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Anticancer Effects of Carvacrol in *In Vitro* and *In Vivo* Models: A Comprehensive Review

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Abstract: Carvacrol is an active phenolic monoterpenoid with enormous anticancerous potential against numerous carcinomas, including prostate, gall bladder, and cervical, and has gained wider recognition in chemotherapeutics. Therefore, this review targeted to study and summarize various *in vitro* and *in vivo* research studies associated with the anticancerous potential of carvacrol with its associated mechanisms in several carcinomas. Carvacrol-treated cancer cells have exhibited significant apoptotic induction, cell cycle arrest, cytotoxicity, antimetastatic activity, and different antiproliferative effects via targeting numerous signaling pathways, including MAPKs, Notch PI3K, mTOR, and AKT. *In vitro*, carvacrol appears to be a highly potent phytoactive compound against several carcinomas. However, more *in vivo* research with better methodology are still needed to elucidate safe and standard dose, determine their toxic effects and elaborate its exact mode of action to develop a potential therapeutic approach for cancer management.

Keywords: carvacrol; monoterpene; bioactive compound; anticancer; therapeutic potential

1. Introduction

According to WHO (World Health Organization, 2020), it has been estimated that one in five people globally faces a diagnosis of a few malignant neoplasms, which is forecast to increase exponentially by 2040 [1]. Cancer is a major worldwide public health issue and has been among the four crucial reasons for premature death in many countries, leading to 8.8 million deaths annually as per the 2019 report of the National Cancer Institute. Several antineoplastic agents have improved the patient survival rate but have posed various side effects and worsened the quality of life of these patients [2-5]. Thus, more research should be focused on exploiting the therapeutic potential of phytocompounds that can further be utilized to develop a more potent and effective treatment against cancer with limited toxic side effects. However, several medicinal plants with potent pharmacological properties have been discovered [6]. However, nature still grips several bioactive compounds without sufficient reports, specifically in cancer biology [7, 8]. Secondary plant metabolites have made major contributions to cancer therapeutics via targeting major signaling pathways, such as vinca alkaloids, perillyl alcohol, limonene (monoterpenes), and paclitaxel [9-11]. In this context, we have summarized our review covering numerous *in vivo* and *in vitro* anticancerous studies

reporting the anticancerous potential of carvacrol (5-isopropyl-2-methylphenol) with its associated mode of action. Carvacrol (monoterpenoid phenol) is one of the main components found in essential oils obtained from various plant species such as *Origanum vulgare*, *Lippia gracilis*, and *Thymus vulgaris* [12-14], that have previously been reported to display medicinal benefits against several diseases, such as cancer [15, 16] (Figure 1).

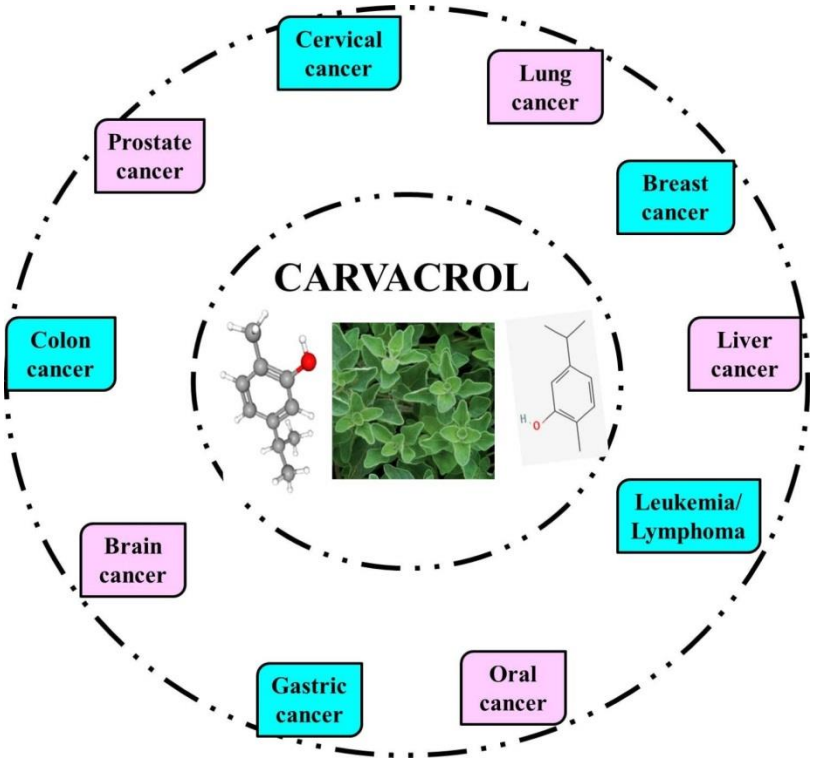

Figure 1. *In vitro* and *in vivo* efficacies of carvacrol in various types of carcinoma.

Carvacrol ($C_{10}H_{14}O$) is a liquid phenolic monoterpenoid, 2-methyl-5-(1-methylethyl)phenol, present in the essential oil of *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Lepidium flavum* (pepperwort), *Citrus aurantium* var. bergamia Loisel (wild bergamot), and several other plants [17, 18]. Commercial carvacrol is synthesized via various biotechnological and chemical methods. It has been recognized as 5-isopropyl-2-methyl phenol by IUPAC (the International Union of Pure and Applied Chemistry) and has a density of 0.976 g/ml at 25 °C. It is lipophilic and insoluble in water but is highly soluble in diethyl ether, ethanol, and acetone [19].

Carvacrol (FDA authorized) has also been included in the list of chemical flavorings used in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, nonalcoholic beverages, and soft candies, according to the Council of Europe. Carvacrol is used as a preservative in various foods, including rice, grape tomatoes, grapes, apple juice, semi-skimmed milk, fresh-cut kiwifruit, and honeydew melon. It has been reported to be strongly effective in reducing the growth of food spoilage and pathogenic bacteria when used alone or in conjunction with other naturally occurring organic molecules. Carvacrol has also been found to be an effective antioxidant in poultry feed, lowering lipid oxidation and so boosting meat nutritional quality. Feed supplementation could be a straightforward and convenient way to get lipid-soluble antioxidants into phospholipid membrane tissues, where they can efficiently suppress oxidative processes in specific places. Concerns about the safety of synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have also prompted increased research into plant ingredients like carvacrol.

As recently reported by numerous researchers, carvacrol exhibited a wider range of biological activities, such as antiviral [20, 21], antibacterial and antifungal [22-24], and anticarcinogenic [25, 26], antioxidant properties [27, 28]. Due to antimicrobial and flavoring activities, carvacrol has also been utilized as a natural food preservative in the food industry [29, 30].

In addition, carvacrol has also presented potent anti-inflammatory and antioxidant potential via reducing inflammation and increasing the non-enzymatic and enzymatic antioxidants in the tumor environment [31, 32]. Hence, this review aims to summarize all the best possible studies reported for carvacrol's antiproliferative and anticancerous potential to provide strong guidance for future carvacrol-based studies.

2. Anticancerous potential of carvacrol against different cancer cell lines

Carvacrol showed significant growth inhibitory potential in the A549 cell line (lung cancer cells) at respective doses of 500 and 1,000 µM with induction of early apoptotic characteristics [33] via inhibition of AXL (tyrosine kinase receptor) expression and enhanced MDA (malondialdehyde) and 8-OHdG (hydroxy-2′-deoxyguanosine) expression levels (8-OHdG) [34, 35]. Carvacrol has also displayed potent antitumor effects in HepG2 cells (hepatocarcinoma) by inducing cell death via the mitochondrial-mediated pathway, accompanied by Bcl-2 inhibition and caspase-3 activation in a dose-dependent manner [36]. Similarly, Meluˇsová *et al*. (2014) have also demonstrated the apoptotic efficacy of carvacrol (650 µM) via growth arrest in G1 and S phases [37]. Caco-2 cancer cells have also exhibited decreased cell viability and increased early apoptotic cells after carvacrol treatment (115 µM) [38]. Carvacrol also has displayed inhibition of HCT116, HT-29 cell proliferation, and reduced metalloproteinases, Bcl-2, p-Akt, p-ERK, and cyclin B1 levels leading to cell cycle arrest at the G2/M phase [39-41].

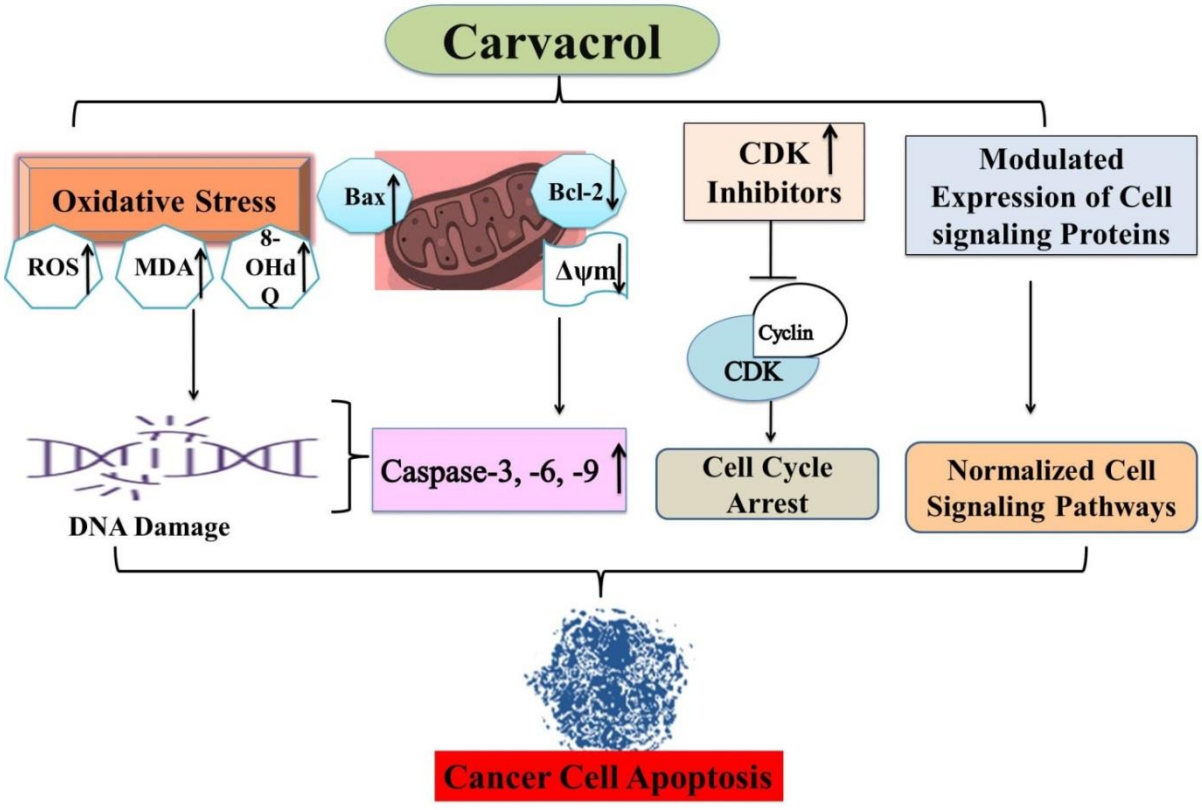

Figure 2. The mechanism associated with the anticancer mode of action of carvacrol.

Numerous research reports have strongly depicted the cytotoxic and pro-apoptotic efficacy of carvacrol against various cancer cells in a time and dose-dependent manner, with significant effects on cell invasion via reduced matrix metalloprotease 2 and 9 expression levels in treated cells [39]. A plethora of cancerous cells, including Hep-2 human larynx carcinoma cells, mouse B16 melanoma, leiomyosarcoma cells, gastric carcinoma cells, A549 non-small-cell lung cancer cells, chronic myeloid leukemia cells, MDA-MB-231 human metastatic breast cancer cells, and human colon cancer cells have been tested with carvacrol and has strongly supported the anticancerous potential of carvacrol [26, 33, 42, 43] (Figure 2). Al-Fatlawi *et al*., 2014 reported dose-dependent downregulation of the Bcl-2 gene and upregulation of the Bax gene in carvacrol-treated chemosensitive MCF-7 breast cancer cells. Carvacrol-treated MCF-7 cells also exhibited upregulated levels of caspase-3, 6, and 9 genes compared to untreated controls. This clearly illustrated the possible mechanism of apoptosis induction in carvacrol-treated cancer cells via p53 and mitochondrial pathway [44].

Arunasree *et al*. 2010 further investigated the molecular mechanism associated with the antitumor potential of carvacrol against MDA-MB-231 (metastatic) breast cancer cells. Clearly, it demonstrated that carvacrol treatment-induced dose-dependent apoptosis in MDA-MB-231 cells and decrease in MMP (mitochondrial membrane potential) of the cells, thereby leading to the cytochrome c release from mitochondria, caspase activation, and PARP (poly-ADP-ribose polymerase) cleavage [26]. FACS (flow cytometric) analysis of carvacrol-treated cells has further shown a significant increase of cells in the G0/G1 phase (apoptotic peak) and a decrease in the S phase, exhibiting apoptosis induction and DNA synthesis inhibition in the S phase [45].

The chemopreventive potential of carvacrol can also be attributed to the effect on hepatic steatosis, which may cause fibrosis, steatohepatitis, and cirrhosis recognized risk factor for HCC (hepatocellular carcinoma). Carvacrol has strong antioxidant properties parallel to butyl hydroxytoluene, ascorbic acid, and vitamin E [46]. Other reports have presented strong evidence about the anticancerous potential of carvacrol against Hep G2 HCC cells via inducing caspase-3 activation, PARP cleavage, and reduced Bcl-2 gene expression [47]. Additionally, carvacrol has been shown to cease cancer cell proliferation via reducing ERK1/2 phosphorylation and activating p38 phosphorylation in a dose-dependent manner [48, 49]. Similar effects have also been reported in DU145 (human prostate cancer) cells, where carvacrol treatment-induced ROS (reactive oxygen species) mediated apoptosis along with cell cycle arrest at G0/G1 in DU145 cancer cells [50].

Interestingly, carvacrol displayed stronger anticancerous potential against HCC cells and lung carcinoma cells, with limited cytotoxicity to normal human fetal liver cells. Recently, the interest in utilizing apoptosis induction as an effective strategy for elucidating potent antitumor drugs has escalated [51].

Table 1. *In vitro* antitumor activities of carvacrol and its possible targets.

Cancer	Cell lines	Doses (Range)	Anticancer mechanism	Molecular targets	References
Breast Cancer	MDA-MB-231 cells	1-10,000 µM	Cell growth, inhibition; apoptosis induction, cell cycle arrest	Cyt c, Bax, cyclin A, B, CDK4	[26, 52-54]
	MCF-7 cells	25-500 µM	Cell cytotoxicity, cell growth inhibition, apoptotic induction, cell cycle arrest	p53, Bax, caspase 3/6/7, cyclin A, B CDK4 and 6, Cyclin D1, Bax, Bcl-2 PI3K/p-AKT	[44, 53-57]

Cancer	Cell lines	Doses (Range)	Anticancer mechanism	Molecular targets	References
	BT-483	25-500 µM	cell growth inhibition, apoptotic induction	cyclin A, B, CDK4	[54]
	BT-474	25-500 µM	cell growth inhibition, apoptotic induction	cyclin A, B, CDK4	[54]
Cervical cancer	HeLa cells	25-800 µM	Cell growth inhibition via apoptotic induction	cyclin D1, BRK1/2, p21, caspase-3, ROS generation	[58-60]
	SiHa cells	25-500 µM	Cell growth inhibition via apoptotic induction	Bax, Bcl-2, p53, caspase-3/6/9	[58, 61]
Choriocarcinoma	JAR cells	50–300 µM	Decreased cell viability, apoptosis induction	PI3K/AKT, p-JNK, p-ERK1/2 p-p38, MMP, ROS	[62]
	JEG3 cells	50–300 µM	Decreased cell viability, apoptosis induction	PI3K/AKT, p-JNK, p-ERK1/2 p-p38, MMP, ROS	[62]
Lung cancer	A549 cells	100-1,000 µM	Reduced Cell growth, apoptosis induction, cell migration inhibition	p38, NF-κB, TNF-α, GSK-3b, Beclin-1, AXL	[33, 35, 63, 64]
	H460 cells	30-300 µM	Reduction in cell proliferation	AXL	[35]
	H1299 cells	25–1800 µM	Decreased cell viability	Cell membrane and DNA damage	[34]
Colorectal cancer	HT-29 cells	25-200 µM	Cell growth inhibition via apoptotic induction	CDK4 and 6 Cyclin D1, Bax, Bcl-2 PI3K/p-AKT	[40]
	Caco-2 cells	100-2,500 µM	Apoptosis induction, Cellular cytotoxicity	DNA damage	[38, 65, 66]
	LoVo cells	100–900 µmol/L	Cell growth inhibition via apoptotic induction and cell cycle arrest	Bcl-2, Bax MMP-2 and -9, Cyclin B1, p-ERK p-JNK p-Akt PI3K/Akt	[39]
	HCT 116 cells	100–900 µmol/L	Cell growth inhibition via apoptotic induction	-	[39, 40]
Prostate cancer	DU145 cells	10-500 µM	Cell growth inhibition via ROS mediated apoptotic induction	-	[50, 56, 67]
	PC-3 cells	25-800 µM	Cell growth inhibition, cell migration and invasion inhibition apoptosis induction, caspase-8/9 activation	Bax, Bcl-2, Notch-1, Jagged-1, MMP-2, p-Akt PI3K/Akt, TRPM7, IL-6 p-STAT3 p-ERK1/2	[56, 67-71]
Melanoma	A375 cells	3.906–1,000 µg/mL	Reduction in cell viability, apoptotic induction	Bcl-2, cell cycle arrest	[72]
	B16-F10 cells	-	Cell cytotoxicity	-	[72]
Gastric adenocarcinoma	AGS cells	100-600 µM	Cell cytotoxicity, apoptosis induction	Bax, Bcl-2, GSH level, Caspase-3 and -9	[43, 73]
Glioblastoma	U87 cells	1–10,000 µM	Anticancer and antioxidant activity, apoptosis induction	PI3K/Akt, MAPK, TRPM7, MMP-2, caspase-3	[52, 74]
	DBTRG-05MG cells	200–1,000 µM	Reduction in cell viability	Caspase-3 and ROS generation	[75]
Liver cancer	Hep G2 cells	100-1000 µM	Cell growth inhibition, DNA damage, membrane damage, apoptosis induction	MAPK p-ERK 1/2 Caspase-3 Bcl-2, p-p38	[25, 36, 47, 48, 63, 65, 66]
	Hep3B cells	1-1000 µM	Antiproliferative and cytotoxic effects	-	[63]
Neuroblastoma	SH-SY5Y cells	12.5-50 µM	Cell growth inhibition	MYCN, Bax, Bcl2, TNFa	[76]
	N2a cells	10–400 mg/L	Anticancer and antioxidant potential	-	[77]
Oral cancer	Tca-8113	10–80 µM	Apoptosis induction	CCND1 CDK4, p21,	[78]

Cancer	Cell lines	Doses (Range)	Anticancer mechanism	Molecular targets	References
				Bcl-2 Bax MMP-2 and -9 COX-2	
	SCC-25	167 µg/mL	Apoptosis induction	CCND1 CDK4, p21, Bcl-2 Bax MMP-2 and -9 COX-2	[78]
	OC2 cells	200–1,000 µM	Decreased cell viability	Caspase-3, ROS generation	[79]
Ovarian cancer	SKOV-3	100-600 µM	Apoptosis induction	-	[80]
Leukemia	HL-60 cells	10–400 µM	Cell growth inhibition	MMP, Bcl-2	[81, 82]
	K562 cells	200-1000 µM	Cell cytotoxicity	-	[82, 83]
	KG1 cells	100-400 µM	Reduction in Cell viability	-	[82]
	CEM cells	0.05–1.25 µM	Cell cytotoxicity	Cell cycle interruption	[55]
	P-815	0.05–1.25 µM	Cell cytotoxicity	Cell cycle interruption	[55, 84]
	Leukemia stem cells (CD123+/ CD34+/CD38+)	160 µg/mL	Cell growth inhibition via apoptotic induction	GSK-3β	[84]

Abbreviations: Atg5/12, autophagy-related 5/12; Bax, Bcl-2 associated X protein; Bcl-2, B cell lymphoma 2; GSK-3β, glycogen synthase kinase 3 beta; LC3-II, light chain 3; PARP, poly (ADP ribose) polymerase; IKK, IκB kinase; MAPK, mitogen-activated protein kinase; MMP, mitochondrial membrane potential; N-myc proto-oncogene protein; NF-κB, nuclear factor-kappa; B ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha.

3. *In vivo* efficacy of carvacrol

Carvacrol administration has displayed significant anticancerous potential in Wistar rats via reduced tumor incidence and enhanced survival rate [42]. Carvacrol-treated animal models with DEN (diethylnitrosamine) induced liver cancer displayed reduced nodules and liver weight and increased body weight. Cells pretreated with carvacrol displayed the disappearance of most tumoral nodules and foci, characterized by some neoplastic cells, thereby validating the chemopreventive efficacy of carvacrol. Whereas carvacrol post-treatment demonstrated distorted cellular architecture, small persistent nodules, and a limited tendency to spread via intrahepatic veins. Moreover, carvacrol treatment resulted in increased levels of SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), GR (glutathione reductase), and GSH (glutathione), along with reduced lipid peroxides and several enzymes such as AST, LDH, ALT, γGT, and ALP in serum [85]. Subramaniyan *et al*. (2014) have further evaluated carvacrol's pre and post-treatment efficacy in a DEN-induced hepatocarcinogenesis rat model and reported stable tumor marker levels, reduced mast cell density, and cell proliferation. Moreover, carvacrol supplementation potentially restored the abnormal activities of liver microsomal xenobiotic-metabolizing enzymes with reduced PCNA (proliferative nuclear cell antigen), MMP-2, and -9 expression validating the antimetastatic efficacy of carvacrol [86]. Hence, carvacrol treatment in DEN (diethylnitrosamine) induced hepatocellular carcinoma rat model resulted in apoptosis induction characterized by DNA fragmentation. Additionally, carvacrol treatment showed a significant reduction in serum levels of AFP (alpha-fetoprotein), AFU (alpha L-fucosidase), VEGF (vascular endothelial growth factor), and reduced GGT (gamma-glutamyl transferase) gene expression [60]. Carvacrol supplementation further displayed significant improvement in the growth rate of DMH (1,2-dimethylhydrazine) induced animal model of colon cancer with lower incidence of pre-neoplastic lesions and tumors, along with reduced oxidative stress damage (increased levels of SOD, GSH, CAT, GPx, and GR) thereby strongly validating the chemopreventive potential of carvacrol [87]. Li *et al*. (2019) further showed limited tumor growth in the carvacrol-treated

DEN-induced hepatocarcinoma mice model, thereby displaying tumor cell reduction, normal cell arrangements, rare mitotic figures microvessels, and reduced peritumor and intrastromal lymphocytes. Similarly, there was reduced DAPK1 (death-associated protein kinase 1) and PPP2R2A (serine/threonine-protein phosphatase 2A) expression in tumor tissues [88]. Another recent study has presented better anticancerous efficacy of carvacrol in DMBA (dimethylbenzanthracene) induced breast cancer in female Holtzman rats by displaying a 75% reduction in tumor frequency, 67% reduction in tumor incidence, and a significant reduction in tumor volume [89]. Additionally, carvacrol has exhibited significant anticancerous potential via regulating numerous cell signaling and apoptotic pathways.

Table 2. *In vivo* antitumor activities of carvacrol and its possible targets**.**

Cancer Model	Cell lines	Doses/treatment	Anticancer mechanism	Molecular targets	References
Breast cancer	DMBA-induced induced breast cancer in the female Holtzman mice	50, 100, and 200 mg/kg carvacrol for 14 weeks orally	Reduction in number of tumors	Antioxidant activity	[89]
Colon cancer	DMH-induced induced colon cancer in male Wistar rats	20, 40, and 80 mg/kg carvacrol daily for 16 weeks i.p.	Tumor growth inhibition	Increased level of GPx, GR, GSH, SOD, CAT	[87]
Liver Cancer	0.01% DEN induced hepatocellular carcinoma in Wistar rats	15 mg/kg carvacrol for 16 weeks orally	Chemopreventive effects and apoptosis induction	Increased serum marker enzymes AST, ALT, ALP, LDH, cGT	[85]
	N-nitrosodimethylamine induced hepatocellular carcinoma in Wistar rats	15 mg/kg carvacrol for 15 weeks orally	Antiproliferative antiangiogenic and apoptosis-inducing effects	Decrease AFP, VEGF, AFU, and GGT PARP, DNA ligase, and polymerase beta	[60]
	0.01% DEN induced hepatocellular carcinoma in Wistar rats	15 mg/kg carvacrol for 16 weeks orally	Decrease tumor growth	Decrease tumor markers, MMP-2 and -9, AgNORs, PCNA	[86]
	DEN-induced hepatocellular carcinoma in C57BL/6 mice	Intragastrically for 20 weeks	Decrease tumor growth	Modulation of DAPK1 and PPP2R2A	[88]

Abbreviations: HPV, human papillomavirus; COX-2, cyclooxygenase-2; I.P., Intraperitoneal; JNK, c-Jun N-terminal kinase; PARP, poly-ADP ribose polymerase; VEGF, vascular endothelial growth factor; ROS, reactive oxygen species;

4. Conclusions

Amongst numerous phyto-compounds, carvacrol contributes to potent anticancerous effects. Carvacrol has been presented to utilize several mechanisms for obstructing carcinogenesis via modulating various deregulated cell signaling pathways associated with apoptosis, autophagy, inflammation, and angiogenesis. Carvacrol has been shown to interfere with several intracellular signaling molecules, including ILs, TNF-α, Bax, VEGF, Beclin, caspases, and Bcl-2. This review has presented several *in vitro* and *in vivo* research studies to validate the strong anticancerous potential of carvacrol via targeting several potential therapeutic targets, including Bcl-2, Bax, NF-κB, p53, caspases, Akt, TNF-α, and GSH. Despite various preclinical mechanistic research reports on the anticancerous potential of carvacrol, the lack of well-designed clinical trials exhibiting the therapeutic efficacies of carvacrol enhanced the urge for elucidating better significant clinical studies. Still, more extensive and elaborative studies are needed to develop a more potent targeted drug delivery system for cancer management. Studies should be aligned towards exploring the novel molecular targets of carvacrol and its associated mechanism in different cancers. Altogether, carvacrol has vast medicinal health potential and, therefore, should be utilized as a potential therapeutic agent via extensive investigation of its anticancerous potential.

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Conflicts of Interest

The authors declare no conflict of interest.

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