Devi, S., Rajaguru, P., & Ramkumar, K. M. (2016). Carvacrol induces mitochondria-mediated apoptosis in HL-60 promyelocytic and Jurkat T lymphoma cells. *Eur. J. Pharmacol., 772,* 92-98. https://doi.org/10.1016/j.ejphar.2015.12.046

Can Baser, K., (2008). Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. *Curr. Pharmaceut. Des., 14,* 3106–3119. https://doi.org/10.2174/138161208786404227

Chen, W. L., Barszczyk, A., Turlova, E., Deurloo, M., Liu, B., Yang, B. B., Rutka, J. T., Feng Z. P., & Sun, H. S. (2015). Inhibition of TRPM7 by carvacrol suppresses glioblastoma cell proliferation, migration and invasion. *Oncotarget. 6*(18), 16321-16340. https://doi.org/10.18632/oncotarget.3872

Dai, W., Sun, C., Huang, S., & Zhou, Q. (2016). Carvacrol suppresses proliferation and invasion in human oral squamous cell carcinoma. *Onco. Targets Ther., 9,* 2297-2304. https://doi.org/10.2147/OTT.S98875

Elbe, H., Yigitturk, G., Cavusoglu, T., Baygar, T., Ozgul Onal, M., & Ozturk, F. (2020). Comparison of ultrastructural changes and the anticarcinogenic effects of thymol and carvacrol on ovarian cancer cells: which is more effective? *Ultrastruct. Pathol., 44*(2), 193-202. https://doi.org/10.1080/01913123.2020.1740366

Eroğlu-Güneş, C., Güçlü, E., Vural, H., & Kurar, E. (2021). Knockdown of lncRNA ZEB2NAT suppresses epithelial mesenchymal transition, metastasis and proliferation in breast cancer cells. *Gene*., *805*, 145904. https://doi.org/10.1016/j.gene.2021.145904

Fan, K., Li, X., Cao, Y., Qi, H., Li, L., Zhang, Q., & Sun, H. (2015). Carvacrol inhibits proliferation and induces apoptosis in human colon cancer cells. *Anti Cancer Drugs., 26,* 813–823. https://doi.org/10.1097/CAD.0000000000000263

Gholijani, N., Gharagozloo, M., Kalantar, F., Ramezani, A., & Amirghofran, Z. (2015). Modulation of cytokine production and transcription factors activities in human Jurkat T cells by thymol and carvacrol. *Adv. Pharm. Bull., 5*(Suppl 1), 653-660. https://doi.org/10.15171/apb.2015.089

Guimarães, A. G., Oliveira, G. F., Melo, M. S., Cavalcanti, S. C. H., Antoniolli, A. R., Bonjardim, L. R., Silva, F. A., Santos, J. P. A., Rocha, R. F., Moreira, J. C. F., Araújo, A. A. S., Gelain, D.P., & Quintans-Júnior, L.J. (2010). Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic Clin. Pharmacol. Toxicol., 107,* 949–957. https://doi.org/10.1111/j.1742-7843.2010.00609.x

Guimarães, A. G., Xavier, M. A., De Santana, M. T., Camargo, E. A., Santos, C. A., Brito, F. A., Barreto, E. O., Cavalcanti, S. C. H., Antoniolli, Â. R., Oliveira, R. C. M., & Quintans-Júnior, L.J. (2012). Carvacrol attenuates mechanical hypernociception and inflammatory response. *Naunyn-Schmiedeberg's Arch. Pharmacol., 385,* 253–263. https://doi.org/10.1007/s00210-011-0715-x

Günes-Bayir, A., Kocyigit, A., & Güler, E. M. (2018a). In vitro effects of two major phenolic compounds from the family Lamiaceae plants on the human gastric carcinoma cells. *Toxicol. Ind. Health., 34*(8), 525-539. https://doi.org/10.1177/0748233718761698

Günes-Bayir, A., Kocyigit, A., Güler, E. M., Bilgin, M. G., Ergün, İ. S., & Dadak, A. (2018b). Effects of carvacrol on human fibroblast (WS-1) and gastric adenocarcinoma (AGS) cells in

vitro and on Wistar rats in vivo. *Mol. Cell. Biochem.,* 1–13. https://doi.org/10.1007/s11010-018-3329-5

Hay, E. D., & Zuk, A. (1995). Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. *Am. J. Kidney Dis., 26*(4), 678–690.

Heidarian, E., & Keloushadi, M. (2019). Antiproliferative and anti-invasion effects of carvacrol on PC3 human prostate cancer cells through reducing pstat3, pakt, and perk1/2 signaling proteins. *Int. J. Prev. Med., 9*(10), 156. https://doi.org/10.4103/ijpvm.IJPVM_292_17

Khan, F., Khan, I., Farooqui, A., & Ansari, I. A. (2017) Carvacrol induces reactive oxygen species (ROS)-mediated apoptosis along with cell cycle arrest at G_0/G_1 in human prostate cancer cells. *Nutr. Cancer., 69*(7), 1075-1087. https://doi.org/10.1080/01635581.2017.1359321

Khan, I., Bahuguna, A., Bhardwaj, M., Pal Khaket, T., & Kang, S. C. (2018a). Carvacrol nanoemulsion evokes cell cycle arrest, apoptosis induction and autophagy inhibition in doxorubicin resistant-A549 cell line. *Artif. Cells Nanomed. Biotechnol., 46* (sup1), 664-675. https://doi.org/10.1080/21691401.2018.1434187

Khan, I., Bahuguna, A., Kumar, P., Bajpai, V. K., & Kang, S. C. (2018b) In vitro and in vivo antitumor potential of carvacrol nanoemulsion against human lung adenocarcinoma A549 cells via mitochondrial mediated apoptosis. *Sci Rep., 8*(1), 144. https://doi.org/10.1038/s41598-017-18644-9

Khan, F., Singh, V. K., Saeed, M., Kausar, M. A., & Ansari, I. A. (2019). Carvacrol induced program cell death and cell cycle arrest in androgen-independent human prostate cancer cells *via* inhibition of Notch signaling. *Anticancer Agents Med. Chem., 19*(13), 1588-1608. https://doi.org/10.2174/1871520619666190731152942

Koparal, A. T., & Zeytinoglu, M., (2003). Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line, A549. *Cytotechnology., 43*, 149–154.

Kuehn, B.M. (2020). Looking to long-term survivors for improved pancreatic cancer treatment. *JAMA., 324,* 2242–2244.

Li, L., He, L., Wu, Y., & Zhang, Y. (2021). Carvacrol affects breast cancer cells through TRPM7 mediated cell cycle regulation. *Life Sci., 266,* 118894. https://doi.org/10.1016/j.lfs.2020.118894

Liang, Y., Yang, A. Y., Liu, M., Cheng, Y. J., Zang, S. B., Huang, J., Tang, Y. Y., & Huang, Z. P. (2022). Effect of carvacrol on the biological behavior of leukemia cells and its mechanism. *Zhongguo Shi Yan Xue Ye Xue Za Zhi., 30*(2), 393-399. https://doi.org/10.19746/j.cnki.issn.1009-2137.2022.02.012

Lim, W., Ham, J., Bazer, F. W., & Song, G. (2019). Carvacrol induces mitochondria-mediated apoptosis via disruption of calcium homeostasis in human choriocarcinoma cells. *J. Cell Physiol., 234*(2), 1803-1815. https://doi.org/10.1002/jcp.27054

Liu, H., Xu, X., Wu, R., Bi, L., Zhang, C., Chen, H., & Yang, Y. (2021). Antioral squamous cell carcinoma effects of carvacrol via inhibiting inflammation, proliferation, and migration related to Nrf2/Keap1 pathway. *Biomed. Res. Int.,* 6616547. https://doi.org/10.1155/2021/6616547

Luo, Y., Wu, J. Y., Lu, M.H., Shi, Z., Na, N., & Di, J. M. (2016). Carvacrol alleviates prostate cancer cell proliferation, migration, and invasion through regulation of PI3K/Akt and MAPK signaling pathways. *Oxid. Med. Cell Longev.,* 1469693. https://doi.org/10.1155/2016/1469693

Peinado, H., Olmeda, D., & Cano, A. (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat. Rev. Cancer., 7*(6), 415–428.
Potočnjak, I., Gobin, I., & Domitrović, R. (2018). Carvacrol induces cytotoxicity in human cervical cancer cells but causes cisplatin resistance: Involvement of MEK-ERK activation. *Phytother. Res., 32*(6), 1090-1097. https://doi.org/10.1002/ptr.6048
Rejhová, A., Opattová, A., Čumová, A., Slíva, D., & Vodička, P. (2018). Natural compounds and combination therapy in colorectal cancer treatment. *Eur. J. Med. Chem., 144*, 582–594. https://doi.org/10.1016/j.ejmech.2017.12.039
Secme, M., Kaygusuz, O., Eroglu, C., Dodurga, Y., Colak, O. F., & Atmaca, P. (2018). Potential anticancer activity of the parasol mushroom, *Macrolepiota procera* (Agaricomycetes), against the A549 Human Lung Cancer Cell Line. *Int. J. Med. Mushrooms., 20*(11), 1075-1086. https://doi.org/10.1615/IntJMedMushrooms.2018028589
Souza, M. T. S., Teixeira, D. F., de Oliveira, J. P., Oliveira, A. S., Quintans-Júnior, L. J., Correa, C. B., & Camargo, E. A. (2017). Protective effect of carvacrol on acetic acid-induced colitis. *Biomed. Pharmacother., 96,* 313–319. https://doi.org/10.1016/j.biopha.2017.10.017
Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. Cancer J. Clin., 71*, 209–249.
Yin, Q., Yan, F., Zu, X. Y., Wu, Y., Wu, X., Liao, M., Deng, S., Yin, L., & Zhuang, Y. (2012). Anti-proliferative and pro-apoptotic effect of carvacrol on human hepatocellular carcinoma cell line HepG-2. *Cytotechnology., 64,* 43–51. https://doi.org/10.1007/s10616-011-9389-y
Yin, X., Chen, H., Chen, S., & Zhang, S. (2022). Screening and validation of a carvacrol-targeting viability-regulating protein, SLC6A3, in liver hepatocellular carcinoma. *Dis Markers., 2022*, 3736104. https://doi.org/10.1155/2022/3736104
Zhang, S., He, L., Shang, J., Chen, L., Xu, Y., Chen, X., Li, X., Jiao, Q., Jin, S., Hu, X., & Liang, W. (2021). Carvacrol suppresses human osteosarcoma cells via the Wnt/β-Catenin signaling pathway. *Anticancer Agents Med. Chem., 22*(9), 1714-1722. https://doi.org/10.2174/1871520621666210901111932