Molecular mechanisms of eugenol as an anti-tumour bioactive compound: A comprehensive review

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Abstract. The eugenol, a biologically active compound found in various plant species, has gained considerable attention in recent years for its anticancer and other medicinal properties. This review aimed at elucidating the current knowledge and the molecular mechanisms underlying eugenol's antitumour effects. Eugenol via two pathways; intrinsic and extrinsic can induce apoptosis, cause cell cycle arrest together with its antioxidant/anti-inflammatory effects against angiogenesis and metastasis. It can modulate various cellular signalling pathways as well. The most commonly reported three are: MAPK/ERK, PI3K/Akt/mTOR and JAK/STAT. These pathways, and others as well, are critical in cellular events associated with oncogenesis. Moreover, it exhibits additive effects in combination with chemotherapy agents, natural compounds and radiotherapy this increasing its therapeutic possibility. Eugenol hits a wide range of molecular targets, with involvement of various proteins (including transcription factors), genes and epigenetic modifications as well as alterations in microRNA levels, implying complex anticancer mechanisms. It also shows markedly improved therapeutic benefits with chemo-drugs, phytochemicals and radiotherapy. The complex anticancer mechanisms of which include interactions with specific proteins, genes and epigenetic modifications as well has been shown to affect microRNA regulation. Yet, and although its actions are suggested, additional investigation is required to clarify the molecular mechanisms of eugenol entirely with potential clinical applications.

1 Introduction

Cancer is still one of the most common causes for mortality all over the world, despite advancements in prevention and treatment strategies developed [1]. The exploration of naturally-derived compounds with anticancer potential has gained substantial attention in recent years, as these compounds often exhibit potent antitumour activities with fewer side

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effects compared to conventional chemotherapeutic drugs [2]. Among the various natural compounds studied for their anticancer properties, eugenol, a phenylpropanoid found in several plant species, particularly in clove oil, has emerged as a promising candidate [3].

Eugenol (4-allyl-2-methoxyphenol, Figure 1) has been largely studied due to its broad pharmacological activities such as anti-inflammatory, antioxidant, antibacterial activities and antinociceptive function in pain management [4, 5]. In addition, accumulating scientific evidence suggest that eugenol exerts prominent anti-cancer potential against various types of cancer; especially breast, lung and colon carcinomas as well as prostate cancer [3, 6–9]. In cancer cells, these pharmacologic effects of eugenol were credited to involve various cellular pathways associated with carcinogenesis such as the modulation of cell cycle and the induction of programmed cell death as well as anti-angiogenic and anti-metastatic effects [7, 10–13].

Considering the rising importance towards eugenol as a promising anticancer candidate, it is imperative to have an in-depth knowledge regarding how exactly eugenol works at molecular level for proper development followed up with clinical translation. Therefore, this literature review describes the present studies on antitumour effects of eugenol and the proposed molecular mechanisms.

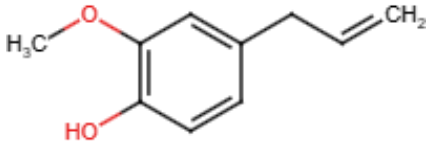

Fig. 1. Molecular structure of eugenol ($C_{10}H_{12}O_2$, 164.20 g/mol). Obtained from Reaxys (ID: 3208596).

2 Apoptosis induction

Apoptosis induction with eugenol has been demonstrated in many cell lines, by both intrinsic mitochondrial and extrinsic death receptor pathways. Caspases, a group of cysteine-dependent aspartate-directed proteases, takes on particular importance during the execution phase of apoptosis [14].

Intrinsic apoptotic cascade is triggered by intracellular stressors such as DNA damage, oxidative stress and endoplasmic reticulum stress [15]. Accumulated evidence has shown that eugenol contributes in the activation of this pathway in multiple cancer cell types. In human melanoma cells, the eugenol reduced cell viability in a dose-dependent manner and also caused alterations to nuclear morphology, enhancement of caspase-3/6 and cleavage of caspase substrates including DFF45, PARP, and lamin A [8, 16]. In another study, apoptosis initiated by eugenol in human promyelocytic leukaemia cells was evidenced to be mediated via induction of reactive oxygen species (ROS) generation and the release of cytochrome c through mitochondrial permeability transition [17,18]. In human osteosarcoma cells, eugenol induced caspase-dependent apoptosis by cleaving PARP, Lamin A and DFF-45 in addition to caspase-3 [19]. The role of the mitochondrial apoptotic pathway was also established in human glioblastoma cells: eugenol induced ROS formation, decreased the potential of mitochondrial membrane, and caused cytochrome c release with activation of caspase-9 and -3 [20].

In addition to intrinsic apoptotic pathway, the extrinsic pathway is triggered by the binding of death ligands to their corresponding cell surface receptors. Although not as well characterised as the intrinsic pathway, there is some evidence that eugenol can activate the extrinsic apoptotic pathway in specific types of cancer cells. In human colon cancer cells, eugenol induced apoptosis through various mechanisms, including activation of caspase-3

and p53 [8, 21]. Since p53 is involved in regulating the expression of death receptors [22], this suggests that eugenol could promote an extrinsic cell-death-induced pathway indirectly in these cells. Nevertheless, further studies are necessary to molecularly elucidate the involvement of the extrinsic pathway in eugenol-induced apoptosis.

Caspases, the principal executioners of apoptosis, are central in both intrinsic and extrinsic apoptotic pathways. Eugenol was reported to activate caspase in many cancer cell death signalling pathways including apoptosis. And, it effectively activates caspase-3 in a time-dependent manner in human cervical carcinoma cells [23]. It also stimulated the activation and cleavage of caspase-3 in human osteosarcomacells [19] and oral squamous cell carcinoma cells [24]. In addition to caspase-3, eugenol has been shown to stimulate a number of other caspases such as caspase-6 [8], and caspase-9 [10, 18, 20] thus providing further evidence that Eugenol induces apoptosis by multiple pathways.

3 Cell cycle arrest

Eugenol can also arrest the cell cycle on various cancer cell lines has been proven by many research, which is important for its anti-tumour activity. This arrest takes place at different phases, G1/S or G2/M and is controlled by a complex network of cell cycle proteins like cyclins as well as cyclin-dependent kinases (CDKs) [25–28].

Several reports have suggested that eugenol can arrest cancer cells in the G1/S phase. For instance, Yan *et al*. [29] reported that eugenol arrested G1/S phase by blocking fatty acid oxidation and oxidative phosphorylation though c-Myc/PGC-1β/ERRα signalling pathway in MCF10A-ras breast cancer cells. Ghosh *et al*. [30], in a separate study, showed that eugenol induced G1/S phase arrest of melanoma based on the inhibition of E2F1 transcriptional activity thereby suppressed growth in melanomas. Furthermore, research also showed that eugenol has a protective effect on breast precancerous lesion through inducing cellular apoptosis and S-phase arrest via HER2/PI3K-AKT pathway [31].

Besides G1/S phase block, eugenol is able to induce G2/M arrest in different cancer cell lines. For instance, methyl eugenol (a derivative of eugenol) affects RB355 human retinoblastoma cells leading to autophagic cell death and G2/M arrest with inhibition the PI3K/mTOR/Akt signalling [32]. Furthermore, Choi *et al*. [28] reported that eugenol led to cell cycle arrest at the S phase in G361 human melanoma cells through altering expression of various key molecules regulating cell cycle.

The induction of cell cycle arrest by eugenol is mediated through the regulation of various cell cycle proteins, such as cyclins and CDKs. Owa *et al*. [33] highlighted the significance of genes in G1 regulation and emphasised the ongoing efforts in developing new chemotherapeutic anticancer agents targeting the G1 phase, including flavopiridol, an effective inhibitor of cyclin-dependent kinases. Research by Manikandan and colleagues [34] showed eugenol's efficacy in mitigating the occurrence of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric tumours in murine model. In this context, the effect was proposed to be related with eugenol's ability of NF-κB inhibition and, consequently its capability to regulate the expression of downstream target genes which are involved in proliferation and survival.

4 Antioxidant and anti-inflammatory activities

The eugenol also has important anti-oxidative and anti-inflammatory activities which contribute to its antitumour activity. These effects were proposed to be achieved by interfering with regulation of the reactive oxygen species (ROS), inhibiting NF-κB signal pathway and downregulation cyclooxygenase-2 (COX-2) [5, 35].

Eugenol also shows high antioxidant activity involved in scavenging free radicals to decrease oxidative stress, a crucial factor in carcinogenesis and tumour promotion [36, 37]. Bezerra *et al*. [38], reported that eugenol has a dual effect on oxidative stress. It acts as an antioxidant and pro-oxidant, depending on the conditions in a cell. And, while eugenol's ability to inhibit oxidative stress may protect against cancer formation, its antioxidant effect could potentially contribute to cancer development once formed. There is a pro-oxidative effect of eugenol, which induces the death of cancer cells [38]. Besides, studies have shown that eugenol can protect against chemically induced skin cancer by preventing oxidative stress and inflammation [39]. Nagababu *et al*. [40] state that eugenol protects against iron-mediated lipid peroxidation better than allopurinol and can be a powerful antioxidant. The antioxidant properties of eugenol have also been ascribed to its ability to form complexes with reduced metals which suppress the chain reaction of a free radical [41].

The NF-κB signalling pathway is important for the control of inflammation and cell survival; its deregulation in cancer development has been well documented [42]. Eugenol has been demonstrated to have anti-inflammatory activities by downregulating NF-κB signalling pathway [43]. For example, Yeh *et al*. [44] reported that eugenol and its derivatives possess an anti-inflammatory potential by suppressing lipopolysaccharide (LPS)-induced nitric oxide (NO) production via the inhibition of inducible nitric oxide synthase in macrophages. This was mediated by the suppression of MAPKs and Akt/IκBα signalling cascades, inhibiting NF-κB and AP-1 genes. Furthermore, a report by Manikandan *et al*. [34] suggested that eugenol treatment significantly lowered the occurrence of MNNG-triggered gastric neoplasms in an experimental model. This effect was attributed to eugenol's capacity to suppress NF-κB activation and modulate the expression of its downstream target genes, which are essential to cellular proliferation and survival pathways.

COX-2 is an enzyme that involved in the inflammation and has been implicated in the development and progression of various cancers [45]. Eugenol has been shown to inhibit COX-2 expression and activity, thereby exerting its anti-inflammatory and anti-tumour effects [46]. Kim *et al*. [47] demonstrated that eugenol suppresses COX-2 expression in lipopolysaccharide-stimulated mouse macrophages, suggesting its potential as an anti-inflammatory and cancer chemopreventive agent. Andrade *et al*. [48] further reported that eugenol has the potential to bind to both COX-2 and 5-lipoxygenase (5-LOX) enzymes, indicating its role as an anti-inflammatory compound. The ability of eugenol to target both COX-2 and 5-LOX pathways suggests its potential as a possible replacement for non-steroidal anti-inflammatory drugs (NSAIDs) in many diseases [48].

5 Angiogenesis and metastasis inhibition

Associations between eugenol and several cancer types have been described in the open literature indicating potent anti-angiogenic/anti-metastatic activities within different experimental models. Angiogenesis, the process of forming new blood vessels from pre-existing ones, is essential to tumour growth and metastasis [49]. Metastasis, the invasion of cancer cells to other organs from a primary tumour site, constitutes one of fundamental cause in most cancers associated mortality [50]. Eugenol may, therefore, be an appealing candidate for cancer therapy based on its multitargeted activities in different pathways involved in these processes.

Vascular endthelial growth factor (VEGF) plays a crucial role in angiogenesis and promotes proliferation, migration and survival of endothelial cells [49]. In various cancer models, eugenol was found to be potentially inhibiting VEGF signalling. In one example, eugenol inhibited the VEGF and VEGFR2 expression in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced malignant gastric carcinoma in rats. This decrease was accompanied by reduced microvessel density and suppressed tumour growth [51]. Also,

when used on human cervical cancer cells with eugenol-encapsulated chitosan nanoparticles, VEGF expression decrease and sequential angiogenesis attenuation achieved [52].

Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, are essential in degrading the extracellular matrix (ECM) during tumour invasion and metastasis [50]. Eugenol has been shown to reduce the expression as well as activity of MMPs in some cancer models. Treatment with eugenol was shown to inhibit MMP-9 activity and expression, an important enzyme in ECM degradation, involved in migrating by human fibrosarcoma cells [53].

EMT (epithelial-mesenchymal transition) is the process by which epithelium cells lose their cell-cell adhesion and apical-basolateral polarity, gain migratory capacities to transform into mesenchymal-like properties [50]. This process is critical in tumour metastasis and is characterised by the reduction of epithelial markers (such as E-cadherin) expression as well as the upregulation of mesenchymal markers (such as vimentin, N-cadherin). Studies have shown that eugenol can suppress EMT in various cancer models. In human cervical carcinoma cells, eugenol caused the inhibition of EMT by downregulating the expression of Snail, a key transcription factor that represses E-cadherin expression, while simultaneously increasing E-cadherin expression [11]. In breast cancer cells, eugenol was found to reverse EMT by enhancing E-cadherin expression and reducing vimentin expression [10]. The ability of eugenol to suppress EMT may contribute significantly to its anti-metastatic effects in cancer.

6 Modulation of signalling pathways

Eugenol has also showed its cancer and tumour preventive action through the alteration in diverse signal transduction pathways as well. MAPK/ERK, PI3K/Akt/mTOR and JAK/STAT are important pathways in cellular proliferation, survival and metastasis which have made them suitable for cancer therapy [54].

One of the most frequently dysregulated pathways in cancer is PI3K/Akt/mTOR pathway. As mentioned before, disturbing this pathway might promote cell growth and prevent apoptosis. Different studies have indicated that eugenol might inhibit the PI3K/ Akt/ mTOR pathway in different cancer cell lines. For example, eugenol induced autophagy and apoptosis in breast cells by inhibiting the PI3K/AKT/FOXO3a pathway [10]. A study by Abdullah *et al*. [10] showed that eugenol-treatment enhanced the levels of apoptosis-associated proteins and autophagy, as determined by up-regulation of LC3 or downregulation in p62 in a concentration-dependent manner. In addition, another research revealed that eugenol could relieve breast precancerous lesions by inducing cell apoptosis and S-phase arrest via HER2/PI3K-AKT pathway [31]. To conclude, the PI3K/Akt/mTOR signalling axis is one of major targets responsible for the anti-cancer effects of eugenol, and particularly in breast cancer.

Another important signalling cascade that controls cell proliferation, differentiation, and survival is the MAPK/ERK pathway. Several studies in different cancer models have been illustrated eugenol-mediate modulation of this pathway. The effects of eugenol and its derivatives (eugenolol & glyceryl-isoeugeno) in macrophages have been demonstrated to inhibit LPS-triggered iNOS expression by decreasing both NF-κB and AP-1 via the suppression of MAPKs signalling pathways as well as AKT/IκBα degradation [44]. The compound eugenol and its derivatives may also mediate anticancer activities through their anti-inflammatory properties described in the same study. In another study, eugenol inhibits triple-negative breast cancer (TNBC) cell proliferation and metastasis by regulating NOD1-NF-κB signalling pathway [55]. Among the candidate target proteins of eugenol for treating TNBC, NF-κB was identified in proteomic analyses.

The JAK/STAT pathway regulates cytokine signalling, and is also a fundamental player in the survival, proliferation, and anti-apoptotic activities of cancer cells. Although the direct evidence on the effect of eugenol in cancer via JAK/STAT pathway is still poor, it has been proposed by some researches that this molecule could indirectly modulate it because of its anti inflammatory and immunomodulatory properties. Eugenol for example has been found to modulate the synthesis of inflammatory cytokines by peripheral blood mononuclear cells (PBMC) in response to stimulation with colon carcinoma cells [56]. Furthermore, since cytokines may activate the JAK/STAT pathway and given that eugenol modulates production of these molecules it may be indirectly impacting this signalling cascade. However, further research is required to determine the specific effects of eugenol on cancer and JAK/STAT signalling.

7 Synergistic effects

Eugenol has shown promising adjuvant efficacy in cancer therapies, with evidence of synergistic effects with conventional chemotherapeutics, natural products and radiation therapy [3, 57].

Combined with gemcitabine, eugenol increased anticancer and anti-inflammatory effects in cervical cancer cells [58], whereas; its synergy effect was seen on HeLa cells when working together with cisplatin and X-ray treatment [23]. Similarly, in TNBC cells, eugenol enhanced the anticancer properties of cisplatin through its inhibitory effects on cancer stem cells via targeting aldehyde dehydrogenase activity and NF-κB signalling pathway [6]. Eugenol intensified the blocking of NF-κB signalling pathway, and subsequently decreased IL6 as well as interleukin 8 by inhibiting the p65 binding to its target sites. Moreover, eugenol exerted the synergy in inhibiting the NF-κB activation with impaired binding to its target sites and suppressing proinflammatory, inflammation-promoting, cytokines IL-6 and IL-8.

Synergistic effects have also been observed when eugenol is combined with other natural compounds. Abdullah *et al*. [59] studied the anti-proliferative and anti-metastatic effect of eugenol on triple negative as well as HER2+ breast cancer cells. They suggested that eugenol could potentially induces late apoptosis in both triple negative (MDA-MB-231) and HER2+ (SK-BR-3) cell lines; thus, exhibiting an anti-cancer effect on breast cancer. Moreover, Sarkar *et al*. [60] demonstrated that eugenol and capsaicin exert anti-metastatic potential due to the regulation of TGF-β signalling pathway in gastric carcinoma, with a higher potency of capsaicin and SMAD4-dependence, compared to eugenol.

8 Molecular targets and mechanisms

Eugenol showed anti-cancer properties at different molecular level. It uses a variety of mechanisms to achieve its effects: acting directly on specific proteins and genes, regulating epigenetic modifications including methylation status and controlling the expression of microRNAs [3].

Eugenol targets a wide range of proteins and genes which contribute to cancer progression. It suppresses the expression of transcription factors c-Myc, E2F1 and H-ras that are involved in cell proliferation and survival [61, 62]. Eugenol also upregulates genes associated with the programmed cell death such as Bcl-2, Bax and survivin resulting to the eventual program cell death of cancer cells [21]. For instance, eugenol has been reported to inhibit the HER2/PI3K-AKT pathway causing cell apoptosis and S-phase arrest in breast cancer [31]. It also blocks the activation of MMPs including MMP-2 and 9, which are important for cancer cell migration as well as that of adhesion protein Paxilin [54, 63]. Eugenol is able to inhibit the NF-κB-regulated TRIM59 pathway in non-small cell lung carcinoma cells, thus resulting its

anti-tumour effect [64]. Additionally, it also induces suppression of oral squamous cell carcinoma via modulation of macrophage migration inhibitory factor (MIF) alters, resulting in increased apoptosis [65].

Post-transcriptional modifications including DNA methylation and histone acetylation are key regulators in the gene expression process, which has been hypothesised to be involved in cancer development [66]. Inhibition of these epigenetic mechanisms have been associated with the anti-cancer effects of eugenol. It downregulates the expression of DNA methyltransferases DNMT1 and DNMT3A in the breast cancer-associated fibroblasts (CAFs) by E2F1 dependent mechanism thus contributes to active breast CAF cell normalization [12]. Therefore, eugenol-based targeting of DNMT1/DNMT3A may also represent a promising approach to normalising the tumour microenvironment. Histone methylation is a essential epigenetic process that regulates chromatin-based processes and, as such, is essentially involved in breast cancer development [67]. Inhibitors of histone methyltransferases, including EZH2 and DOTL, have shown great promise in preclinical oncology studies [68]. The direct effect of eugenol on histone methylation remains no fully clear, however, its ability to modulate such epigenetic mechanism supports the necessity for further investigation.

miRNAs are short noncoding RNAs implicated in post-transcriptional gene regulation and play important roles in cancer biology [69, 70]. Eugenol has been shown to modulate the expression of several miRNAs, contributing to its anti-tumour effects. In colorectal cancer cells, eugenol alters the expression of miRNAs involved in the regulation of APC, p53, and KRAS genes, thereby exhibiting its antitumour activities by inducing specific metabolic pathways in the cell's metabolome profile [71]. However, the specific miRNAs targeted by eugenol in this context have not been identified, and further research is needed to elucidate the precise mechanisms.

9 Conclusions

To sum up, eugenol has proven to be an antitumour compound having various molecular mechanisms such as cell cycle arrest, apoptosis, antioxidant and anti-inflammation effects, inhibition of angiogenesis or metastasis pathway along with modulation of many signalling pathways. It also synergizes with chemotherapies, natural compounds and radiation therapy as well. Its molecular targets include: proteins, genes and gene products, epigenetic alterations and microRNA regulation Further investigations are required to elucidate the exact mechanism that makes eugenol a therapeutic candidate and anti-cancer target at molecular level and uses it for clinical management.

References

1. The global challenge of cancer, Nat. Cancer **1**, 1–2 (2020).
2. A. Naeem, P. Hu, M. Yang, J. Zhang, Y. Liu, W. Zhu, Q. Zheng, Natural products as anticancer agents: Current status and future perspectives, Molecules **27**, 8367 (2022).
3. I. Padhy, P. Paul, T. Sharma, S. Banerjee, A. Mondal, Molecular mechanisms of action of eugenol in cancer: Recent trends and advancement, Life **12**, 1795 (2022).
4. A. Abdou, A. Elmakssoudi, A. El Amrani, J. JamalEddine, M. Dakir, Recent advances in chemical reactivity and biological activities of eugenol derivatives, Med. Chem. Res. **30**, 1011–1030 (2021).
5. J.N. Barboza, C. da Silva Maia Bezerra Filho, R.O. Silva, J.V.R. Medeiros, D.P. de Sousa, An overview on the anti-inflammatory potential and antioxidant profile of eugenol, Oxid. Med. Cell. Longev. 1–9 (2018).

6. S.S. Islam, I. Al-Sharif, A. Sultan, A. Al-Mazrou, A. Remmal, A. Aboussekhra, Eugenol potentiates cisplatin anti-cancer activity through inhibition of ALDH-positive breast cancer stem cells and the NF-κB signaling pathway, Mol. Carcinog. **57**, 333–346 (2018).
7. P. Choudhury, A. Barua, A. Roy, R. Pattanayak, M. Bhattacharyya, P. Saha, Eugenol emerges as an elixir by targeting β-catenin, the central cancer stem cell regulator in lung carcinogenesis: an in vivo and in vitro rationale, Food Funct. **12**, 1063-1078 (2021).
8. S.K. Jaganathan, A. Mazumdar, D. Mondhe, M. Mandal, Apoptotic effect of eugenol in human colon cancer cell lines, Cell Biol. Int. **35**, 607–615 (2011).
9. E. Dervis, A. Yurt Kilcar, E.I. Medine, V. Tekin, B. Cetkin, E. Uygur, F.Z.B. Muftuler, In vitro incorporation of radioiodinated eugenol on adenocarcinoma cell lines (Caco2, MCF7, and PC3)., Cancer Biother. Radiopharm. **32**, 75–81 (2017).
10. M.L. Abdullah, O. Al-Shabanah, Z.K. Hassan, M.M. Hafez, Eugenol-induced autophagy and apoptosis in breast cancer cells via PI3K/AKT/FOXO3A pathway inhibition, Int. J. Mol. Sci. **22**, 9243 (2021).
11. P. Happy Kurnia, E. Andika Bachtiar, Q. Faqrizal Ria, F. Fadhil, D. Anita, Eugenol isolated from Syzygium aromaticum inhibits HeLa cancer cell migration by altering epithelial-mesenchymal transition protein regulators, J. Appl. Pharm. Sci. **11**, 49–53 (2021).
12. L.A. Al-Kharashi, T. Bakheet, W.A. AlHarbi, N. Al-Moghrabi, A. Aboussekhra, Eugenol modulates genomic methylation and inactivates breast cancer-associated fibroblasts through E2F1-dependent downregulation of DNMT1/DNMT3A, Mol. Carcinog. **60**, 784–795 (2021).
13. A.T. Zari, T.A. Zari, K.R. Hakeem, Anticancer properties of eugenol: A review, Molecules **26**, 7407 (2021).
14. S. Elmore, Apoptosis: A review of programmed cell death, Toxicol. Pathol. **35** 495–516 (2007).
15. D. Westaby, J.M. Jimenez-Vacas, A. Padilha, A. Varkaris, S.P. Balk, J.S. de Bono, A. Sharp, Targeting the intrinsic apoptosis pathway: A window of opportunity for prostate cancer, Cancers (Basel). **14** 51 (2021).
16. G.-C. Kim, D.-S. Choi, J.-S. Lim, H.-C. Jeong, I.-R. Kim, M.-H. Lee, B.-S. Park, Caspases-dependent apoptosis in human melanoma cell by eugenol, Korean J. Anat. 245–253 (2006).
17. C.-B. Yoo, K.-T. Han, K.-S. Cho, J. Ha, H.-J. Park, J.-H. Nam, U.-H. Kil, K.-T. Lee, Eugenol isolated from the essential oil of Eugenia caryophyllata induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells, Cancer Lett. **225**, 41–52 (2005).
18. U.-H. Kil, K.H. Lee, K.-T. Lee, J.-Y. Jin, Eugenol induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cell, Korean J. Hematol. **40**, 65 (2005).
19. S.-H. Shin, J.-H. Park, G.-C. Kim, B.-S. Park, Y.-G. Gil, C.-H. Kim, The mechanism of apoptosis induced by eugenol in human osteosarcoma cells, J. Korean Assoc. Oral Maxillofac. Surg. **33**, 20-27 (2007).
20. W.-Z. Liang, C.-T. Chou, S.-S. Hsu, W.-C. Liao, P. Shieh, D.-H. Kuo, H.-W. Tseng, C.-C. Kuo, C.-R. Jan, The involvement of mitochondrial apoptotic pathway in eugenol-induced cell death in human glioblastoma cells, Toxicol. Lett. **232**, 122–132 (2015).

21. S.K. Jaganathan, E. Supriyanto, Antiproliferative and molecular mechanism of eugenol-induced apoptosis in cancer cells, Molecules **17**, 6290-6304 (2012).
22. J.S. Fridman, S.W. Lowe, Control of apoptosis by p53, Oncogene **22**, 9030–9040 (2003).
23. M. Fathy, M.A. Fawzy, H. Hintzsche, T. Nikaido, T. Dandekar, E.M. Othman, Eugenol exerts apoptotic effect and modulates the sensitivity of HeLa cells to cisplatin and radiation, Molecules **24**, 3979 (2019).
24. Y.-H. Kim, B.-S. Park, The effect of eugenol on the induction of apoptosis in HSC-2 human oral squamous cell carcinoma, J. Korean Soc. Dent. Hyg. **15**, 523–529 (2015).
25. K. Thu, I. Soria-Bretones, T. Mak, D. Cescon, Targeting the cell cycle in breast cancer: towards the next phase, Cell Cycle **17**, 1871-1885 (2018).
26. Y. Niu, J. Xu, T. Sun, Cyclin-dependent kinases 4/6 inhibitors in breast cancer: Current status, resistance, and combination strategies, J. Cancer, **10**, 5504–5517 (2019).
27. L. Ding, J. Cao, W. Lin, H. Chen, X. Xiong, H. Ao, M. Yu, J. Lin, Q. Cui, The roles of cyclin-dependent kinases in cell-cycle progression and therapeutic strategies in human breast cancer, Int. J. Mol. Sci. **21**, 1960 (2020).
28. B.-B.R. Choi, S.-H. Shin, U.-K. Kim, J.-W. Hong, G.-C. Kim, S phase cell cycle arrest and apoptosis is induced by eugenol in G361 human melanoma cells, Int. J. Oral Biol. **36**, 129-134 (2011).
29. X. Yan, G. Zhang, F. Bie, Y. Lv, Y. Ma, M. Ma, Y. Wang, X. Hao, N. Yuan, X. Jiang, Eugenol inhibits oxidative phosphorylation and fatty acid oxidation via downregulation of c-Myc/PGC-1β/ERRα signaling pathway in MCF10A-ras cells, Sci. Rep. **7**, 12920 (2017).
30. R. Ghosh, N. Nadiminty, J.E. Fitzpatrick, W.L. Alworth, T.J. Slaga, A.P. Kumar, Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity, J. Biol. Chem. **280**, 5812–5819 (2005).
31. M. Ma, Y. Ma, G.-J. Zhang, R. Liao, X.-F. Jiang, X.-X. Yan, F.-J. Bie, X.-B. Li, Y.-H. Lv, Eugenol alleviated breast precancerous lesions through HER2/PI3K-AKT pathway-induced cell apoptosis and S-phase arrest, Oncotarget **8**, 56296–56310 (2017).
32. L. Yin, Z. Sun, Q. Ren, X. Su, D. Zhang, Methyl eugenol induces potent anticancer effects in RB355 human retinoblastoma cells by inducing autophagy, cell cycle arrest and inhibition of PI3K/mTOR/Akt signalling pathway., J. Buon. **23**, 1174–1178 (2018).
33. T. Owa, H. Yoshino, K. Yoshimatsu, T. Nagasu, Cell cycle regulation in the G1 phase: a promising target for the development of new chemotherapeutic anticancer agents, Curr. Med. Chem. **8**, 1487-1503 (2012).
34. P. Manikandan, G. Vinothini, R. Vidya Priyadarsini, D. Prathiba, S. Nagini, Eugenol inhibits cell proliferation via NF-κB suppression in a rat model of gastric carcinogenesis induced by MNNG, Invest. New Drugs **29**, 110–117 (2011).
35. S. Mohammadi Nejad, H. Özgüneş, N. Başaran, Pharmacological and toxicological properties of eugenol, Turkish J. Pharm. Sci. **14**, 201–206 (2017).
36. İ. Gülçin, Antioxidant activity of eugenol: A structure-activity relationship study, J. Med. Food **14**, 975–985 (2011).
37. S.M. Yaseen, H.A. Abid, M.A. Al-Obaidi, Disturbed levels of non-enzymatic antioxidants and malondialdehyde among makeup users, J. Tech. **2**, 42–48 (2020).

38. D. Bezerra, G. Militão, M. de Morais, D. de Sousa, The dual antioxidant/prooxidant effect of eugenol and its action in cancer development and treatment, Nutrients **9**, 1367 (2017).
39. G. Kaur, M. Athar, M.S. Alam, Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis, Mol. Carcinog. **49**, 290–301 (2010).
40. E. Nagababu, J.M. Rifkind, S. Boindala, L. Nakka, Assessment of antioxidant activity of eugenol in vitro and in vivo, in: Methods Mol. Biol., pp. 165–180, (Humana Press, 2010).
41. M. Ito, K. Murakami, M. Yoshino, Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation, Food Chem. Toxicol. **43**, 461–466 (2005).
42. B. Hoesel, J.A. Schmid, The complexity of NF-κB signaling in inflammation and cancer, Mol. Cancer **12**, 86 (2013).
43. Y. Murakami, A. Kawata, S. Fujisawa, Expression of cyclooxygenase-2, nitric oxide synthase 2 and heme oxygenase-1 mRNA induced by bis-eugenol in RAW264.7 cells and their antioxidant activity determined using the induction period method, In Vivo (Brooklyn). **31**, 819–831 (2018).
44. J.L. Yeh, J.H. Hsu, Y.S. Hong, J.R. Wu, J.C. Liang, B.N. Wu, I.J. Chen, S.F. Liou, Eugenolol and glyceryl-isoeugenol suppress LPS-induced iNOS expression by down-regulating NF-κB and AP-1 through inhibition of MAPKs and Akt/IκBα signaling pathways in macrophages, Int. J. Immunopathol. Pharmacol. **24**, 345–356 (2011).
45. N. Hashemi Goradel, M. Najafi, E. Salehi, B. Farhood, K. Mortezaee, Cyclooxygenase-2 in cancer: A review, J. Cell. Physiol. **234**, 5683–5699 (2019).
46. A. Marchese, R. Barbieri, E. Coppo, I.E. Orhan, M. Daglia, S.F. Nabavi, M. Izadi, M. Abdollahi, S.M. Nabavi, M. Ajami, Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint, Crit. Rev. Microbiol. **43**, 668–689 (2017).
47. S.S. Kim, O.-J. Oh, H.-Y. Min, E.-J. Park, Y. Kim, H.J. Park, Y. Nam Han, S.K. Lee, Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells, Life Sci. **73**, 337–348 (2003).
48. F. das Chagas Pereira de Andrade, A.N. Mendes, Computational analysis of eugenol inhibitory activity in lipoxygenase and cyclooxygenase pathways, Sci. Rep. **10**, 16204 (2020).
49. K. Barefoot, D. Curtis, W. Jolliff, J.R. Nicholson, R. Omohundro, Antiangiogenic Agents in Cancer Therapy, (Humana Press, Totowa, NJ, 1999).
50. K.M. Cook, W.D. Figg, Angiogenesis inhibitors: Current strategies and future prospects, CA. Cancer J. Clin. **60**, 222-243(2010).
51. P. Manikandan, R.S. Murugan, R.V. Priyadarsini, G. Vinothini, S. Nagini, Eugenol induces apoptosis and inhibits invasion and angiogenesis in a rat model of gastric carcinogenesis induced by MNNG, Life Sci. **86**, 936–941 (2010).
52. P. Happy Kurnia, T.R. Dhanang Puruhita, H. Muhammad Nazhif, C. Rizq Threevisca, Eugenol-loaded chitosan nanoparticle induces apoptosis, inhibits cell migration and epithelial to mesenchymal transitionprocess in human cervical cancer cell line hela cells, Eur. J. Mol. Clin. Med. **7**, 1184-1197 (2020).
53. H. Nam, M.-M. Kim, Eugenol with antioxidant activity inhibits MMP-9 related to metastasis in human fibrosarcoma cells, Food Chem. Toxicol. **55**, 106-112 (2013).

54. R. Sever, J.S. Brugge, Signal transduction in cancer, Cold Spring Harb. Perspect. Med. **5**, a006098–a006098, (2015).
55. X. Shi, W. Zhang, X. Bao, X. Liu, M. Yang, C. Yin, Eugenol modulates the NOD1-NF-κB signaling pathway via targeting NF-κB protein in triple-negative breast cancer cells, Front. Endocrinol. (Lausanne). **14** (2023).
56. M. Djaldetti, H. Bessler, Eugenol as a modulator of the immune dialogue between human mononuclears and colon cancer cells, J. Mol. Pathophysiol. **6**, 1 (2017).
57. M.R. Charan Raja, Versatile and synergistic potential of eugenol: A review, Pharm. Anal. Acta **06** (2015).
58. A. Hussain, K. Brahmbhatt, A. Priyani, M. Ahmed, T.A. Rizvi, C. Sharma, Eugenol enhances the chemotherapeutic potential of gemcitabine and induces anticarcinogenic and anti-inflammatory activity in human cervical cancer cells, Cancer Biother. Radiopharm. **26**, 519–527 (2011).
59. M.L. Abdullah, M.M. Hafez, A. Al-Hoshani, O. Al-Shabanah, Anti-metastatic and anti-proliferative activity of eugenol against triple negative and HER2 positive breast cancer cells, BMC Complement. Altern. Med. **18**, 321(2018).
60. A. Sarkar, S. Das, A. Rahaman, A. Das Talukdar, S. Bhattacharjee, D.P. Mandal, Eugenol and capsaicin exhibit anti-metastatic activity via modulating TGF-β signaling in gastric carcinoma, Food Funct. **11**, 9020–9034 (2020).
61. D. Pal, S. Banerjee, S. Mukherjee, A. Roy, C.K. Panda, S. Das, Eugenol restricts DMBA croton oil induced skin carcinogenesis in mice: Downregulation of c-Myc and H-ras, and activation of p53 dependent apoptotic pathway, J. Dermatol. Sci. **59,** 31–39 (2010).
62. I. Al-Sharif, A. Remmal, A. Aboussekhra, Eugenol triggers apoptosis in breast cancer cells through E2F1/survivin down-regulation, BMC Cancer **13**, 600 (2013).
63. J. Baharara, T. Ramezani, M. Mousavi, K. Kouhestanian, Eugenol suppressed metastasis of breast carcinoma cells and migration by regulation of MMP-9 & paxilin gene expression, Sch. J. Agric. Vet. Sci. **2**, 125–130 (2015).
64. Z. Cui, Z. Liu, J. Zeng, L. Chen, Q. Wu, J. Mo, G. Zhang, L. Song, W. Xu, S. Zhang, X. Guo, Eugenol inhibits non-small cell lung cancer by repressing expression of NF-κB-regulated TRIM59, Phyther. Res. **33**, 1562–1569 (2019).
65. Y. Duan, X. Huang, B. Qiao, R. Ma, J. Li, Eugenol inhibits the biological activities of an oral squamous cell carcinoma cell line SCC9 via targeting MIF, Anticancer. Agents Med. Chem. **22**, 2799–2806 (2022).
66. Y. Keneda, Atsushi; Tsukada, DNA and Histone Methylation as Cancer Targets, (Springer International Publishing, Cham, 2017).
67. P. Trojer, Histone methylation modifiers in medical therapeutics, in: Med. Epigenetics, (Elsevier, pp. 693–720, 2021).
68. Y. Song, F. Wu, J. Wu, Targeting histone methylation for cancer therapy: enzymes, inhibitors, biological activity and perspectives, J. Hematol. Oncol. **9**, 49, (2016) .
69. R. Rupaimoole, H.-D. Han, G. Lopez-Berestein, A.K. Sood, MicroRNA therapeutics: principles, expectations, and challenges, Chin. J. Cancer 30 (2011) 368–370.
70. A.R. Raheem, O.F. Abdul-Rasheed, O.S. Khattab, A.Z. Alsammarraie, H. Al-Aubaidy, H.A. Abid, Circulating miRNA-373 and vascular endothelial growth factor as potential biomarkers for early detection of breast cancer, Indian J. Clin. Biochem. (2024).
71. E. Ghodousi-Dehnavi, R.H. Hosseini, M. Arjmand, S. Nasri, Z. Zamani, A metabolomic investigation of eugenol on colorectal cancer cell line HT-29 by

modifying the expression of APC, p53, and KRAS genes, Evidence-Based Complement. Altern. Med. **2021**, 1–9 (2021).