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# Exploring the Potential of Rosemary Derived Compounds (Rosmarinic and Carnosic Acids) as Cancer Therapeutics: Current Knowledge and Future Perspectives

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## Abstract

Cancer is a global health challenge with high morbidity and mortality rates. However, conventional cancer treatment methods often have severe side effects and limited success rates. In the last decade, extensive research has been conducted to develop safe, and efficient alternative treatments that do not have the limitations of existing anticancer medicines. Plant-derived compounds have shown promise in cancer treatment for their anti-carcinogenic and anti-proliferative properties. Rosmarinic acid (RA) and carnosic acid (CA) are potent polyphenolic compounds found in rosemary (*Rosmarinus officinalis*) extract. They have been extensively studied for their biological properties, which include anti-diabetic, anti-inflammatory, antioxidant, and anticancer activities. In addition, RA and CA have demonstrated effective anti-proliferative properties against various cancers, making them promising targets for extensive research to develop candidate or leading compounds for cancer treatment. This review discusses and summarizes the anti-tumor effect of RA and CA against various cancers and highlights the involved biochemical and mechanistic pathways.

**Key Words:** Rosmarinic acid, Carnosic acid, Anticancer, Plant-derived compound, *Saliva Rosmarinus*

## INTRODUCTION

Cancer is a disease characterized by aberrant cellular proliferation resulting from dysfunctional checkpoint control mechanisms. Despite the availability of various treatments, including chemotherapy, immunotherapy, and current personalized approaches, it remains one of the leading causes of death worldwide, with an estimated ten million deaths in the past two years (Upadhyay, 2021). Unfortunately, common side effects associated with the existing treatments include hair loss, fatigue, fever, and immunosuppression, which can significantly impact the patient's quality of life and limit the treatment's effectiveness (Jain *et al*., 2016). Moreover, the potential decrease in effectiveness, high toxicity, and cost of these treatments pose significant challenges in clinical practice. Consequently, numerous studies are underway to develop alternative strategies to overcome these limitations and improve patient outcomes (Jain *et al*., 2016).

Since ancient times, medicinal plants have been utilized as natural remedies for different health conditions (Menon and

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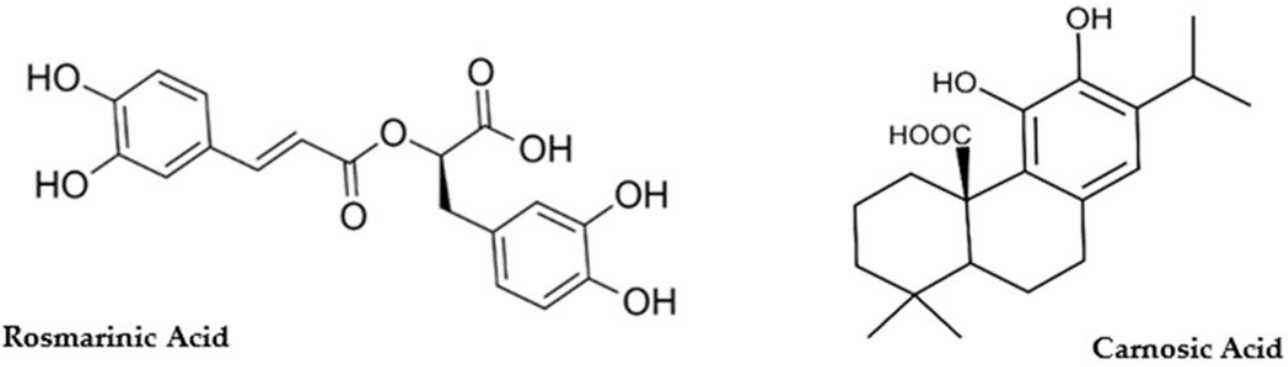


**Fig. 1.** Chemical structure of rosmarinic acid and carnosic acid (Illustrated using ChemDraw [Chemdraw Ultra 14.0, Cambridge Soft Corp., Cambridge, MA, USA]).

Povirk, 2014). With advancements in the medical field and our knowledge of the chemical compounds found in these plants, medicinal plants have recently attracted interest in therapeutic research (Veeresham, 2012). Many of these plants contain alkaloids and secondary metabolites with exceptional antioxidant, anti-proliferative, chemoprotective, anti-diabetic, anti-inflammatory and many other pharmacological properties. Likewise, their ability to be less toxic to normal cells while displaying increased potency toward cancer cells has made them a promising source for developing plant-derived drugs for cancer treatment (Roy *et al*., 2017; El-Huneidi *et al*., 2020, 2021, 2022).

Rosmarinic acid (RA) and carnosic acid (CA) are polyphenols derived from the plants *Rosmarinus officinalis* and *Salvia rosmarinus*, both of which belong to the Lamiaceae family (Bulgakov *et al*., 2012; Loussouarn *et al*., 2017). RA is a hydroxylated ester compound synthesized from the combination of caffeic acid and 3,4-dihydroxy phenyl lactic acid (Fig. 1). It is derived from the amino acids, phenylalanine, and tyrosine. CA is a phenolic diterpene containing two hydroxyl groups. It is well-known for its radical scavenging activity (Fig. 1) (Birtić *et al*., 2015). Both compounds have been shown to possess a wide range of antioxidant, anti-inflammatory, anti-diabetic, and anticancer biological properties.

These compounds offer several advantages in the fight against cancer. Firstly, their anti-inflammatory and antioxidant properties are crucial in preventing cancer cell growth by reducing oxidative stress and inflammation. Secondly, they possess anti-proliferative properties that can slow cell cycle progression and potentially halt cancer cell proliferation. Moreover, carnosic and rosmarinic acids induce apoptosis in cancer cells, triggering a natural process that leads to their death. Additionally, they exhibit anti-angiogenic effects by limiting the growth of new blood vessels necessary for tumor development. Their selective cytotoxicity (specifically targeting cancer cells while preserving healthy cells) minimizes the side effects commonly associated with traditional cancer treatments. Furthermore, carnosic acid and rosmarinic acid have the potential to enhance the effects of chemotherapy drugs when used in combination, thus synergistically improving cancer therapy outcomes (Bulgakov *et al*., 2012; Loussouarn *et al*., 2017).

This review aims to summarize recent findings on the tumor-suppressive role of two diterpenes, RA and CA, against various cancers, and highlight the underlying mechanism and signaling pathways contributing to their effects. Tables 1 and 2 present a comprehensive summary of the effect of RA on various cell types and animal models. In addition, Tables 3 and 4 outline the outcomes of CA in laboratory settings (*in vitro*) and living organisms (*in vivo*), respectively.

## THE EFFECT OF ROSMARINIC ACID AND CARNOSIC ACID ON DIFFERENT CANCERS

### Hepatocellular carcinoma (HCC)

Experimental studies have shown that RA can inhibit the growth of the Hep-G2 liver cancer cell line in a dose-dependent manner (Jin *et al*., 2020); however, a dose-independent effect was observed in the SMMC-7721 hepatocellular carcinoma cell line (Cao *et al*., 2016; Han *et al*., 2018; Wang *et al*., 2019).

Hep-G2 cells treated with RA from 0 to 320 μM ($IC_{50}$ 14 μM) exhibited a decrease in cell viability, inhibited cell migration, and induced apoptosis as evidenced by high caspase-3 and caspase-9 which play a crucial role in apoptosis (Jin *et al*., 2020). Similarly, RA-treated SMMC-7721 cells induced apoptosis by down-regulating protein expression involved in the G1/S phase, Cyclin D1, and Cyclin E, eventually triggering G1 arrest (Wang *et al*., 2019). In both cancer cell lines, apoptosis was mediated by the elevated expression of Bax (pro-apoptotic protein) and reduced Bcl-2 (anti-apoptotic protein) expression in both cancer cell lines (Wang *et al*., 2019; Jin *et al*., 2020).

In SMMC-7721 cells, RA suppresses the PI3K/AKT/mTOR pathway, which is known to promote cell survival and proliferation (Wang *et al*., 2019). Furthermore, RA has been shown to impede Hep-G2 cell migration and invasion by downregulating the expression of metalloproteases (MMP) 2/9 (An *et al*., 2021). MMPs are enzymes that play a crucial role in degrading the surrounding matrix to facilitate invasion and metastasis by cancer cells (Kessenbrock *et al*., 2010). Furthermore, high levels of the pro-apoptotic protein Bax and cleaved caspase-3 and 9 were observed in Hep-G2 treated with RA and the mechanism underlying the observed effect was associated with PI3K/Akt/NF- κB pathway (An *et al*., 2021).

*In vivo* studies have demonstrated the effectiveness of RA against liver cancer. For instance, intragastric administration

**Table 1.** Mechanisms induced by rosmarinic acid in different cancer cell lines (*in vitro*)

| Cancer type | Cell Line | Treatment concentrations | Mechanism of action | References |
|---|---|---|---|---|
| HCC | Hep-G2 | 0-320 μM | Induction of apoptosis, caspase activation and inhibition of cell migration and invasion | Jin *et al*., 2020 |
| | SMMC-7721 | 0-100 μmol/L | Inhibit PI3K/AKT pathway | Wang *et al*., 2019 |
| | Hep-G2 | 100, 200 and 400 μM | Inhibits PI3K/Akt/NF- κB pathway | An *et al*., 2021 |
| CRC | CT26<br>HCT116 | 0-200 μM | Activation of AMPK | Han *et al*., 2018 |
| | HT-29 | - | Inhibition of p38/AP-1 pathway and EMT by miR-1225-5p | Yang *et al*., 2021 |
| | HCT-8<br>HCT-116 | 0,75 and 150 μmol/L | Suppression of miR-155 leading to an anti-Warburg effect | Xu *et al*., 2016 |
| Pancreatic cancer | PATU-8988<br>MIA PaCa-2<br>Panc-1<br>BxPC-3 | 0-600 μM | Inhibition of Gli1 expression and target proteins | Zhou *et al*., 2022 |
| | Panc-1<br>SW1990 | 0-200 μM | Elevated expression of miR-506 | Han *et al*., 2019 |
| Breast cancer | MDA-MB-468<br>MDA-MB-231 | 0-500 μM | Modulating extrinsic and intrinsic caspase-independent apoptosis | Messeha *et al*., 2020 |
| | MCF-7 | 1.5, 15 and 50 μM | Hindering MDM2, negative regulator of p53 | Juskowiak *et al*., 2018 |
| Ovarian cancer | OVCAR-3 | 0-160 μM | Regulation of lncRNA MALAT-1 | Zhang *et al*., 2018 |
| | SKOV-3<br>TOV-21G | 0, 20 and 40 μM (RAME) | Inhibiting FOXM1 and FOXM1 target genes | Lim *et al*., 2020 |
| Cervical cancer | HeLa<br>SiHa | 0-80 μM (RAME) | Inhibition of mTOR/S6K1 pathway | Nam *et al*., 2019 |
| Renal carcinoma | 786-O | 0, 25, 50, and 100 μM | Downregulated FAK phosphorylation | Su *et al*., 2016 |
| NSCLC | A549<br>A549 DPP (cisplatin-resistant) | 0-200 μg/mL | Activation of JNK pathway | Liao *et al*., 2020 |
| Gastric cancer | SGC7901/5-Fu | 12.5 to 100 μg/mL | Reversal of chemoresistance by inhibiting miR-6785-5p | Yu *et al*., 2019 |
| | MKN45 | 0-600 μM | miR-155 mediated inhibition of the Warburg effect | Han *et al*., 2015 |
| Prostate cancer | PC-3<br>DU-145 | 25-300 μM | Inhibition of HDAC2 | Jang *et al*., 2018 |
| Oral cancer | SCC-15 | 0-180 μM | Oxidative ER stress | Luo *et al*., 2020 |
| Glioma | U251<br>U343 | 0, 100, 200 and 400 μM | Inhibition of PI3K/Akt/NF-κB | Liu *et al*., 2021 |
| | U-78 MG | 0-1000 μM | HSP27 suppression | Şengelen and Önay-Uçar, 2018 |
| Osteosarcoma | MG63<br>U2OS | 0, 12.5, 25, and 50 μg/mL | Suppression of oncogene DJ-1 | Ma *et al*., 2020 |
| Melanoma | A375 | 0, 50, 100 and 200 μg/mL | Suppress ADAM17 expression | Huang *et al*., 2021 |
| Leukemia | NP4 | 40 μM | Enhance ARTA-induced macrophage differentiation | Heo *et al*., 2015 |
| | CCRF-CEM<br>Resistant CEM/ADR500 | 3-100 μM | Inhibition of NF- κB | Wu *et al*., 2015 |

**Table 2.** Mechanisms induced by rosmarinic acid in different cancer models (*in-vivo*)

| Cancer type | Animal models | Treatment concentrations | Mechanism of action | References |
|---|---|---|---|---|
| HCC | H22-xenograft mice | 75, 150 and 300 mg/kg | Inhibition of NF-κB p65 | Cao *et al*., 2016 |
| | SMMC-7721 inoculated mice | 0, 5, 10 and 20 mg/kg | Inhibition of PI3K/AKT pathway | Wang *et al*., 2019, 2021 |
| CRC | DMH-induced rat | 4, 8 and 16 mg/kg | Antioxidant activity by acting on cytochrome P450 | Furtado *et al*., 2015 |
| | DMH-induced rat | 5 mg/kg | | Venkatachalam *et al*., 2016 |
| | AOM/DSS-induced CAC mice | 30 mg/kg | Suppression of TLR4-mediated NF-κB and pSTAT3 | Jin *et al*., 2021 |
| Pancreatic cancer | Panc-1 inoculated mice | 10 mg/kg<br>50 mg/kg | Elevated expression of miR-506 | Han *et al*., 2019; Zhou *et al*., 2022 |
| | PDAC xenograft mice | 50 mg/kg | Inhibition of Gli1 expression | Zhou *et al*., 2022 |
| Breast cancer | Ehrlich induced mice | 50 and 100 mg/kg | Impeding NF-κB-p53-Caspase-3 pathway | Mahmoud *et al*., 2021 |
| Gastric cancer | MKN45 inoculated mice | 2 mg/kg | Inhibition of Warburg effect | Han *et al*., 2015 |

of RA in H22-inoculated tumor mice or injection into nude mice with hepatocellular cancer cell SMMC-7721 led to a significant reduction in tumor size (Cao *et al*., 2016; Wang *et al*., 2019). Notably, the latter study showed significant apoptotic effects only at 10 and 20 mg/kg. Furthermore, in SMMC-7721 mice, RA exhibited its suppressive effect through the PI3K/AKT/mTOR pathway, while the NF-κB intrinsic pathway mediated the anti-tumor effects observed in H22-inoculated mice (Wang *et al*., 2019). Furthermore, RA inhibited angiogenic and inflammatory factors, including IL-1β, TNF-α, and TGF-β, in H22 xenografts (Cao *et al*., 2016).

CA treatment has been shown to inhibit proliferation and induce apoptosis in multiple hepatoma carcinoma cell lines, including Hep-G2, SK-HEP1, MHCC97-H, and Bel7402 (Min *et al*., 2014; Jung *et al*., 2015; Xiang *et al*., 2015; Hasei *et al*., 2021). In Hep-G2 cells, CA treatment (5-100 μM) resulted in activation of caspase-3, PARP cleavage, high Bax/Bcl2 ratio levels and cytochrome c release, all of which are indicative of the mitochondrial-mediated apoptosis pathway (intrinsic). Akt inhibition has been identified as a key mechanism for CA's suppressive effects (Xiang *et al*., 2015). CA treatment in low doses (10 μM) in Hep-G2 elevated tumor suppressor p53 and inhibited mTORC1 through AMPK activation (Hasei *et al*., 2021). In liver cancer cells, MHCC97-H and Bel7402, CA nanoparticle (NP) treatment induced apoptosis as proven by the high levels of cytochrome-c, Apaf-1, caspase 9 caspase-3 and suppression of Bcl-2 mediated by Bad pathway. Furthermore, CA NPs also induced G0/G1 cell cycle arrest by upregulating p21 and downregulating Cyclin D1 and Cyclin D3. CA NPs suppressed NF-κB, IKK-α, and IκB-α, resulting in the downregulation of cytokines IL1-β and IL18. The suppressive effect of CA in liver cancer cells was similar to the results observed upon Akt knockout. *In vivo* studies in MHCC97-H and Bel7402-inoculated mice showed that CA reduced tumor growth, induced apoptosis, and enhanced cell cycle arrest in Akt knockout tumor mice (Tang *et al*., 2016). The CA's effect on Hep-G2 resulted in high levels of sestrin2, MRP2 and Nrf2, which are proteins associated with the Nrf2/ARE (antioxidant response element) pathway. The Nrf2/ARE pathway protects cells from oxidative stress and carcinogens and suppresses cancer progression (Tong *et al*., 2017). Additionally, CA was found to facilitate TRAIL-mediated apoptosis, as evidenced by the enhanced levels of Bim, PUMA (pro-apoptotic markers), death receptor 5 (DR5), CHOP, and decreased levels of Bcl-2 and C-flip (anti-apoptotic markers) (Jung *et al*., 2015). Alternatively, CA induced autophagy in Hep-G2 cells, as indicated by the increased level of LC3 II (autophagosome marker), which was mediated by the inhibition of Akt/mTOR pathway(Gao *et al*., 2015). CA treated at 6.25-50 μg/mL caused inhibition of Hep-3B cells (Yesil-Celiktas *et al*., 2010). Collectively, the anti-tumor effect of CA on hepatoma carcinoma cancer cell lines is regulated by multiple pathways, with the Akt/mTOR pathway being the most prominent (Gao *et al*., 2015; Xiang *et al*., 2015).

### Colorectal cancer (CRC)

The proliferation of CT26 and HCT116 colon cancer cell lines was found to be inhibited in a concentration-dependent manner upon exposure to RA. The observed cell cycle arrest at G0/G1 phase was consistent with the decreased expression of Cyclin D1 and Cyclin-Dependent Kinase 4 in RA-treated CRC cells. RA-induced apoptosis in CT26 and HCT116 via intrinsic pathway by downregulating the expression of anti-apoptotic proteins Bcl-xL and Bcl-2 and via extrinsic pathway by activating its associated caspases (Han *et al*., 2018). Epithelial-Mesenchymal Transition (EMT) mechanism was inhibited in both cell lines, CT26 and HCT116 (Han *et al*., 2018). EMT is a process observed in cancer cells where densely packed epithelial cells lose their innate characteristics, such as cell junctions and cell polarity, and transform into motile mesenchymal cells. They acquire migratory properties with increased expression of specific mesenchymal markers such as N-cadherin (Lai *et al*., 2020). Furthermore, the expression of MMP 2 and 9 was also repressed. RA exerted its effects on CT26 and HCT116 by activating AMPK pathway, which regulates vital processes associated with tumors, such as metastasis, invasion and EMT (Han *et al*., 2018).

In HCT-8 and HCT-116 cells treated with RA, the effects of

**Table 3.** Mechanisms induced by carnosic acid in different cell lines (*in vitro*)

| Cancer type | Cell Line | Treatment concentrations | Mechanism of action | References |
|---|---|---|---|---|
| HCC | Hep-G2 | 5-100 μM | Inhibition of Akt | Xiang *et al*., 2015 |
| | Hep-G2 | 0-30 μM | AMPK activation | Hasei *et al*., 2021 |
| | MHCC97-H<br>Bel7402 | 20 and 30 μM | Inhibition of NF-κB | Tang *et al*., 2016 |
| | Hep-G2 | 0-100 μM | Nrf2 Upregulation | Tong *et al*., 2017 |
| | SK-HEP1 | 20, 40 and 60 μM | | Min *et al*., 2014 |
| | SK-HEP1 | 20 μM | Sensitize TRAIL-mediated apoptosis | Jung *et al*., 2015 |
| | Hep-G2 | 20-100 μM | Inhibition of Akt/m-TOR | Gao *et al*., 2015 |
| | Hep-3B | 6.25-50 μg/mL | | Yesil-Celiktas *et al*., 2010 |
| CRC | HCT116<br>SW480<br>HT-29 | 5, 20, 50 and 100 μM | Inhibition of STAT3 | Kim *et al*., 2016 |
| | HCT116<br>SW480 | 0-100 μM | Activation of Nrf2/Sestrin-2 pathway | Yan *et al*., 2015 |
| | HT-29 | 1-10 μM | Inhibition of Akt | Kim *et al*., 2014 |
| | HT-29 | 12.5 μg/mL | Elevated ER stress and unfolded protein response | Valdes *et al*., 2015 |
| | HT-29 | 0-33.2 μg/mL | ROS accumulation and enhanced GSH levels | Valdes *et al*., 2014 |
| | HT-29<br>SW480 | 30 and 60 μg/mL | | Borrás-Linares *et al*., 2015 |
| | Caco-2 | 6.25, 12.5, 25, 50 and 100 μg/mL | Upregulation of GLCL and down-regulation of COX-2 | Khella *et al*., 2022 |
| | SLW620<br>DLD-1 | - | Upregulation of GCNT3 and Suppression of miR-15b | Gonzalez-Vallinas *et al*., 2014 |
| Pancreatic cancer | MIA PaCa-2<br>PANC-1 | - | - | Gonzalez-Vallinas *et al*., 2014 |
| Breast cancer | MDA-MB-231 | 20 μM | Sensitize TRAIL-mediated apoptosis | Jung *et al*., 2015 |
| | MDA-MB-361 | 20, 40 and 60 μM | | Min *et al*., 2014 |
| | T47D<br>MCF-7 | 0-40 μM | Activation of TRAIL/p53 pathway | Han *et al*., 2017 |
| | SKBR3<br>BT474 | 27.5 μM<br>37.5 μM | Dephosphorylation of Akt | D'Alesio *et al*., 2017 |
| | MCF-7 | 6.25, 12.5, 25, 50 and 100 μg/mL | Upregulation of GLCL and down-regulation of COX-2 | Khella *et al*., 2022 |
| | MCF-7 | 6.25-50 μg/mL | | Yesil-Celiktas *et al*., 2010 |
| Cervical cancer | CaSki<br>SiHa | 0-100 μM | Activation of JNK pathway | Su *et al*., 2016 |
| Esophageal cancer | KYSE-150 | 10, 20 and 40 μM | Inhibition of MAPK signaling | Jiang *et al*., 2021 |
| Renal cell carcinoma | Caki | 20, 40 and 60 μM | ER stress | Min *et al*., 2014 |
| | Caki<br>ACHN<br>A498 | 20 μM | Sensitize TRAIL-mediated apoptosis | Jung *et al*., 2015 |
| | Caki | 20, 50 and 100 μM | Inactivation of STAT3 | Park *et al*., 2016 |
| Non-small cell lung cancer | A549 | 0-200 μM | Inhibition of PI3K/AKT/m-TOR pathway | Zhao *et al*., 2019 |
| | NCI-H460 | 40-320 μM | | Corveloni *et al*., 2020 |

miRNA-155-5p reversed, suppressing tumor inflammation and the Warburg effect by inhibiting IL-6/STAT-3 signaling. MicroRNAs are non-coding RNAs, derived from precursor RNA transcripts and are essential in regulating gene expression. They are involved in the fundamental biological process, including cell proliferation, cell cycle and apoptosis (Yang *et al*., 2021). Dysregulation of miRNA is associated with the pathogenesis of various diseases (Liu *et al*., 2014). The overexpression of miRNA-155-5p has been associated with tumor progression by activating signal transducer and activator of transcription

**Table 3.** Continued

| Cancer type | Cell Line | Treatment concentrations | Mechanism of action | References |
|---|---|---|---|---|
| | H358<br>HCC827 | 20 μM | Activation of TRAIL/Caspase pathway | Shi *et al*., 2017 |
| Gastric cancer | AGS<br>MKN45 | 0-200 μg/mL | Inhibition of PI3K/mTOR/Akt pathway | El-Huneidi *et al*., 2021 |
| Prostate cancer | LNCaP<br>22Rv1 | 0-45 μM | ER-stress-mediated Proteasomal degradation of Androgen Receptor | Petiwala *et al*., 2016 |
| | LNCaP | 10 μM | Activation of EpRE/ARE system | Linnewiel-Hermoni *et al*., 2015 |
| | PC-3<br>DCU-145 | 6.25-50 μg/mL | | Yesil-Celiktas *et al*., 2010 |
| Oral cancer | SCC9 -DPP<br>CAL27 -DPP | 1-100 μM | Inactivation of Nrf2/HO-1/xCT pathway | Han *et al*., 2022 |
| | SCC9<br>CAL27 | 0-80 μM | Induction of Mitochondrial apoptotic pathway | Min *et al*., 2021 |
| Glioma | U251 MG | 17.5-40 μM | Proteasomal degradation of SOX2, Rb and Cyclin B | Cortese *et al*., 2016 |
| | U251<br>LN2299 | 10 μM | Potentiated TMZ-induced effects by inhibiting PI3K/Akt pathway | Shao *et al*., 2019 |
| Neuroblastoma | SH-SY5Y | 10 μM | Suppression of caspase cascade | Meng *et al*., 2015 |
| | SH-SY5Y | 2 μM | Activation of AMPK | Liu *et al*., 2016 |
| | SH-SY5Y | 1 μM | Upregulation of Nrf2 | de Oliveira *et al*., 2018 |
| | SH-SY5Y | 1 μM | Activation of PI3K/Akt/Nrf2 pathway | de Oliveira *et al*., 2015, 2016 |
| | SH-SY5Y | 1 μM | Activation of Nrf2/HO-1 pathway | de Oliveira *et al*., 2017 |
| Astrocytes | U373MG | 50 μM | Activation of TACE | Yoshida *et al*., 2014 |
| Melanoma | B16F10 | 0-100 μM | Enhance BCNU- and CCNU-mediated cytotoxicity | Lin *et al*., 2018 |
| | B16F10 | 2.5-10 μmol/L | Inhibition of EMT | Park *et al*., 2014 |
| Leukemia | CML KBM-7 | 0-50 μM | Suppression of miRNA-708 | Liu *et al*., 2018 |
| | HL60<br>U937<br>MOLM-13 | 10 μM | Enhanced the effects of 1,25-D | Nachliely *et al*., 2016 |

3 (STAT3) in various cancers. Activated STAT3 contributes to maintaining the tumor microenvironment and promoting tumor inflammation. Additionally, dysregulated miRNA-155-5p influences the Warburg effect, a phenomenon where tumor cells undergo lactic acid fermentation even in the presence of sufficient oxygen, leading to chemoresistance (Xu *et al*., 2016). Interleukin-6 (IL-6) is a pro-inflammatory cytokine that results in inflammation-associated cancer by activating STAT3 (Xu *et al*., 2016). Another study has shown that the overexpression of miRNA-1225-5p inhibited metastasis of HT-29 cells by altering p38/AP-1 signaling. p38 is a MAP kinase that assists tumor progression. Upon treatment of HT-29 cells with RA, a change in the expression of MMP 1, 3 and 9 and inhibition of metastasis were observed. Additionally, it was found that the antitumor effects of RA against HT-29 cells also involved inhibition of EMT and abated p38 MAPK signaling through the regulation of miRNA-1225-5p. Silencing of miRNA-1225-5p was hindered the suppressive effects of RA on the HT-29 cancer cell line, indicating its crucial role in mediating RA's inhibitory effects on cancer cells (Yang *et al*., 2021).

In animal studies using 1,2-dimethylhydrazine (DMH) to induce colon cancer in rats, treatment with RA at doses of 4, 8, and 16 mg/kg effectively prevented DNA damage and eliminated dose-independent pre-cancerous lesions called aberrant crypti foci (ACF).

This protective effect of RA may be attributed to its antioxidant activity, as DMH is known to cause carcinogenesis by generating excessive reactive free radicals. RA may also have a suppressive effect on cytochrome P450, which metabolizes DMH (Furtado *et al*., 2015). A study that used DMH-induced rats found that RA administered orally at 5 mg/kg activated caspase-3, leading to apoptosis (Venkatachalam *et al*., 2016). Moreover, Venkatachalam *et al*., found that DMH-induced alteration in the NF-κB pathway was inhibited by RA (Venkatachalam *et al*., 2016). Further studies on a mice model of inflammation-associated colon cancer induced by azoxymethane (AOM)/ Dextran sulfate sodium (DSS) revealed that oral administration of RA at a dose of 30 mg/kg/day effectively reduced tumor weight. RA treatment was shown to reduce the expression of IL-6 and phosphorylated STAT3 in tumor rats. In severe inflammation-associated tumors, Toll-like receptor 4 (TLR4) mediates the activation of the NF-κB pathway and STAT3. RA inhibited TLR4, preventing the nuclear translocation of NF-κB and pSTAT3. This led to reduced inflammation

**Table 4.** Mechanisms induced by carnosic acid in different cancer models (*in-vivo*), (NA, Not available)

| Cancer type | Animal models | Treatment concentrations | Mechanism of action | References |
|---|---|---|---|---|
| HCC | MHCC97-H inoculated mice<br>Bel7402 inoculated mice | 10 and 20 mg/kg | NA | Tang *et al*., 2016 |
| CRC | AOM-induced high-fat diet mice | 0.01-0.02% | Inhibition of Akt | Kim *et al*., 2014 |
| Breast cancer | MCF-7 inoculated mice | 30 mg/kg | Activation of Caspase-3 | Han *et al*., 2017 |
| Lung cancer | HCC827 inoculated mice<br>H358 inoculated mice | 30 mg/kg | Activation of TRAIL/Caspase pathway | Shi *et al*., 2017 |
| | Cisplatin Resistant Lewis Lung cancer Mice | 10 mg/kg | Inhibition of Myeloid-Derived suppressor cells | Wen *et al*., 2018 |
| Prostate cancer | 22Rv1 inoculated mice | 100 mg/kg | Decreased expression of AR | Petiwala *et al*., 2016 |
| Oral cancer | CAL27 inoculated mice<br>SCC9 inoculated mice | 20 mg/kg | Induction of Mitochondrial apoptotic pathway | Min *et al*., 2021 |
| Melanoma | B16F10 inoculated mice | 50 mg/kg | Enhance BCNU- and CCNU anti-tumor effect | Lin *et al*., 2018 |
| Leukemia | K562/A02 inoculated NOD/SCID mice | 1 % (V/V) | NA | Wang *et al*., 2015 |

and suppression of tumorigenesis in a mouse model of AOM/DSS-colitis-associated colon cancer. Similar effects were observed *in vitro* cell lines cultured in an inflammatory microenvironment (Jin *et al*., 2021).

HT-29, HCT-116 and SW480 cells treated with CA (0-100 μM) were shown to cause a significant dose-dependent reduction in cell viability with enhanced apoptosis (Yan *et al*., 2015; Kim *et al*., 2016). The Apoptosis was mediated by a high Bax/Bcl-2 ratio, activation of caspases 3 and 9, and PARP cleavage. The tumor suppressor p53 expression was elevated by CA treatment, facilitating the degradation of MDM2. CA treatment also caused ROS accumulation, contributing to cell death. The suppressive effects of CA were achieved by impeding STAT3 activation induced by preventing the phosphorylation of JAK and Src kinases. The expression of STAT3 target proteins, D-type cyclins, and survivin was also hampered (Kim *et al*., 2016). High levels of Nrf2 and phosphorylation of PERK were observed in HCT-116 and SW480 cells treated with CA (Yan *et al*., 2015). In addition to the marked reduction in cell proliferation in HT-29 cells, treatment with CA resulted in a cell cycle arrest in the S phase that was attributed to a decrease in the expression of Cyclin D1 and CDK4. Furthermore, the expression of the pro-apoptotic protein Bax increased while the expression of the anti-apoptotic proteins Bcl-xL and Akt decreased.

Because colorectal cancer is linked to obesity, the effects of CA therapy on HT-29 cells cocultured with adipocytes were studied. CA therapy reduced triacylglycerol accumulation and adipocyte development, hence inhibiting tumor growth assisted by adipocytes (Kim *et al*., 2014). CA, treated at 12.5 μg/mL in HT-29, has been found to induce cell proliferation and ROS accumulation and hamper cell cycle progression. This inhibitory effect was mediated by activation of Nrf2 coupled with high levels of unfolded protein response (UPR), proteins XBP1 and Ire1, as well as ER stress proteins, Atf4 and PERK. Interestingly, CA treatment is linked with cholesterol accumulation, as evidenced by the high levels of very low-density lipoprotein receptors (VLDL) (Valdes *et al*., 2015). Transcriptomic analysis revealed that CA induces the expression of detoxifying enzymes in HT-29, while a metabolomics study showed elevated glutathione (GSH), which is involved in chemoprevention by functioning as an antioxidant (Balendiran *et al*., 2004; Valdes *et al*., 2014). CA also stifled N-acetyl putrescine expression in HT-29, a polyamine that accelerates cancer progression (Valdes *et al*., 2014). Pure CA (98.7%) obtained from the fractionation of rosemary extract showed the most potent cytotoxic properties in contrast to other purified fractions when treated against HT-29 and SW480 (Borrás-Linares *et al*., 2015). In Caco-2 cells, CA loaded onto bovine serum albumin nanoparticles (CA-BSA-NPs) showed a significant reduction in cell viability, with an $IC_{50}$ of 2.60 μg/mL as opposed to $IC_{50}$ of 8.29 μg/mL using free CA. This dramatic reduction may be attributed to the improved cellular uptake of CA when loaded onto nanoparticles. CA-BSA-NPs induced apoptosis which was found to be orchestrated by the downregulation of Bcl-2 and cyclooxygenase-2 (COX-2) and elevated Glutamate cystyl ligase catalyzed subunit gene (GCLC) (Khella *et al*., 2022). GCLC is involved in glutathione synthesis and modulates tumorigenesis, while COX-2 promotes cancer cell survival by causing resistance to apoptosis, angiogenesis, inflammation, and invasion, among many other properties (Hashemi Goradel *et al*., 2019; Sun *et al*., 2019a). G2/M phase cell cycle arrest was also induced (Khella *et al*., 2022). Alternatively, in SLW620 and DLD-1, the reduced cell viability induced by CA was attributed to heightened expression of glycosyltransferase GCNT3 and downregulation of micro-RNA miR-15b (Gonzalez-Vallinas *et al*., 2014). GCNT3 is a tumor suppressor that prevents cancer metastasis and progression (Gonzalez-Vallinas *et al*., 2015).

In an AOM-induced colorectal cancer mice model fed with a high-fat diet (HFD) supplemented with 0.01-0.02% CA, the average number of tumors was reduced compared to control mice fed with HFD. Supplementation with CA significantly suppressed the elevated insulin levels, insulin-like growth factor 1 (IGF-1), and leptin observed in the HFD tumor model. Additionally, CA reduced the expression of insulin and leptin receptors (Ob-R). Similar to the *in vitro* studies, CA exerted its suppressive effect on tumors by decreasing the expression of Cyclin D1 and Bcl-xL and interfering with the activation of STAT3, Erk, and Akt (Kim *et al*., 2014).

### Pancreatic cancer

*In vitro* studies have shown that RA can inhibit the proliferation of pancreatic ductal adenocarcinoma cell lines (PDAC) in a dose-dependent manner. RA treatment at doses ranging from 0-600 μM inhibited cell proliferation and induced G0/G1 cell cycle arrest in PDAC cell lines with K-ras mutations (which are commonly present in pancreatic cancer), including PATU-8988, MIA PaCa-2, PANC-1, and BxPC-3 wild-type K-ras. Inhibition of metastasis and invasion were also observed, and the expression of MMP-9 was downregulated, while EMT was inhibited. RA achieved this inhibition by modulating the expression of Gli1 and its target genes, including VEGFC, VEGFD, Cyclin D1, and Snail1. Gli1 is a key protein in the Hedgehog signaling pathway, which is abnormally expressed in cancer cells. RA promotes proteasomal degradation of Gli1 and prevents its nuclear translocation. Furthermore, knockdown of Gli1 increased RA's anti-tumor effects, causing cell cycle arrest and upregulated cell cycle inhibitors p21 and p27, while overexpression of Gli-1 abated RA-induced effects. Overall, RA exerted its cytotoxicity and induced intrinsic apoptosis in PDAC cell lines by regulating Gli-1 expression (Han *et al*., 2019; Zhou *et al*., 2022). Alternatively, RA's effects on other pancreatic cell lines, Panc-1 and SW1990, were studied by inducing cytotoxicity in a dose-dependent manner (0-200 μM). It was found that micro-RNA, miR-506 mediated RA's inhibitory effect on metastasis, invasion, and EMT. MiR-506 functions by repressing MMP 2 and 16, which are involved in degrading extracellular matrix and assisting EMT in cancer cells. Therefore, RA imparts its suppressive effect by modulating the miR-506/MMP2/16 axis in pancreatic cancer cells (Han *et al*., 2019).

Animal model studies investigating the effects of RA on pancreatic cancer have shown promising results. For instance, PDAC xenograft mouse models treated orally with RA at 50 mg/kg and xenograft mouse models injected with Panc-1 and treated with 10 and 50 mg/kg of RA demonstrated inhibited xenograft growth and decreased tumor size (Han *et al*., 2019; Zhou *et al*., 2022). In the PDAC xenografts, the underlying mechanism involved increased levels of E-cadherin and Bax and decreased expression of Gli1, Bcl-2, and MMP 9, which was in line with the *in vitro* studies. Conversely, increased levels of miR-506 were thought to be responsible for the inhibitory effects observed in Panc-1 injected mouse models (Han *et al*., 2019; Zhou *et al*., 2022). Furthermore, the inhibitory effect of CA on pancreatic cell lines MIA PaCa-2 and PANC-1 was more pronounced when combined with carnosol than when treated with CA alone (Gonzalez-Vallinas *et al*., 2014).

### Breast cancer

Recent studies have explored the effects of RA on two triple-negative breast cancer (TNBC) cell lines, MDA-MB-231 and MDA-MB-468, and have demonstrated a dose-dependent reduction in cell viability, with MDA-MB-468 showing more significant effects. RA achieved its cytotoxicity by up-regulating the expression of pro-apoptotic proteins, including HRK, BNIP3, TNF-α, GADD45A, and BNIP3. HRK and BNIP3, members of the BCL-2 family, induce apoptosis by blocking anti-apoptotic proteins and inhibiting cell proliferation, respectively. The downregulation of the gene BIRC5 may be responsible for RA's differential response and effectiveness towards MDA-MB-468 breast cancer cells. BIRC5, when overexpressed in breast cancer cells, is known to suppress apoptosis by inhibiting caspases.

Therefore, the observed anti-tumor effects of RA on MDA-MB-468 cells may be attributed to the reduction in BIRC5 expression, which promotes apoptosis in these cells. Additionally, RA treatment resulted in cell cycle arrest at G0/G1 for MDA-MB-231 cells and at S-phase for MDA-MB-468 cells, possibly due to increased expression of the pro-apoptotic gene GADD45A (Messeha *et al*., 2020, Yin *et al*., 2020). In the MCF-7 cell line ($ER^+$, $PR^+$), RA-induced cell death, when combined with the chemotherapeutic drug, doxorubicin (DOX), and RA also decreased the expression of MDM2. Under normal conditions, an elevated tumor suppressor gene (p53) activates MDM2, which ubiquitinates p53 for proteasomal degradation. Therefore, the reduced expression of MDM2 by RA upregulates p53 (Juskowiak *et al*., 2018). Overall, RA has shown significant cytotoxic effects in triple-negative breast cancer and the luminal A subtype model (ER+, PR+). However, further studies are necessary to investigate other key signaling pathways affected in RA-treated MCF-7 cells (Juskowiak *et al*., 2018).

*In vivo* studies using a mice model with an Ehrlrich-induced mammary tumor showed that the cumulative effect of RA and the FDA-approved chemotherapeutic drug, Paclitaxel (PTX) was more potent against cancer than treatment with RA or Paclitaxel alone. RA was administered orally to a group of mice at a dose of 50 mg/kg/day before tumor induction and continued until the end of the experiment. Another group of mice was treated with RA at 100 mg/kg/day after tumor formation. Both RA-treated groups showed elevated expression of tumor suppressor p53 and pro-apoptotic Bax protein, repressed expression (Bcl2), and reduced tumor size. This is indicative of the chemopreventive potential of RA.

In addition, RA's anti-tumor effect is associated with hindering the NF-κB pathway, causing a significant reduction of the angiogenic marker, VEGF and the pro-inflammatory cytokine, TNF-α. Mice that were only treated with PTX also had suppressed tumor growth with reduced VEGF and TNF-α. However, a combination of PTX and RA showed the most enhanced anti-angiogenesis and anti-inflammatory effects coupled with significantly decreased tumor size (Mahmoud *et al*., 2021). Overall, RA is an effective adjuvant that can improve the efficacy of chemotherapeutic drugs.

CA has demonstrated potential in reducing cell viability in various breast cancer cell lines, such as MDA-MB-361, MDA-MB-231, and MCF7, while having a protective effect on normal cells (Yesil-Celiktas *et al*., 2010; Min *et al*., 2014; Jung *et al*., 2015; Han *et al*., 2017). Furthermore, CA's cytotoxicity has been noted to be most effective when combined with chemotherapeutic drugs. For example, in T47D, MCF-7 cells and MCF-7 inoculated xenografts, CA, in combination with tamoxifen (TAM), caused significant inhibition of proliferation and tumor growth.

Moreover, it was observed that the combination resulted in a more potent inhibition of migration and invasion in T47D and MCF-7 cells than treatment using chemotherapeutic drugs alone. This combinational therapy-induced apoptosis in TAM-resistant MCF-7 cells through enhanced expression of the pro-apoptotic proteins Bax, Bad, Bak, and cleaved caspase-8, and a marked decrease of anti-apoptotic proteins Bcl-2 and Bcl-xl. In combination with TAM, CA promoted high expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and decoy receptors, DcR1 and DcR2. TRAIL is as-

sociated with inhibiting tumor progression by interacting with DcR1 and DcR2, causing the activation of caspase-8, which in turn activates caspase-3. Both *in vitro* (MCF-7) and *in vivo* models showed stimulated expression of cleaved caspase 3, PARP, p53 and p73 levels. p73 modulates apoptosis and cell cycle arrest. Taken together, these findings indicate that CA potentiates tamoxifen in inducing apoptosis in breast cancer cell lines by modulating the activation of TRAIL/p53 pathway (Han *et al*., 2017).

Similarly, in ERBB2$^+$ cell lines SKBR3 and BT474, CA, combined with Trastuzumab (Tz), was found to reduce cell survival, and enhance G0/G1 cell cycle arrest compared to CA or Trastuzumab monotherapy. Cell cycle arrest was accompanied by the upregulation of CDK inhibitor p27$^{KIP1}$ (D'Alesio *et al*., 2017). Autophagy, which inhibits cancer progression and promotes tumor survival (Bhutia *et al*., 2013), is also interfered with by CA alone and in combination with Tz, leading to the inhibition of cancer survival by increasing expression of p62 and LC3 II (autophagosome markers) in SKBR3 and BT474 cells. In Trastuzumab resistant SKBR3 cell line (Tz-Res-SKBR3), combination treatment caused a dramatic reduction in cell survival, assisted by the Akt's dephosphorylation. Reduction of ERBB2$^+$ receptor was also noted in Tz-Res-SKBR3 treated with CA and Trastuzumab (D'Alesio *et al*., 2017). Interestingly, when MCF-7 cells were treated with CA-loaded BSA nanoparticles, a reduced $IC_{50}$ of 6.02 μg/mL from 27.43 mg/mL was observed. Induction of apoptosis and G2/M cell cycle arrest, consistent with free CA treatment, was also detected. Furthermore, CA-BSA-NP resulted in an upregulated expression of GCLC and a repressed expression of Bcl-2 and cyclooxygenase-2 (COX-2) (Khella *et al*., 2022).

### Ovarian cancer

Apoptosis-associated morphological changes and suppression of cell migration were observed in RA-treated OVCAR-3 cell line (Zhang *et al*., 2018). MALAT-1 is a long noncoding RNA that is markedly linked to cancer progression. It was reported that MALAT-1 regulates key signaling pathways such as NF- κB, MAPK/ERK, and PI3K/AKT, which affect tumor progression. OVCAR-3 cells exhibited a higher expression of MALAT-1 in treated tumor cells compared to normal and untreated tumor cells (Zhang *et al*., 2018).

The effect of the rosmarinic acid derivative, rosmarinic acid methyl ester (RAME), was studied against ovarian cancer cells. It showed potential anticancer effects by targeting FOXM1-associated target genes (Lim *et al*., 2020). Forkhead box protein M1 (FOXM1), a transcription factor and a proto-oncogene, is involved in cell cycle progression, DNA repair, tissue homeostasis, and apoptosis. Therefore, its abnormal expression inhibits the apoptotic effect in cancers and increases their metastatic abilities and drug resistance. Ovarian cancer cells SKOV-3 and TOV-21G, treated with RAME at 20 and 40 μM, were found to diminish the expression of FOXM1 and its associated target genes CCNB1, CENPF, TOP2A and UBE2C. RAME also inhibited the expression of these genes by preventing the binding of FOXM1 to their respective promoters. Ultimately, invasion and migration were also inhibited in a dose-dependent manner in both cell lines. In other studies on cisplatin-resistant TOV-21G cell lines (high levels of FOXM1), RAME showed similar effects in reducing the expression of FOXM1 and its target genes but it also induced apoptosis. Overall, RAME induces anti-tumor effects by targeting FOXM1 in ovarian cancer cells and also shows immense potential to be used in sensitizing drug-resistant cancer cells (Lim *et al*., 2020).

### Cervical cancer

Studies using human CaSki and SiHa cervical cancer cell lines showed that CA (0-100 μM) exerts an inhibitory effect on cell growth in a dose-dependent manner. The elevated expressions of Bax, Bak, Bad and cleaved caspases-3 and 9 contributed to CA-induced apoptosis. Cell cycle arrest at the G2/M phase and ROS production leading to ER stress were also observed. In addition to *in vitro*, in the *in vivo* models with CaSki inoculated mice, CA treatment resulted in high levels of ER stress proteins ATF2, ATF4, XBP1, CREB and iNOS, leading to reduced tumor growth. The molecular targets of CA in both *in vivo* and *in vitro* studies were found to be through the activation of JNK, p38 and ERK1/2 (MAPK pathway), which are involved in apoptosis-mediated cell death (Su *et al*., 2016).

### Esophageal cancer

Esophageal cancer, an aggressive type of cancer, is found to be highly incident among males. It has a low survival rate due to its poor prognosis. The two types of esophageal cancer are adenocarcinoma and squamous cell carcinoma (SCC), with SCC being the most common (Abbas and Krasna, 2017). Treatment of the KYSE-150 cell line with CA (more than 25 μM) resulted in suppression of cell growth, migration, and invasion, which were mediated by impeding JNK, p38 and ERK1/2 pathways. CA caused G2/M phase arrest, which was correlated to the decreased expression of cyclin B1, MDM2, CDC2 and the induction of DNA damage (Jiang *et al*., 2021). The elevated levels of P53 binding protein 1 (53BP1), a protein involved in the double-strand repair pathway, and γ-H2AX that serves as a marker for the double-strand break, revealed that apoptosis in treated cells was assisted by DNA damage (Menon and Povirk, 2014; Jiang *et al*., 2021). Furthermore, heightened expression of pro-apoptotic Bax protein, and cleaved caspase-3 with low levels of Bcl 2 was also observed (Jiang *et al*., 2021).

### Renal carcinoma (RCC)

RCC is kidney cancer and it arises from the renal tubules' epithelium. The wide diversity of the subtypes of RCC coupled with its poor prognosis have made it challenging to manage this disease (Moch, 2013). Treatment of RA on renal cell carcinoma (RCC) 786-O resulted in a dose-dependent decrease in cell proliferation, while it exerted a protective effect in normal HK-2 cells. In combination with cisplatin, RA produced pronounced cytotoxic effects in 786-O cells by inhibiting migration and invasion and inducing G2/M phase cell cycle arrest. Apoptosis induced by this combination therapy was attributed to increased expression of cleaved PARP and inhibition of focal adhesion kinase (FAK) phosphorylation (Su *et al*., 2016). FAK is implicated in tumor metastasis and invasion and thus promotes tumor progression (Chou *et al*., 2020).

CA inhibited cell viability and induced apoptosis in human renal carcinoma Caki cells while it showed a protective effect when treated in normal mouse TMCK-1 kidney cells and normal human skin fibroblasts. In Caki cells, high doses of CA (40 μM) induced apoptosis; however, at low doses, cell death was not observed (Min *et al*., 2014; Jung *et al*., 2015). CA treatment at 40 μM led to intracellular ROS accumulation and in-

creased expression of ATF4 and CHOP proteins, indicative of ER stress (Min *et al*., 2014). Furthermore, CA was found to induce apoptosis mediated by an intrinsic and extrinsic pathway evident by the induced expression of cleaved caspase-3, 7, 9 and PARP. The upregulated levels of death receptors, DR4 and DR5, also mediated the extrinsic pathway. The pro-apoptotic Bax protein was elevated while the expression of Bcl-2 was diminished upon CA treatment. Additionally, CA mediated the downregulation of MDM2, increasing the expression of tumor suppressor, p53, and cell cycle regulator, p27. Furthermore, STAT3 activation was hampered, reducing survivin, D-type cyclins, and c-myc (Park *et al*., 2016). C-myc is an oncogene, while survivin is an inhibitor of apoptosis (Jaiswal *et al*., 2015; Lin *et al*., 2018). In further studies on renal carcinoma cell lines (Caki, ACHN and A498), low doses of CA enhanced TRAIL-mediated apoptosis by promoting proteasomal degradation of c-FLIP and Bcl-2. At the same time, Bim, PUMA and DR5 were upregulated. CA treatment with TRAIL induced ER stress, as seen by elevated $Ca^{2+}$, CHOP and ATF4 (Jung *et al*., 2015).

## Lung cancer

Lung cancer originates within the bronchi or alveoli and can be classified into small-cell lung cancer and non-small-cell lung cancer (NSCLC) (Lundin and Driscoll, 2013). Rosmarinic acid treated at 0-200 μg/mL against non-small cell lung cancer (NSCLC) A459, and cisplatin (DPP)- resistant cell line A459DPP exhibited a reduction of cell viability in a dose-dependent manner. RA's inhibitory concentration ($IC_{50}$) was 14.05 μg/mL and 46.47 μg/mL for A459 and A459DPP, respectively. Cell cycle arrest at G1 phase and induction of apoptosis was observed in the RA-treated cisplatin-resistant cell line A459DPP (combination therapy). Apoptosis was mediated by the increased expression of p53, cleaved caspase-3 and Bax, while BCL-2 was downregulated. RA subdued the expression of MDR1gene and P-glycoprotein (P-gp), which are known to be present at high levels in drug-resistant cancer cells. The multi-drug resistance gene 1 (MDR1) codes for the P-glycoprotein (P-gp) protein, a member of the ATP-binding cassette (ABC) transporter family. P-gp is responsible for the efflux of a wide range of chemotherapeutic drugs, leading to chemoresistance in cancer cells. The overexpression of the MDR1 gene drives this mechanism and the subsequent upregulation of P-gp, which results in the reduction of drug accumulation within the cancer cells and, subsequently, decreased efficacy of chemotherapy. The effects of RA on A459DPP were mediated by the activation of the JNK pathway, which reduces MDR1 gene expression. Alternatively, *in vivo* studies showed that a combined therapy of RA at 10 mg/kg and DPP had the most significant tumor reduction (Liao *et al*., 2020).

Treatment of CA on NSCLC cell lines A459 and NCI-H460 induced apoptosis and suppressed cell proliferation (Zhao *et al*., 2019; Corveloni *et al*., 2020). In the A459 cell line, CA (12, 2.5 and 50 μM) caused significant inhibition of invasion and metastases, as evidenced by the decreased expression of MMP-9. In addition, CA was found to exert its cytotoxicity by hampering PI3K/AKT/m-TOR pathway (Zhao *et al*., 2019). In comparison, the cytotoxic effects of CA in NCI-H460 were more prominent only at a higher concentration of 160 μM. An induction of apoptosis, achieved by enhanced expression of PUMA, and an elevated G1 cell cycle arrest were also observed (Corveloni *et al*., 2020). However, this study revealed that CA showes inhibitory effects in human fetal fibroblasts IMR-90 as opposed to its protective effect on normal lung fibroblasts MRC-5 (Shi *et al*., 2017; Zhao *et al*., 2019; Corveloni *et al*., 2020). This raises concerns about CA's potential selective toxicity. CA (6.25-50 g/mL) also reduced cell viability in NCI-H82 small-cell lung cancer (Yesil-Celiktas *et al*., 2010).

Furthermore, CA exhibits potent cytotoxicity in lung cancer cells when combined with fisetin, a flavonoid with reported anti-tumor properties. Used in combination with fisetin, CA caused a significant reduction in cell viability in H358 and GCC827 cell lines, and in tumor growth of *in vivo* tumor models. The enhanced induction of apoptosis in both models was attributed to the activation of the caspases cascade, with increased expression of Bax and Bad and decreased levels of Bcl-2 and Bcl-xl. Furthermore, the combination treatment significantly increased the levels of TRAIL and its associated proteins, DR4, DR5, and FADD, in the *in vitro* experiments. In contrast, the tumor suppressor protein, p53 was upregulated in tumors inoculated with H358 and HCC827 cells. CA and fisetin exert their potent cytotoxicity by modulating the TRAIL/caspase pathway (Shi *et al*., 2017). Notably, *in vivo* studies on mice affected by cisplatin-resistant Lewis's lung cancer (LLC) demonstrated CA's ability to potentiate the effect of cisplatin. In addition to the significant reduction in tumor growth, cisplatin (1 mg/kg) with CA (10 mg/kg) also induced apoptosis, as correlated with the elevated levels of activated caspase 3. Furthermore, carnosic acid showed remarkable potential in ameliorating anti-tumor response by promoting CD8+ T cell infiltration and suppressing myeloid-derived suppressor cells (MDSCs). MDSCs are known to prevent anti-tumor immune response, prompted by increased expression of arginase 1 (arg 1) and iNOS that are involved in inhibiting T cells. MDSCs also produce MMP-9, thus promoting tumor progression (Wen *et al*., 2018).

## Gastric cancer

The incidence of gastric cancer is geographically variable, as it is correlated with diet intake and lifestyle, among other factors. Currently, surgery is the only effective treatment strategy (Zali *et al*., 2011). However, RA has been found to exhibit an anti-carcinogenic property in gastric cancer cell lines by modulating micro-RNAs. Treatment with RA (12.5 to 100 μg/mL) produced dose-dependent cytotoxicity in chemo-resistant gastric cancer cell line SGC7901/5-Fu by targeting two miRNAs: miR-642a-3p and miR-6785-5p. For example, gastric cancer patients often develop resistance to the chemotherapeutic drug 5-Fluorouracil (5-FU) (Yu *et al*., 2019). RA showed the potential to reverse this chemoresistance by downregulating the expression of the two miRNAs, miR-642a-3p and miR-6785-5p, which are found to be present in elevated levels in 5-Fu-resistant cells SGC7901/5. The miR-6785-5p is a negative regulator of FOXO4, which is involved in regulating the cell cycle and apoptosis. RA reduces the $IC_{50}$ of 5-FU, increasing the sensitivity of 5-FU-resistant cells. In combination with 5-FU, RA abolishes miR-6785-5p expression, increasing FOXO4 levels and reversing chemoresistance. In addition, the expression of P-gp is depleted, with an upregulated Bax expression in RA-treated SGC7901/5-Fu cells compared to untreated ones (Yu *et al*., 2019). Similarly, RA attenuated the Warburg effect in gastric carcinoma MKN45 and *in vitro* models by repressing the expression of miR-155-5p. Cell viability was reduced in a dose-dependent manner with $IC_{50}$ at 240.2

μM. RA inhibited key processes involved in the Warburg effect, such as glucose uptake by cancer cells, lactose generation, and Hypoxia-inducible factor-α expression. Furthermore, pro-inflammatory cytokines, such as IL-6 production, was abated and RA prevented STAT3 activation. Therefore, the suppressive effect of RA in MKN45 is promoted by the IL-6/STAT3 pathway (mediated by the inhibition of miR-155-5p) which is essential in the Warburg effect (Han *et al*., 2015).

Treatment of AGS and MKN45 with carnosic acid (0-200 μg/mL) showed dose-dependent inhibition of cell viability and induced G1-phase arrest. CA (20 μg/mL) also enhanced apoptosis, effects evidenced by the increased expression of caspases-3,8,9, and cleaved PARP-1 in treated cells. CA produced its anticancer effect in AGS and MKN45 cells by inhibiting PI3K/mTOR/Akt pathway. In addition, gastric cancer cells treated with carnosic acid downregulated survivin expression, aiding in apoptosis (El-Huneidi *et al*., 2021). Survivin is a protein overexpressed in cancer, promoting cell proliferation while blocking apoptosis.

**Prostate cancer**

In prostate cancer, the predominant type of tumor in men, RA showed anti-tumor effects in PC-3 and DU-145 cell lines. The natural mechanism by which RA exerts its effects involves inhibiting the histone deacetylase (HDAC) enzyme (Jang *et al*., 2018). HDAC removes acetyl groups from histones and causes tight wrapping of the DNA leading to the differential regulation of the genes, including cell cycle and apoptosis-related genes. Therefore, the abnormal expression of HDACs is linked to tumor development (Ropero and Esteller, 2007). Furthermore, RA has been reported to decrease cell viability and cause apoptosis induction in both cell lines parallel to the effects induced by suberoylanilide hydroxamic acid (SAHA), which is an HDAC inhibitor. However, significant cytotoxicity was observed at a higher dose of 200 μM for RA and at merely 1 μM for SAHA. In contrast, RA can modulate the expression of apoptosis-related genes more effectively than SAHA by increasing the expression of Bax/Bcl-2, caspase-3 and cleaving PARP-1. Furthermore, it has been observed that apoptosis can be induced in both prostate cancer cell lines via the intrinsic pathway mediated by p53. As a result, rosmarinic acid has the potential to serve as an HDAC inhibitor while causing cytotoxicity in cancer cells (Jang *et al*., 2018).

Several studies reported that CA inhibited cell proliferation in prostate cancer cell lines LNCaP, 22Rv1, PC-3, and DCU-145 in a dose-dependent manner (Yesil-Celiktas *et al*., 2010; Petiwala *et al*., 2016). In LNCaP and 22Rv1, induction of apoptosis and an elevated expression of BiP, IRE1α and CHOP/GADD135, indicative of ER stress, were observed (Petiwala *et al*., 2016). Bip, an ER chaperone, and IRE1α are involved in unfolding protein response activation (Kopp *et al*., 2019). CHOP/GADD135, also known as C/EBP homologous protein, is a transcription factor responsible for inducing ER stress-mediated apoptosis (Oyadomari and Mori, 2004). Furthermore, Petiwala's study revealed that CA exhibited suppressive effects in prostate cancer by promoting proteasomal degradation of the androgen receptor (AR) by inducing ER stress (Petiwala *et al*., 2016). The androgen receptor is a molecular target for prostate cancer treatment; however, these receptors can develop resistance by increasing AR levels or inducing AR mutations for survival after androgen therapy (Guerriero *et al*., 2021).

Moreover, *in vivo* studies on athymic nude mice treated with CA at 100 mg/kg for 14 days showed a 53% reduction in tumor size, decreased expression of AR, and elevated levels of CHOP compared to the control group. Conversely, the prostate epithelial cell lines treated with CA failed to increase the expression of CHOP, preventing ER stress, which indicates that CA is selectively toxic to cancer cell lines (Petiwala *et al*., 2016). In addition, treatment of LNCap with carnosic acid alone at 10 μM, or in combination with phytonutrients, repressed the androgen receptor gene and prostate-specific antigen (PSA) secretion (Linnewiel-Hermoni *et al*., 2015). PSA is a protein-specific antigen used as a molecular marker for prostate cancer due to its high level presence in prostate cancer patients (Lilja *et al*., 2008). Additionally, CA was reported to inhibit cell proliferation in LNCap and cause synergistic activation (CA with phytonutrients like curcumin) of the EpRE/ARE transcription system. The electrophile/antioxidant response element transcription (EpRE/ARE) causes the transcription of genes involved in oxidative stress, such as detoxifying enzymes, thus reducing cancer risk (Linnewiel *et al*., 2009; Linnewiel-Hermoni *et al*., 2015).

**Oral cancer**

Experimental studies on oral cancer cell line SCC-15, exposed to RA at 0-180 μM, showed dose-dependent cytotoxicity with an $IC_{50}$ ranging between 20 and 40 μM. Additionally, the selective inhibitory property of RA towards cancer cells was observed by comparing its exposure to normal oral cells hTERT-OME with cancer cell line SCC-15. RA induced apoptosis in SCC-15 by elevating the expression of pro-apoptotic protein Bax and cleaved caspase-3, while Bcl-2 expression was reduced. Moreover, RA had an anti-migratory effect and inhibited the invasion of SCC-15 by decreasing the expression of metalloproteases MMP-2 and MMP-9 (Luo *et al*., 2020; Liu *et al*., 2021). Cell cycle arrest in oral cancer cells was detected at the G2/M phase, which correlated with the decrease in cyclin B1. Furthermore, the increased expression of ER-stress markers indicated that RA functions in oral cancer cells by inducing oxidative ER-stress. The endoplasmic reticulum (ER) is an essential organelle that functions in the folding, packaging, and translocation of proteins (Luo *et al*., 2020). ER-stress, caused due to misfolding of proteins, DNA damage, or other external factors is known to promote apoptosis which can help induce cancer cell death (Yadav *et al*., 2014).

When tested on oral squamous cancer cell (OSCC), lines SCC9 and CAL27, CA (1-100 μM) resulted in a dose-dependent decrease in cell viability, while no significant inhibition was observed in normal oral keratinocyte NHOK. Importantly, when treated with CA, cisplatin-resistant cell lines SCC9-DPP and CAL27-DPP had an increased cell death rate induced by ferroptosis. This is in accordance with decreased levels of glutathione (GSH), an antioxidant that protects the cell from ferroptosis (Li *et al*., 2022), and elevated levels of the reactive oxygen species (ROS) and lipid peroxidation that were observed in treated SCC9-DPP and CAL27-DPP cells (Han *et al*., 2022). The nuclear factor erythroid 2–related factor 2 (Nrf2) protects the cell from oxidative stress by promoting the expression of the antioxidant protein heme oxygenase 1 (HO-1). In SCC9-DPP and CAL-27DPP, the Nrf2/HO-1/signaling is enhanced upon CA treatment. Moreover, CA reduced the expression of xCT, a cysteine/glutamate antiporter involved in modulating ferroptosis (Han *et al*., 2022; Jyotsana *et al*.,

2022). Similarly, another study showed apoptosis by CAL27, and SCC9 cells, and tumor xenografts treated with CA. However, apoptosis was mediated by the intrinsic pathway, which was indicated by the upregulation of cleaved PARP-1 and cleaved caspases-3 and 8 in treated cells and tumor xenografts. An increased expression of Bax and Bad, with a reduced Bcl-2 expression, was observed in both *in vivo* and *in vitro* models. Furthermore, in CAL27 and SCC9 cells, CA decreased the mitochondrial membrane potential while the ROS accumulation and $Ca^{2+}$ influx increased. $Ca^{2+}$ is a crucial secondary messenger that increases ROS production. Overall, CA treatment was noted to also disrupt the mitochondrial structure, inhibiting OSCC proliferation (Min *et al*., 2021).

## Brain cancers

Brain and central nervous system (CNS) cancers are a diverse group of malignancies that arise from the brain and its surrounding structures. For example, the gliomas are tumors in glial cells, such as astrocytes, oligodendrocytes, and others in the brain or the central nervous system (Davis, 2016). In glioma cells, U251 and U343, RA was reported to reduce the cell viability dose-dependently, while no significant toxic effect was seen in normal human astrocytes (NHA) (Liu *et al*., 2021). Furthermore, apoptosis was induced by increasing Bax/Bcl-2 ratio, while metastasis was inhibited by downregulating the expression of MMP 2 and 9. The mechanism underlying these effects induced by RA was found to be correlated with PI3K/Akt/NF-κB signaling pathway. The PI3K/Akt/NF-κB pathway is usually activated in cancer cells, causing tumor progression. In addition, a tyrosine (kinase Fyn), usually overexpressed in glioma cancer cells, was inhibited by RA, which impedes the PI3K/Akt/NF-κB pathway (Liu *et al*., 2021).

Further studies on the treatment of glioma cells U-78 MG with RA correlated the effect induced by RA with heat shock protein 27 expression (hsp27) (Şengelen and Önay-Uçar, 2018). Hsp27 is a chaperone protein involved in the correct folding of misfolded proteins and protects the cells during stress. However, the abnormal expression of hsp27, as reported in brain tumors, leads to cancer cell survival by preventing apoptosis and inducing drug resistance. In this study, treatment with RA at 0-1,000 μM for 48 h exhibited cytotoxic effects in U-78 MG cells and downregulated expression of hsp27. Quercetin, a known hsp27 inhibitor, was used as a positive control. RA treated at 80 and 215 μM showed 28.8 and 46.7% reduction of hsp27, respectively. Furthermore, RA treatment in hsp27-silenced U-78MG cells showed high caspase-3 activity compared to unsilenced RA-treated, unsilenced quercetin-treated, or hsp27-silenced quercetin-treated cells. Therefore, it can be concluded that RA is a potential hsp27 inhibitor, and, in combination with silenced hsp27, produces evident caspase-3 dependent apoptosis in glioma cell U-78 MG (Şengelen and Önay-Uçar, 2018; Choi *et al*., 2019).

It was further observed that sarnosic acid treatment inhibited cell growth, apoptosis, and cell cycle arrest at the G2 phase in glioblastoma (GMB) cell line U251 MG and patient cells. CA exerted its anti-carcinogenic effect by inducing proteasomal degradation of crucial proteins -cyclin B, retinoblastoma (Rb), SOX2, glial fibrillary acidic protein (GFAP)- and increasing the expression of cell cycle inhibitor p21 (Cortese *et al*., 2016). Retinoblastoma regulates the cell cycle, while SOX 2 allows cancer cells to evade apoptosis (Cortese *et al*., 2016; Zhang *et al*., 2020). GFAP is an intermediate filament that is known to be associated with glioma (Santos *et al*., 2009). However, its toxicity towards normal astrocytes limits CA's potential use in glioma (Cortese *et al*., 2016). Interestingly, CA can potentially be used in combination with temozolomide (TMZ), which is often used as a chemotherapeutic drug for brain cancer. In U251 and LN229 cells, low doses of CA with TMZ inhibited cell proliferation, migration and enhanced apoptosis as opposed to TMZ alone. Furthermore, CA improved the effects of TMZ, increasing the expression of cleaved PARP, caspase-3 and cyclin B1. In addition to apoptosis, the use of the CA and TMZ combination resulted in the overexpression of LC3-II and the downregulation of p62, indicative of autophagy. The suppressive effect produced by CA and TMZ was found to be mediated by the negative regulation of PI3K/AKT pathway (Shao *et al*., 2019). On the other hand, CA exhibits a neuroprotective effect in neuroblastoma cell lines SH-SY5Y. CA subdued apoptosis and caspases activation caused by amyloid β (Aβ) treatment in the SH-SY5Y cell line (Meng *et al*., 2015). Furthermore, CA suppressed Aβ toxicity by decreasing ROS accumulation while inducing autophagy, mediated by the activation of AMPK (Liu *et al*., 2016). In SH-SY5Y exposed to $H_2O_2$, Cacrnosic acid was also found to prevent mitochondrial dysfunction, modulated by elevated levels of GSH and upregulation of Nrf2 (de Oliveira *et al*., 2018). Carnosic acid has the ability to attenuate the effects induced by certain toxic compounds, namely methylglyoxal and paraquat, mediated by the PI3K/Akt/Nrf-2 pathway (de Oliveira *et al*., 2015, 2016, 2017). In addition to Nrf-2 activation by CA, HO-1 also reduced the neurotoxicity induced by paraquat (de Oliveira *et al*., 2017). Overall, CA is implicated in neuroblastoma as an antioxidant and neuroprotective agent, crucial in averting neurodegenerative diseases while inducing cytotoxic effects in glioblastoma. In astrocytoma cells U373MG, CA reduced the Aβ levels, specifically Aβ40 and Aβ42, which was found to be correlated with the upregulated level of α-secretase tumor necrosis factor converting enzyme (TACE). TACE is essential in the cleavage of amyloid precursor protein by α-secretase, preventing Aβ deposition, which leads to Alzheimer's (Yoshida *et al*., 2014).

## Osteosarcoma

Osteosarcoma is an aggressive tumor of the bone that often occurs in children and adolescents, affecting the metaphysis of long bones, including the distal femur (Lindsey *et al*., 2017). Treating the osteosarcoma cell line MG63 and U2OS with RA (12.5, 25 and 50 μg/mL) inhibited cell proliferation and induced apoptosis dose-dependently. Both intrinsic and extrinsic pathways of apoptosis were activated in both the cell lines, contributed by the upregulation of caspase-9 (intrinsic), caspase-3 and caspase-8 (extrinsic). Bax/Bcl-2 ratio was also increased. Cell cycle arrest was observed at the G2/M phase owing to the ablated expression of its associated proteins, cyclin B1, cdc2, and CDC25c. In addition to inhibiting EMT, rosmarinic acid also decreased the migration and invasion by downregulating the expression of MMP 2 and 9. These effects were achieved by suppressing oncogene DJ-1, which allows the activation of the tumor suppressor gene, PTEN, subsequently inhibiting cell proliferation by the PI3K/Akt/mTOR pathway (Ma *et al*., 2020).

## Melanoma

Melanoma is a metastatic skin cancer originating from the melanocytes. A study on melanoma cancer cells A375 report-

ed that rosmarinic acid caused a significant decline in cell viability and inhibited cancer cell invasion and migration by hindering ADAM17/EGFR/AKT/GSK3β axis (Huang *et al*., 2021). ADAM17 is an integrin and metalloprotease that has been reported to be involved in tumor progression by regulating EGFR and TNF-α (Düsterhöft *et al*., 2019). Upon treatment with RA, A375 cells showed decreased expression of EGFR, AKT and GSK3β along with ADAM17. In addition, impediment with ADAM17 inhibitor TPD enhanced the effect of rosmarinic acid, while the overexpression of ADAM17 counteracts the cytotoxicity induced by RA. Although this study has revealed the potential underlying the mechanism of RA on melanoma cells, it is essential to confirm these results by investigating different melanoma cell lines and ensure the selectivity of RA by examining its effects on normal melanoma cells (Huang *et al*., 2021).

Moreover, CA was found to inhibit cell growth, migration and induce cell cycle arrest in B16F10 cells (Park *et al*., 2016; Lin *et al*., 2018). Cell cycle arrest at G0/G1 phase in treated cells was revealed to be associated with a high level of p21, which is a CDK inhibitor (Lin *et al*., 2018). Additionally, CA exhibited the potential to inhibit migration in B16F10 cells, caused by the decrease in metastasis-associated proteins MMP-9, TIMP1, urokinase-type plasminogen activator (upA) and adhesion protein VCAM-1. CA's ability to prominently inhibit EMT in melanoma cancer cells is correlated to the decrease in EMT-associated transcription factors in the snail, and slug, coupled with elevated levels of E-cadherin. Moreover, the mesenchymal markers, vimentin, and N-cadherin, were repressed. Carnosic acid prevented the phosphorylation of Akt and Src/Fak, which were found to be key signaling pathways involved in EMT (Park *et al*., 2014). Intriguingly, CA also enhances the cytotoxic effect of chemotherapeutic drugs carmustine (BCNU) and lomustine (CCNU). Animal studies in B16F10 inoculated mice showed that CA (50 mg/kg) reduced tumor growth, with a more pronounced effect when treated in combination with BCNU or CCNU (Lin *et al*., 2018).

### Leukemia

Leukemia is a hematological cancer that originates in the bone marrow and affects the lymphoid or myeloid cells resulting in abnormal white blood cells. Primarily, the different types of leukemia are classified as acute or chronic, such as the acute myeloid leukemia and chronic lymphocytic leukemia, among many others (Davis *et al*., 2014; Dong *et al*., 2020). Human acute promyelocytic leukemia NP4 cells treated with RA (40 μM) and all-trans retinoic acid (ARTA) promoted differentiation of macrophages, evident by the elevated expression of CD11b and CD14 (macrophage markers). Furthermore, the differentiated macrophages depicted the ability to release ROS and have elevated expression of chemokine receptors CCR-1, CCR-2 and ICAM-1. This RA and ARTA synergistic effect is regulated by NF-κB activation (Heo *et al*., 2015). In acute lymphoblastic leukemia (ALL) cell line CCRF-CEM and multi-drug resistant leukemia cell line CEM/ADR5000, treatment with RA caused a significant reduction in cell viability, while normal lymphocytes were tolerant. RA induced G2/M phase arrest, apoptosis, and necrosis in CCRF-CEM cells modulated by PARP cleavage and NF-κB inhibition. Disruption of mitochondrial membrane potential also contributes to cell death induced by RA (Wu *et al*., 2015).

Treatment of carnosic acid on chronic myeloid leukemia cells CML KBM-7 caused apoptosis and inhibition of cell proliferation. Furthermore, significant inhibition of invasion and cell cycle arrest at the G2/M phase were observed. The cytotoxic effect of CA was mediated by the repression of miRNA-708 (Liu *et al*., 2018). miRNA-708 has been previously reported to be associated with the pathogenesis of many cancers, such as colorectal cancer (Sun *et al*., 2019b). Vitamin D3 (1,25-dihydroxy vitamin D3, 1,25-D) is shown to induce monocyte differentiation; however, its use is limited as it results in hypercalcemia. Carnosic acid treatment at 10 μM with low doses of 1,25-D exhibits the potential to enhance the differentiation effect of 1,25-D in acute myeloid leukemia cell lines HL60, U937 and MOLM-13. This combined treatment also elevated vitamin D protein receptor levels (Nachliely *et al*., 2016). CA inhibited the cell viability of K-562 in a dose-dependent manner (Yesil-Celiktas *et al*., 2010). *In vivo* studies on K562/A02 inoculated NOD/SCID mice (acute myeloid leukemia) revealed that CA potentiated the effects of Adriamycin, increased apoptosis and prolonged mice survival. The high levels of MDR1 (multi-drug resistance receptor) observed in K562/A02/SCID mice were significantly repressed when treated with CA and Adriamycin (Wang *et al*., 2015).

## PHARMACOKINETICS AND BIOAVAILABILITY OF ROSMARINIC AND CARNOSIC ACIDS

The pharmacokinetics of rosmarinic acid (RA) can be better understood by evaluating the metabolic pathways involved after uptake. *In vivo* studies conducted on mice and rats have revealed the breakdown of RA into caffeic acid, coumaric acid as well as m-hydroxybenzoic acid. Further metabolism involves processes such as sulfation, methylation, glucose conjugation, and glucuronic acid conjugation (Guo *et al*., 2019). In a study of human liver microsomes, researchers identified 14 metabolites within 19 minutes of incubation with RA produced through various metabolic pathways, with glucoridantion being a major pathway (Su *et al*., 2020).

The absorption of orally ingested rosmarinic acid in the intestine occurs by paracellular transport (Domínguez-Avila *et al*., 2017). It was found that when Sprague-Dawley rats were injected with RA intravenously, the acid was distributed from the blood to different tissues including kidney, liver, lung and heart tissues. However, the concentration of RA in the brain was relatively low owing to the blood-brain barrier preventing its penetration (Chen *et al*., 2021). In rats treated orally with RA (12.5, 25 and 50 mg/kg), RA was rapidly absorbed as evident by high plasma concentration of 215.21, 361.57, and 790.96 ng/mL. This study also demonstrated poor absolute bioavailability of RA, as low as 1% (Wang *et al*., 2017). The elimination of RA occurs renally, being excreted with urine (Nunes *et al*., 2017). The low bioavailability of RA is attributed to its poor water solubility and reduced permeability due to its acidic nature (pH=2.9) (Chaitanya *et al*., 2022).

A number of studies attempted to determine the pharmacokinetic profile where CA is administered orally at an average dose of 64.3 mg/kg in *in vivo* models. After absorption into the blood stream, CA is distributed to liver, muscle, and intestinal tissues, with its elimination through the fecal route. The bioavailability of CA after 360 minutes was found to be 40.1% (Doolaege *et al*., 2011). Alternatively, Sprague-Dawley rats treated with CA intragastrically at 90 mg/kg exhibited a

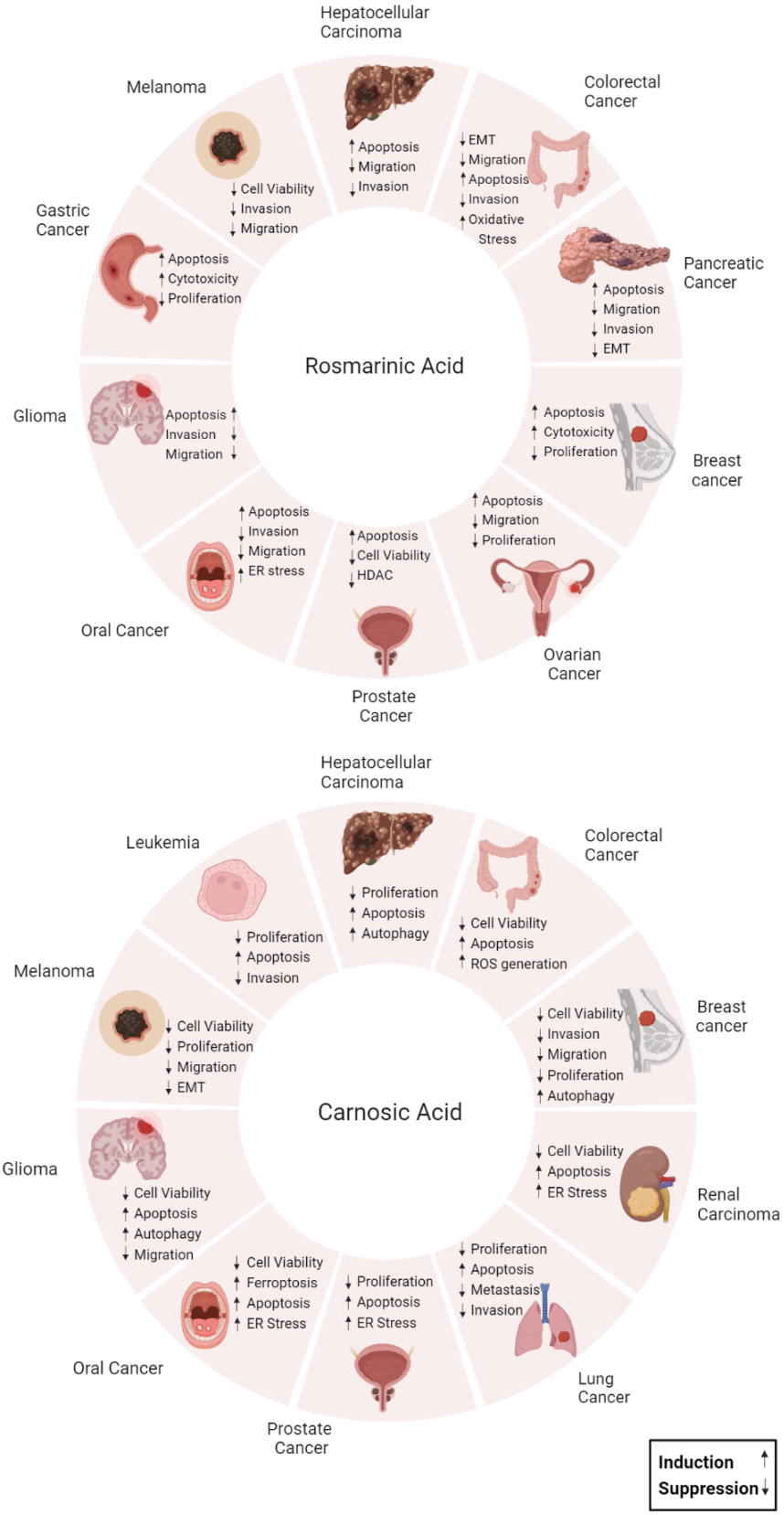


**Fig. 2.** Summary of anticancer effects of RA and CA in different cancers.

bioavailability of 65.09% (Yan *et al*., 2009). Additional studies on mice treated orally with a physiologically relevant dose of 50 mg/kg/daily of CA demonstrated its presence in the serum as well as in the heart, spleen and liver. Furthermore, at low doses (50 μM-75 μM), CA was passively absorbed in a Caco-2 monolayer model, while higher doses (100 μM) resulted in mild efflux (Chen *et al*., 2021). Metabolism studies on CA using *in vitro* and *in vivo* models showed that oxidation, glucuronidation and methylation are the primary metabolic pathways of CA. Moreover, the high oral bioavailability of CA compared to RA is attributed to the increased formation of glucuronidated metabolites (Song *et al*., 2014).

## CONCLUSION AND PROSPECTS

RA and CA exhibited pro-apoptotic effects while inhibiting migration, invasion, and proliferation of cancer cells (Fig. 2). The cytotoxicity and therapeutic potential of rosmarinic acid (RA) and carnosic acid (CA) in numerous types of cancer, including resistant cells, make them promising candidates for anti-cancer therapies. The selective toxicity, cost-effectiveness, and synergistic effects with chemotherapeutic medications further support their potential application in cancer treatment. Furthermore, its abundance makes rosemary a convenient and accessible source for possible medicinal development.

Looking ahead, several avenues for future research and exploration can be identified. Firstly, further research is needed to understand the long-term effects of RA and CA, including their impact on normal cell lines and tissues, as well as the possibility for selective cancer cell targeting. Important concerns include assessing the safety profile of RA and investigating the interaction of CA with cytochrome P450 enzymes.

Furthermore, in-depth investigation on the modification of microRNAs by RA and CA, as well as the development of genomic tools such as the miRNA microarray, might provide vital insights into their mechanism of action and allow for the identification of specific miRNA targets for distinct malignancies. This understanding can help in the development of therapeutic strategies to counteract drug resistance.

It is critical to address the constraints caused by the variability in the metabolic profile and limited absorption of RA and CA. Formulation strategies including lipid nanoparticles, nano-emulsions, and cyclodextrins can boost their bioavailability and anti-cancer benefits at lower dosages. Understanding the interaction between these polyphenols and gut flora may also help to maximize their therapeutic benefits and influence the design of future clinical trials.

Despite their potential, RA and CA have significant limitations that must be addressed. The varying concentrations of these chemicals, which are impacted by extraction processes, geographical origin, and harvest time, cause repeatability issues. To achieve constant bioactive content, extraction techniques must be standardized, and quality control measures implemented.

In conclusion, while RA and CA show significant potential as anti-cancer medicines, further study is needed to understand their long-term effects, improve dosing regimens, establish their safety profiles, and address bioavailability and variability constraints. By moving forward in these areas of research, RA and CA can be developed into practical and cost-effective therapeutics, bringing us closer to effective cancer treatments.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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