ROSMARINIC ACID: A REVIEW OF ITS ANTICANCER ACTION

Md. Shahadat Hossan¹, Shahnaz Rahman², A.B.M. Anwarul Bashar¹, Rownak Jahan², Abdullah Al-Nahain¹, Mohammed Rahmatullah¹*

¹Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh
²Department of Biotechnology & Genetic Engineering University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh

Article Received on 6 June 2014,
Revised on 19 July 2014,
Accepted on 13 August 2014

*Correspondence for Author
Dr. Mohammed Rahmatullah
Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh

ABSTRACT
Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenylactic acid commonly found in plants belonging to the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae family. The compound has a number of important biological activities, e.g. antiviral, antibacterial, antiinflammatory, anticancer and antioxidant. This review shall focus on the reported anticancer activities of rosmarinic acid and discuss its therapeutic potential against a variety of cancers including colon and skin cancer.

Key Words: Rosmarinic acid, anticancer, skin cancer, colon cancer.

INTRODUCTION
Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid commonly found in plants belonging to the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae family. It is a naturally occurring phenolic compound. The compound has been reported to have a number of interesting biological activities, e.g. antiviral, antibacterial, anticancer, antiinflammatory and antioxidant activities. [¹] The compound is formally known as(R)-α-[[3-(3,4-dihydroxyphenyl)-1-oxo-2E-propenyl]oxy]-3,4-dihydroxy- enzenepropanoic acid and has a molecular formula of C₁₈H₁₈O₈. Rosmarinic acid was originally isolated in 1958 from the rosemary plant (Rosmarinus officinalis). Some of the plants from which this compound has been recently reported include Rosmarinus officinalis, [²] Thymus mastichina, [³] Forsythia koreana, [⁴] Ocimum sanctum, [⁵] Hyptis pectinata, [⁶] and plants belonging to the Agastache genus of the Lamiaceae family. [⁷] The structure of the compound is shown in...
Rosemary and its various extracts containing carnosol, carnosic acid, ursolic acid, and rosmarinic acid can suppress the development of tumors in several organs including the colon, breast, liver, stomach, as well as melanoma and leukemia cells. [8] This review shall focus on the reported anticancer activities of rosmarinic acid as collected from various database searches like PubMed and SCOPUS.

Reported Anticancer Activities Of Rosmarinic Acid

Colon/Colorectal Cancer

In colon cancer HT-29 cells, rosmarinic acid (RA) at doses of 5, 10 and 20 micromol/L reportedly reduced the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced (cyclooxygenase-2) COX-2 promoter activity and protein levels. RA also reduced TPA-induced transcription from a control activator protein-1 (AP-1) promoter-luciferase construct and repressed binding of the AP-1 factors, c-Jun and c-Fos to COX-2 promoter oligonucleotides harboring a cAMP-response element (CRE). RA also antagonized the activation of the extracellular signal-regulated protein kinase-1/2 (ERK1/2). Thus the anticancer effect of RA may be due to its ability to inhibit COX-2 activation by AP-1 inducing agents. [9] Notably, it has been reported that alterations in the COX-2 pathway and increased levels of its enzymatic product prostaglandin E2 (PGE2) plays a major role in the development and progression of colorectal cancer. COX-2 can be activated through activation of the ERK-signaling pathway; PGE2 can stimulate cell proliferation at least partly through stimulation of beta-catenin/TCF4 activity. [10] COX-2 is induced and expressed in neoplastic growths; anti-inflammatory agents like aspirin and NSAIDS (non-steroidal anti-inflammatory drugs) can reduce the risk of colon cancer and promote tumor regression in both human and
animal models of colorectal tumors. As such, rosmarinic acid can have an anti-colon cancer effect through its anti-inflammatory properties. Mechanisms of rosmarinic acid to prevent colon cancer following different routes are presented in Figure 2.

![Mechanism Of Rosmarinic Acid As Anticancer Agent Against Colon Cancer.](image)

**Figure 2. Mechanism Of Rosmarinic Acid As Anticancer Agent Against Colon Cancer.** [9-12]

**Inhibition Of ERK**

Phosphorylation and consequent activation by RA has also been seen in human colon carcinoma-derived cell line HCT15. In another human colon carcinoma-derived cell line, CO115, RA had no effect on PI3K/Akt signaling pathways. RA induced apoptosis in both cell lines and the apoptotic effect has been attributed to ERK signaling inhibition. Although the role of ERK in apoptosis is not very clear, it has been observed that ERK inhibitors can induce apoptosis and enhance the diallyl-disulfide-induced apoptotic effect in human CNE2 nasopharyngeal carcinoma cells. RA inhibited tumor metastasis in Ls174-T human colon carcinoma cells both *In vitro* and *in vivo*, and the effect has been shown through modulation of the ERK signaling pathway, although RA also demonstrated antioxidant effect and repressed the activity and expression of matrix metalloproteinase (MMP)-2,9 in the cells. The results are also interesting from the view point that phenolic antioxidants like RA, caffeic acid, rutin, and quercetin can inhibit tumor growth. A 50% ethanol extract of the plant, *Melissa officinalis* demonstrated decreased cell viability in human colon cancer cell line, HCT-116. Bioactive fractionation led to isolation of RA, which at a dose of 1,000 microg/ml was cytotoxic to the cancer cells even at 24h. The chemopreventive potential of RA has been assessed in 1,2-dimethylhydrazine (DMH) rat colon carcinogenesis model. DMH-treated rats showed large numbers of colonic tumors, decreased lipid peroxidation and antioxidant status, and elevated cytochrome P450 and p-nitrophenol hydroxylase activities.
RA significantly reversed the above parameters. In DMH-treated rats, RA at 10 mg/kg body weight, p.o. everyday reduced the formation of aberrant crypt foci and aberrant crypt foci multiplicity found in DMH-treated rats. RA supplementation also prevented alterations in circulatory antioxidant enzymes. Chemopreventive effect of RA has also been examined on the development of intestinal adenomas in the Apc(Min) mouse model of colorectal carcinogenesis. RA was found to inhibit the growth of APC10.1 cells derived from Apc(Min) mouse adenomas with an IC$_{50}$ value of 43 microM. The frequency of large adenomas was significantly decreased by RA following dietary administration for 8 weeks.

**Skin Cancer And Melanoma**

In a two stage carcinogenesis murine model where cancer was initiated by application of 7,12-dimethylbenz[a]anthracene (DMBA) and promoted by application of 12-tetradecanoylphorbol 13-acetate (TPA), the compounds induced marked neutrophil infiltration in skin, increased myeloperoxidase activity, increases in intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mRNA expressions, increases in synthesis of the chemokines KC and macrophage inflammatory protein-2, induction of COX-2 mRNA expression, increases in reactive oxygen radical production and increases in 8-hydroxy-2’-deoxyguanosine production. These effects were reversed by topical application of RA, which suggests that RA acted against skin tumors by exerting an anti-inflammatory as well as an antioxidant effect. The concentrations of circulating ICAM-1 and VCAM-1 has been observed to be markedly increased in nasopharyngeal carcinoma, so RA may also play a beneficial role in this type of cancer. Increased ICAM-1 expression correlates with poor prognosis in pancreatic cancer and has been reported to play a role in tumor growth and metastasis. As such, RA can also play a beneficial role in various types of cancer, including pancreatic cancer, where there is increased expression of ICAM-1. VCAM-1 reportedly provides survival advantage to breast cancer cells, so RA can also play a protective role in breast cancer through decreasing VCAM-1 expression. Mechanisms of rosmarinic acid to prevent skin cancer following different routes are presented in Figure 3.
Melanoma is considered the most dangerous form of skin cancer, which occurs due to unrepaired DNA damage to skin cells, and which damage can be from excessive exposure to UV rays from sunshine. Melanogenesis is the formation of melanin, which can play a crucial protective role against skin photocarcinogenesis. In B16 melanoma cells, RA caused increased tyrosinase expression and melanin content. The results have been attributed to activation of protein kinase A (PKA), which occurs upstream of cAMP and which results in activation of cAMP response element (CRE) and phosphorylation of CRE-binding protein CREB. Tyrosinase enzymes are the rate limiting step in the formation of melanin, and melanogenesis occurs in human epidermal melanocytes through the PKA/CREB/MITF (microphthalmia-associated transcription factor), which can lead to increased melanin content in cells, so it is probable that RA acts in melanomas by inhibiting this pathway, though it is yet to be studied. In another study with B16 melanoma cells, RA was found to increase tyrosinase activity and its expression levels along with demonstrating antioxidant activity, which may also play a role in RA-induced melanogenesis. In B16F10 melanoma cells, RA was found to be a radiosensitizer and increased cellular death by 42%. Thus RA has the dual capability, namely that of protecting normal cells against radiation but sensitizing melanomas to radiation. This is a feature of RA that needs to be studied more so that a therapeutic benefit of RA can be established against melanomas. The antitumor effect of orally administered RA was evaluated in 7,12-dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. 100% tumor formation was observed with topical application of DMBA alone for 15 weeks. RA administration reduced tumor formation, favorably modulated the antioxidant status, and normalized the DMBA-induced changes in the apoptotic markers (p53, Bcl-2, caspase-3 and caspase-9).
Lung Cancer
RA showed 50% inhibition of A549 lung carcinoma cells and inhibited COX-2 activity in these cells. The inhibitory effect on cell proliferation was attributed to COX-2 inhibition through hydrogen bonding to Arg120 and Ser353 in COX-2 active site residues. [29]

Oral Cancer
The inhibitory effect of RA was studied in 7,12-dimethylbenz(a)anthracene (DMBA)-induced oral tumors in golden Syrian hamsters. 100% tumor formation was noted in hamsters treated with DMBA in their buccal pouches, which was brought to zero percent with administration of RA at a dose of 100 mg/kg body weight. It was further observed that RA improved antioxidant status, and down-regulated the expressions of p53 and Bcl-2 during DMBA-induced oral carcinogenesis. [30]

Leukemia
In human leukemia U937 cells, RA reportedly sensitized TNF-alpha-induced apoptosis through the suppression of tumor necrosis factor-alpha (TNF-alpha)-induced nuclear transcription factor-kappaB (NF-kappaB) activation and reactive oxygen species (ROS) generation. Activation of caspases was also noted following RA treatment. RA suppressed NF-kappa B activation through inhibition of phosphorylation and degradation of IkappaBalpha, and nuclear translocation of p50 and p65. This was correlated with suppression of NF-kappaB-dependent anti-apoptotic proteins (IAP-1, IAP-2, and XIAP). [31]

Hepatoma
In human hepatoma HepG2 cells, RA increased the expression of apoptosis-related genes and induced apoptosis, thus showing to be a promising candidate against hepatocellular carcinoma. [32]

Breast Cancer
In human breast cancer MCF7 cell line, RA reportedly inhibited DNA methyltransferase activity. [33] In cancer, DNA methylation pattern undergoes aberrant changes causing a wide variety of tumor suppressor genes to undergo promoter hypermethylation and become transcriptionally silent, leading to tumor formation. As such, inhibition of DNA methyltransferase can reverse this effect and can be of potential therapeutic value against cancer. It has been reported that besides RA, other plant-derived compounds like curcumin and resveratrol can also inhibit DNA methyltransferase activity. [34] RA also has been found
to dose-dependently inhibit the migration of MDA-MB-231BO human bone-homing breast cancer cells, which effect can prevent skeletal disorders found in breast cancer metastasis. From the obtained results, it has been suggested that RA may inhibit bone metastasis from breast carcinoma via the pathway of the receptor activator of NF kappaB ligand (RANKL)/RANK/osteoprotegerin (OPG) and by simultaneously suppressing the expression of interleukin-8 (IL-8). [35] Osteoprotegerin is a pro-angiogenic factor, and inhibition of OPG can be of benefit in inhibiting metastasis of cancer cells. [36] Elevated levels of IL-8 expression by breast cancer cells has been implicated in the osteolysis associated with metastatic breast cancer. [37] RA also showed toxicity against two human breast cancer cell lines, namely Adriamycin-resistant MCF-7/Adr and wild-type MCF-7/wt. [38] Mechanisms of rosmarinic acid to prevent breast cancer following different routes are presented in Figure 4.

![Figure 4. Mechanism Of Rosmarinic Acid As Anticancer Agent Against Breast Cancer.](image)

**Ovarian Cancer**

Human ovarian cancer cells demonstrated sensitivity against RA, which was enhanced with a combination of RA and cisplatin. Various phases of the cell cycle were blocked leading to inhibition of cell proliferation. Additionally there was induction of apoptosis. [39]

**Anticancer Activities Of Rosmarinic Acid Derivatives**

In a number of different plant cell cultures (like *Eritrichium sericeum* [40]), RA is one of the first secondary metabolites that can be found in yield as high as 19% of cell dry weight. More complex derivatives of RA like rabdosin and lithospermic acid B are obtained at later periods of cell culture. RA derivatives, like RA have promising applications in cognitive performance improvement, prevention of development of Alzheimer’s disease, cardioprotective effects, reducing the severity of kidney diseases, antioxidant, antiallergic,
and cancer chemoprevention. [41,42] Lithospermic acid B has been reported to inhibit the proliferation of vascular smooth muscle cells. [43] Rosmanol (Figure 5), with a structure quite similar to RA, has been shown to induce apoptosis in human colon adenocarcinoma COLO 205 cells through both the mitochondrial apoptotic pathway and the death receptor path. [44]

![Figure 5. Structure Of Rosmanol](image)

**Medicinal Preparations Containing Rosmarinic Acid**

Since a number of plants contain RA as one of their phytochemical constituents, many traditional medicinal preparations containing extracts or decoctions of these plants will contain RA. The list of medicinal plants containing RA is indeed huge, particularly for plants belonging to the Boraginaceae and the Lamiaceae/Labiatae families. In fact, analysis of 29 species of Labiatae family plants including *Salvia officinalis*, *Salvia limbata*, *Salvia virgata*, *Salvia hypoleuca*, *Salvia macrosiphon*, *Salvia chloroleuca*, *Melissa officinalis*, *Origanum vulgare*, *Lavandula angustifolia*, *Rosmarinus officinalis*, *Thymus daenensis*, *Thymus citriodorous*, *Thymus pubescens*, *Thymus vulgaris*, *Zataria multiflora*, *Mentha piperita*, *Mentha pulegium*, *Mentha longifolia*, *Mentha spicata*, *Mentha aquatica*, *Mentha crispa*, *Perovskia artemisoides*, *Zhumeria majdae*, *Satureja hortensis*, *Satureja khuzistanica*, *Satureja bachtirica*, *Satureja atropatana*, *Satureja mutica* and *Satureja macrantha* found RA in most of these species with the highest amount of RA in plants belonging to the *Mentha* genus, particularly *Mentha spicata*. [45] Thus any medicinal preparations (containing plants having RA as one of its phytoconstituents) will have RA, although the medicinal preparation may not be knowingly used as an anticancer agent but may be primarily used for treatment of other diseases. A case in point is the traditional Uighur herbal medicinal preparation, Abnormal Savda Munziq (ASMq), which is used for the treatment and prevention of diabetes, cardiovascular diseases, chronic asthma and cancer. The preparation has been found
to demonstrate antiproliferative activity against HL-60 cells and contain RA as one of its constituents. [46] Eight commercial herbal tinctures (names not mentioned) obtained from fresh and dried lemon balm (*Melissa officinalis*) used by Western herbal practitioners for treatment of Herpes simplex infection as well as for carminative, sedative and diaphoretic actions have been found to contain RA, although these tinctures were not used for cancer treatment. [47] *Ocimum sanctum* is sold as an Ayurvedic drug, namely ‘Tulsi’ and is recommended for common cough, cold, viral hepatitis, asthma, nausea, and stress-related diseases, but not for cancer. However, the plant reportedly contains RA. [5] Such examples can be found in numerous herbal preparations throughout the world, and it may be productive for anticancer therapy to analyze the anticancer effect of these preparations.

**CONCLUSION**

As discussed earlier, RA has been found to be effective against a number of cancer cell lines and so is a potential therapeutic agent against these cancer types. The mechanisms of anticancer activity of RA needs further studies; initial studies indicate that RA may act through various mechanisms including anti-inflammatory and antioxidant actions as well as inhibiting cell proliferation, migration, and inducing selectively cancer cell apoptosis. Moreover, the compound has been shown to have antiangiogenic activity as demonstrated by inhibition of proliferation, migration, adhesion, and tube formation of human umbilical vein endothelial cells, [48] which can be beneficial in preventing tumor growth and metastasis. RA has also been found to reverse multi-drug resistance in SGC7901/Adr cells and increase the intracellular accumulation of adriamycin and rhodamine 123, and decrease the transcription of MDR1 gene and the expression of P-gp in SGC7901/Adr cells. [49] Taken together, RA can be an excellent therapeutic agent against treatment of various cancers, particularly colon and skin cancer.

**REFERENCES**


47. Sanchez-Medina A, Etheridge CJ, Hawkes GE, Hylands PJ, Pendry BA, Hughes MJ, Corcoran O: Comparison of rosmarinic acid content in commercial tinctures produced
