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ROLE OF CARVACROL IN PROLIFERATIVE CELL SIGNALLING AND ISCHEMIC STROKES: A DETAILED REVIEW

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ABSTRACT. Plant-based molecules have played important roles in treatment of human diseases as therapeutic agents since ancient times. Carvacrol [2-methyl-5-isopropyl phenol] is among the most studied of the isoprenoid group of bioactive compounds, and has diverse applications in the pharmaceutical, food, biotechnological industries and others. Discovery of its antimicrobial, anticancer, antispasmodic, immunomodulatory, anti-inflammatory, antioxidant and mitogenic effects, has made it a hot topic of numerous research projects. Molecular mechanisms underlying these various effects are still subjects of ongoing researches and existing literatures are quite few on this subject. This review work sought to study the few existing literatures on the carvacrol-cell signalling pathway relationship in cell survival and death, and the emerging future directions for basic and translational research. Carvacrol is known to activate several cell signalling pathways essential to cell survival and death, such as ERK1/2 MAPK signalling, p38 MAPK signalling and JNK MAPK signalling of the MAPK signalling family, PI3K/AKT signalling, IL6/STAT3 signalling, eNOS signalling, etc. Carvacrol exerts different biological actions to influence up-/down-regulation of key cell cycle proteins expressions such as cyclin D1, cyclin B1; CDK4, CDK6, pRb, etc, were either. Mitochondrial-mediated dose-dependent apoptosis and cytoprotection are a major hallmark of mechanism of carvacrol action.

Keywords: *Carvacrol, tissue repair, cell signalling pathway, ischemia-reperfusion*

KARVAKROL'ÜN PROLİFERATİF HÜCRE SİNYALİZASYONUNDA VE İSKEMİK STROKLARDAKİ ROLÜ: DETAYLI BİR İNCELEME

ÖZET. Bitki bazlı moleküller, antik çağlardan beri terapötik ajanlar olarak insan hastalıklarının tedavisinde önemli roller oynamıştır. Karvakrol [2-metil-5-izopropil fenol], biyoaktif bileşiklerin izoprenoid grubu arasında en çok çalışılanlardan biridir ve farmasötik, gıda, biyoteknolojik endüstriler ve diğer alanlarda çeşitli uygulamalara sahiptir. Antimikrobiyal, antikanser, antispazmodik, immünomodülatör, antiinflamatuar, antioksidan ve mitojenik etkilerinin keşfi, karvakrolü çok sayıda araştırma projesinin önemli bir konusu haline getirmiştir. Bu çeşitli etkilerin altında yatan moleküler

mekanizmalar halen devam eden araştırmaların konusudur ve bu konuda mevcut literatür oldukça azdır. Bu derleme çalışması, hücre sağkalımı ve ölümü ile karvakrol-hücre sinyal yolu ilişkisi ile temel ve translasyonel araştırmalar için ortaya çıkan ve gelecekteki olası çalışmalar hakkındaki mevcut literatürü incelemeyi amaçlamıştır. Karvakrolün, MAPK sinyal ailesinin; ERK1/2 MAPK sinyali, p38 MAPK sinyali ve JNK/MAPK sinyali, PI3K/AKT sinyali, IL6/STAT3 sinyali, eNOS sinyali gibi hücrenin hayatta kalması ve ölümü için gerekli olan birkaç hücre sinyal yolunu aktive ettiği bilinmektedir. Karvakrol, siklin D1, siklin B1; CDK4, CDK6, pRb gibi anahtar hücre döngüsü proteini ekspresyonlarının aşağı ve yukarı regüle edilmesini etkileyen farklı biyolojik etkiler gösterir. Mitokondriyal aracılı doza bağımlı apoptoz ve sitoproteksiyon, karvakrol etki mekanizmasının önemli bir özelliğidir.

Anahtar Kelimeler: *Karvakrol, doku onarımı, hücre sinyal yolağı, iskemi reperfüzyon*

INTRODUCTION

Plants and plant-based bio-active molecules have played important roles in treatment of human diseases since ancient times [1]. One such essential class of distinctive functionally and structurally biochemical natural substances are the isoprenoids [terpenoids or terpenes] group, with diverse applications ranging from pharmaceutical, food, biotechnological, industrial uses and several others. Isoprenoids are also biologically indispensable in photosynthesis, respiration, membrane fluidity, regulation of growth and development as primary metabolites. The primary metabolites mostly included steroids, carotenoids and quinines whereas the secondary metabolites usually are for medicinal purposes.

Isoprenoids comprise over fifty thousand (50,000s) compounds including both primary and secondary metabolites. A comprehensive chemical database of all characterised natural products known as the 'Dictionary of Natural Products' [http://dnp.chemnetbase.com] has about 33 percent [33 %] of its collection being made up of terpenome, which is the chemical collection of all known terpenoids (isoprenoids) [2]. Such wide structurally-related but functionally-different compounds should exist, is a puzzling subject. The most probable clue assumingly lies in their multiple structural composition of a basic 5-carbon unit, isoprene [2-methyl 1, 3-butadiene, isopentene] as their fundamental chemical building block. Isoprene ranks among the most found organic chemical compounds on earth [3].

The carbon unit [isoprene] is chemically modified through rearrangement, repetitive motifications, cyclisation, further oxidation [4], and addition of functional groups such as hydroxyl or carbonyl, all contribute to increasing the structural and functional diversity in isoprenoids. Importantly, cyclases as the tailoring enzymes involved in cyclisation reactions, are particularly responsible for the structural diversity of this chemical class [5]. A derivative of a single isoprene unit is termed terpene. Classification of a particular isoprenoid is defined by the number of terpene unit [carbon atom]: a single isoprene (C5) unit-containing isoprenoid compound is called hermiterpenoid, double (C10) monoterpenoid, (C15) sesquiterpenoid, (C20) diterpenoid, and triterpenoid C30, tetraterpenoid (C40) and polyterpenoid (C5)n where n=8, [6, 7].

Terpenes have increasingly gained much attention due to their wide industrial usage, and recently in biomedical and pharmacological research works for their anti-inflammatory, anti-carcinogenic and neuroprotective effects. Several terpenoids [prenyl-bound proteins, ubiquinones, steroids, cholesterol, retinoids, and carotenoids] are essential structural components of proteins executing important cellular functions, which included targeting a number of signalling molecules and pathways, such as; mitogen-activated protein kinase (MAPK), nuclear factor k-light-chain-enhancer of activated B cells (NF-κB), c-Jun N-terminal kinase (JNK), cytokines production {interleukin-6 (IL-6)} and tumour necrosis factor α (TNF-α) [8].

Monoterpenes include a large group with various important biological activities [9], owing to their antitumour, antioxidant, anti-inflammatory, hepatoprotective, cardioprotective and anti-diabetic as well as neuroprotective effects [10]. They are usually composed of two isoprene units, ın the form of diphosphate (pyrophosphate) esters; dimethylallyl diphosphate (DMAPP) and iso-pentyl diphosphate (IPP). On that basis, monoterpenes can be grouped into 4 main classes; acyclic, monocyclic, bicyclic and iridoid glycosides [11].

Carvacrol (2-methyl-5-isopropyl phenol) usually found in the essential oils of oregano (Kekik in Turkish) [1] (Origanum vulgare), thyme (Thyme vulgaris) and other thyme spp, Savory (Satureja hortensis) and Corydothymus species [12, 13], is a natural monocyclic monoterpene phenol compound isomeric to thymol, with substantial benefits as a cosmetic ingredient, safe food additive, and used as a flavour in the bakery, confectionary and beverage industries [14].

Biological Effects and Resulting Signalling Pathways in Pathophysiology

Essential oils containing carvacrol have proved to be cell growth inhibitors (biostatic) and/or cell killers (biocidal) against cells of bacteria, yeast and fungi in vitro [15]. It was showed that the biocidal activity caused the cell membrane to be damaged and resulted in an increased permeability of the membrane to potassium ions and protons, which caused the consumption of the intracellular ATP pool and disrupted proton-motive force [16].

Numerous studies of carvacrol, in vivo and in vitro have observed a range of different bioactivities, including but not limited to antimicrobial, anticancer, antispasmodic, immunomodulatory, antitussive, expectorant, antioxidant and mitogenic effects. In ruminants, it was observed to modify rumen microbial fermentation and reduce methane emission. In addition, its chemopreventive effects against cancer, neurodegenerative diseases, chemically-induced injuries and other pathologies have been reported [17, 18, 19, 20].

The hydroxyl group (OH·) in the chemical structure of carvacrol confers on it the antioxidant effect [21] and by far its chemopreventive and/or cytoprotective abilities [22]. Suganthi et al. (2013) demonstrated this potent antioxidant effect of carvacrol via its [OH·], which is able to scavenge free radicals in both in-vivo and in-vitro models [22]. Mechanism of the chemopreventive action of carvacrol is mediated by inducing phase II (drug metabolising and detoxifying) enzymes which usually evoke carcinogen

metabolites, thereby causing tumour regression in hepatocarcinogenesis [23]. Carvacrol pre-treatment had significant antioxidant and hepatoprotective effects against diethylnitrosamine-induced hepatocellular carcinoma [24] and against D galactosamine-induced injury [25] in rats.

Molecular mechanisms underlying these various effects are still subjects of ongoing research and existing literatures are quite few. This work thus sought to review the few existing literature on the carvacrol-cell signalling pathway relationship in cell survival [proliferation] and cell death [apoptosis], examine the molecular complexity of these interactions in regeneration models and the emerging future prospects for basic and translational research.

Carvacrol affects cell proliferation and survival functionally, via the regulation of differential expression of cell cycle control and checkpoints proteins such as cyclins, cyclin-dependent kinases (CDKs), etc, usually resulting in cell cycle arrest. CDKs and cyclins complexes play very important roles in regulation of cell cycle, causing partial phosphorylation of retinoblastoma (Rb), an essential cell cycle regulator in the G0/G1 phase [26, 27]. CDK4, CDK6, cyclin D1, cyclin B1 and phosphorylated Rb (pRb) were therefore shown to have altered or abnormally higher expression levels in numerous human cancer cells, which is directly proportional to uncontrolled proliferation and tumourigenesis.

Carvacrol was observed to cause cell cycle arrest by down-regulating certain cyclin proteins. For example, carvacrol in reducing cyclin B1 expression caused the arrest of the cell cycle at the growth phase (G2) / mitosis (M) phase to suppress proliferation, migration and invasion in colon cancer cells [28]. This suppression was finally determined to be a result of decreased matrix metalloproteases (MMP) expression: [MMP-2 and MMP-9], by which the colon cancer cells migrated and invaded.

In human oral squamous cell carcinoma (OSCC9 Tca-8113 cell treated with carvacrol, CDK regulators (CCND1 and CDK4) were down-regulated and CDK inhibitor (p21) up-regulated, inducing a cell cycle arrest at G1/S phase. These characteristic actions in Tca-811 3 underlie the possible molecular mechanism of carvacrol treatment. A subsequent cascade of molecular reaction chains result in the inhibition of phosphorylation of focal adhesion kinase, negatively regulating Tca-8113 cancer cell adhesion to fibronectin. In a dose-dependent manner of carvacrol treatment, expression of epithelial-mesenchymal transition (EMT)-related transcription factors β-catenin and ZEB-126, and that of MMP-9 and MMP-2 is decreased, thus causing a significant inhibition of the migrating and invading OSCC [29].

Increased cyclin D1, pRb, CDK4 and CDK6 expressions typical of breast cancer cells (MCF-7), were counteracted by carvacrol treatment. The treatment also caused a peak of sub-diploid DNA distributed within the cell cycle to accumulate at the stage of cell growth arrest [G0] / growth phase 1 (G1), hence inhibiting progression into S and G2/M phase. Down-regulation of PI3K/AKT signalling was thus significant in causing the observed cell cycle arrest at G0/G1 phase and followed by apoptotic cell death [30].

Uncontrolled cellular proliferation as an important hallmark of carcinogenesis can be potentially repressed through effectively targeting or inhibiting the expressions of cyclin D1, pRb, CDK4 and CDK6 proteins. Furthermore PDK1, AKT, P70S6K, and GSK3β which are major AKT substrates and players in regulation of cell survival and cell cycle progression, indicate the central role of PI3K/AKT pathway in tumourigenesis and development of human malignancies [31, 32].

Carvacrol thus elicits various molecular mechanisms and pathways to exert both chemopreventive and chemotherapeutic functions. Deregulated PI3K activity, also directly linked to abnormal proliferation, is well implicated in various cancers; breast cancer, lung cancer, melanomas, and leukaemia. MCF-7 cells treated with carvacrol showed significantly reduced expression of PI3K and p-AKT levels thereby clearly exhibiting the chemotherapeutic effects of carvacrol against breast carcinogenesis [13].

Mitogen-activated protein kinase (MAPK) signalling is known to play a major role in extracellular signal transduction to cellular responses. Three MAPK families have been clearly characterised in mammalian cells; extracellular-regulated protein kinase ERK (the classical MAPK), C-Jun N-terminal kinase/ stress-activated protein kinase (JNK/SAPK) and p38 kinase, all of which lie within protein kinase cascades. MAPK pathways detect signals from a variety of receptors, building up accurate cellular responses; proliferation, differentiation, development, apoptosis and inflammatory responses in the mammalian cells [33].

Roles of MAPKs as key players in progression of the cell cycle and MAPK pathway signalling in hepatocellular carcinoma (HCC) have been extensively documented. Of the MAPKs proteins, (ERK), p38 and (JNK) especially have been shown to play various roles in maintaining the cell survival [proliferation] and cell death [apoptosis] balance [34, 35, 36, 37]. ERK activation promotes cell proliferation [38], while that of JNK and/or p38 could induce apoptosis [39, 40, 41].

In reference to the above, Yin and colleagues concluded that carvacrol decreased ERK1/2 MAPK phosphorylation and activated p38 phosphorylation in HepG2 cells. Such an increase in pro-apoptotic signals sent the cancer cells to apoptosis. Bcl-2 expression was downregulated and Bax upregulated to induce both anti-proliferative [decreased ERK pathway phosphorylation] and apoptosis [p38 pathway activation] [42].

Bax is a known pro-apoptotic protein and induces apoptosis upon an increased expression. Bcl-2 is an anti-apoptotic protein located within the outer membrane of mitochondria and suppresses apoptosis upon increased expression. This Bax/Bcl-2 ratio is an important balance in cell survival [43, 44]. Magnitude of the Bax/Bcl-2 threshold is one of the hallmarks of cells undergoing apoptotic cell death [45, 46]. Bcl-2/Bax protein ratio is also important in the mitochondrial apoptosis pathway [47].

In the maintenance of cell survival and cell death homeostasis where Bcl-2 and Bax play important roles, induction of Bcl-2 and Bax differential expressions by carvacrol was observed to cause mitochondria-mediated apoptosis in a dose-dependent manner. Reduced Bcl-2 expression and an increase in the expression of Bax protein levels were demonstrated in carvacrol-treated MCF-7 cells. Similarly in Tca-8113 cells treated with

carvacrol, downregulation of Bcl-2, Cox-2, and upregulation of Bax protein levels were observed.

Bcl-2 and Bax protein levels play a key role in brain injury in the neonatal hypoxia-ischemia (H/I) model [48, 49]. Carvacrol is able to inhibit pro-apoptotic signalling pathways in neonatal H/I brain injury.

The molecular mechanism of action of carvacrol in inducing apoptosis in cancer cells has been less discussed in literature [42, 50]. Studies showed that carvacrol induced apoptosis in both human metastatic breast cancer (MDA-MB-231) and non-small cell lung cancer [NSCLC, A549] cell lines, in the characteristic fashion of mitochondrial potential decline, cytochrome c release from mitochondria, caspase activation and cleavage of poly ADP ribose polymerase (PARP), cytoplasmic blebbing and cell shape irregularity [30, 50].

This indicates that carvacrol mode of apoptosis induction is mostly mitochondria-mediated. Mitochondrial respiratory chain complex I (NADH ubiquinone oxidoreductase) being the first enzyme of the mitochondrial respiratory chain, oxidises the NADH produced from the Krebs cycle in the mitochondrial matrix, transferring two electrons to ubiquinone to form ubiquinol [51]. Ubiquinone inhibition by carvacrol in lower micromolar range has been reported [52], and this was in agreement with previous studies that showed rotenone to have caused apoptosis through mitochondrial respiratory chain complex I inhibition, probably by increasing reactive oxygen species [ROS] formation and inducing mitochondrial membrane potential decrease [53, 54].

Nuclear Factor-Erythroid 2-Associated Factor 2 (Nrf2) as a cap'n'collar (CNC) family of basic region-leucine zipper transcription factors, tightly-bound to its repressor protein, kelch-like ECH-associated protein 1 [keap1] to form the Nrf2/Keap1 complex. The Nrf2/Keap1 signalling pathway mainly regulates the transcription of genes involved in cellular antioxidant response system, redox homeostasis and metabolic balance [55]. In response to oxidative stress, Nrf2 is released from the Nrf2/Keap1 complex and translocated to the nucleus where it readily binds the antioxidant response elements (ARE) to synthesise downstream antioxidant molecules such as catalase [CAT], quinone oxidoreductase (NQO-1), superoxide dismutase (SOD), glutathione peroxidase (GSH.Px) haemoxygenase-1 (HO-1) [56, 57, 58].

Carvacrol by inhibiting the Keap1/Nrf2/HO-1 pathway in OSCC cells, was shown to both distinctly weaken its proliferative and migrated capacities, NALP3 inflammasome, and EMT activation [29]. Banik et al., [2019] in a study exhibited that carvacrol increased expression of Nrf2 protein to protect against cadmium toxicity in PC-12 cell culture through oxidative stress alleviation, GSH increase, glucocorticoid receptor (GR) upregulation, DNA damage reduction and a co-ordinated modulation of ERK-1 MAPK, Nrf2, Nf-kB, mTOR and Akt expressions [59]. Also, in CCI-induced neuropathic pain reduction, carvacrol was seen to inhibit NLRP3 and activated autophagy through the Keap1/Nrf-2/p62 signalling [60], and ensured quality mitochondrial dynamics [61]. Through the induction of Nrf-2/HO-1 signalling pathway to protect against diabetes-induced testicular damage in rats [62], ethanol-induced neuronal impairment [63] and hydrogen peroxide-induced damage in human neuroblastoma (SH-SY5Y) [64],

carvacrol exerted antioxidant and anti-inflammatory effects to guard the cellular integrity and homeostasis.

Maintenance of calcium homeostasis is essential in cell signalling and metabolism as cytosolic free calcium, Ca^{2+} level [$(Ca^{2+})i$] is involved in many signalling pathways and functions in processes such as muscle contraction-relaxation, secretion, neural transmission, enzyme cofactor, proliferation, apoptosis and also involved in DNA replications [65, 66, 67]. Changes in the endoplasmic reticulum (ER)/mitochondrial coupling process serve as an alternative apoptosis-activating pathway. The ER in releasing an overload of Ca^{2+} into the mitochondria, causes a significant change in the Ca^{2+} homeostasis and effectively contributes to apoptosis [68]. Several effects of carvacrol on Ca^{2+} homeostasis have been studied in diverse cell types. For instance, carvacrol concentration dependently elicits Ca^{2+} mobilisation intracellularly in Jurkat T cells and THP-1 monocytic cells [69], suggesting the need for a further study of this process in order to fully understand the mechanism of action of carvacrol and its toxicity [70]. Other studies have indicated that carvacrol induced apoptosis in human glioblastoma and oral cancer cells by increasing Ca^{2+} concentration [70, 71].

Tissue Repair, Organ Ischemia-Reperfusion and Regeneration

Carvacrol has been shown to possess numerous beneficial properties in scientifically proven in vivo studies. Studies on carvacrol in ischemia-reperfusion (I/R) and regeneration models are scarce in terms of its protective role in cellular proliferation, and the molecular interactions between the numerous cellular signalling pathways involved in its mechanism of biological action as depicted in **fig. 1**.

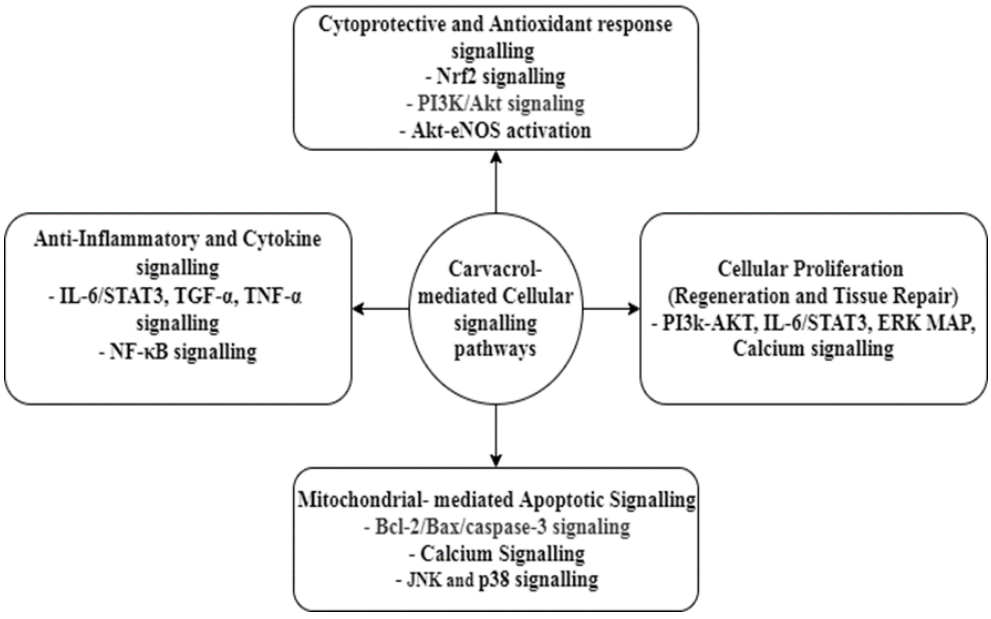

Fig. 1: *Molecular Mechanisms of Carvacrol action and Cellular signalling pathways*

Various degrees of injuries during I/R may occur in remote organs, as a result of limited or total lack of blood flow [72]. Renal I/R-induced liver injury may be considered as a typical example, and acute renal defects have been implicated in the

cause and/or origin of diseases of the liver [73]. I/R activates several protein kinase pathways [74]. The primary protein kinase pathways that are potentially activated by I/R include the MAP kinases, ERK 1/2, JNK 1/2, p38 MAPK alpha/ beta; and the cell survival kinase, Akt. [75]

Carvacrol increased the regeneration in the liver and had a protective effect on remote organ [liver] damages due to renal I/R injury [76]. In accordance with the findings of [24, 77] that exogenous antioxidants had positive effects on CAT, SOD and GSH enzyme activity in the liver tissue. Similar reports of antioxidant enzyme activity were seen after carvacrol treatment in renal I/R [78]. Such protective effect was needed against negative changes; oxidative stress, necrotic cell death and/or apoptosis seen in the remote organ [liver] histology after 45 or 60 min of renal I/R, as demonstrated [79]. Sinusoidal congestion and vacuolization were rather reported [78], and found that a 25 mg/kg carvacrol dose provided full protection while a 50 mg/kg carvacrol dose was partially protective against liver injury after renal I/R. Furthermore, carvacrol was observed to be tolerated at relatively higher doses in the liver [non-hepatotoxic], and hepatoprotective against I/R injury in rats. In similarity to silymarin [80], carvacrol caused no change to the genetic make-up of the hepatocytes [1].

Acetylsalicylic acid (ASA) commonly known as aspirin, was also observed to possess cardioprotective effects against I/R injury via activating phosphatidylinositol 3-kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK) signalling pathways [81]. Numerous other signalling pathways including PI3K/Akt/Nrf2, p38 MAPK [82], and ERK1/2 [83] play essential roles in survival of cardiomyocytes post-I/R injury.

In a similar mode of action like aspirin, carvacrol also showed the ability to protect the heart against myocardial I/R injury. Carvacrol, via activation of MAPK/ERK signalling pathway, had protective effects in myocardial I/R model by significantly increasing antioxidant [SOD and CAT] enzymes activity, reducing malondialdehyde levels and infarct volumes, and inhibiting caspase-3 and bax [cardiomyocyte apoptosis] [84].

Chen and colleagues reported that carvacrol particularly at the 0.6 mM concentration, aided the recovery of cardiac function post hypoxia-reperfusion through reduced mitochondrial injury and protected the heart by apoptotic inhibition through activation of MAPK/ERK pathway [84]. Carvacrol also exerted its cardioprotective effects after I/R injury through the Akt-eNOS activation. Carvacrol pre-treatment was observed to cause a significant increase in p-Akt and p-eNOS phosphorylation levels in hypoxia-reperfusion group compared to the control. Carvacrol was additionally seen to partially block hypoxia-reperfusion-induced myocardial apoptosis via Akt-eNOS activation, as previous studies had indicated Akt-mediated eNOS phosphorylation led to increase in NO production, an important downstream effector in survival signalling in myocardial ischemia and reperfusion [85].

Transient receptor potential melastatin 7 (TRPM7) is a Ca2+-permeable, non-selective cation channel belonging to the melastatin-related subfamily of TRP channels. It is always expressed in the brain and almost all other body tissues [86] as well as cancer tissues. TRPM7 has recently been gaining more importance as an important

mediating factor of anoxic neuronal death [87,88] and by virtue of the potential role of this pathway in ischemic neuronal injury. Upon oxygen-glucose deprivation (OGD) or hypoxic-ischemic conditions, increased brain TRPM7 mRNA and protein expression was seen to occur following cerebral ischemic injury and in hippocampal neurons [89].

Carvacrol possessed neuroprotective effects in cells over-expressing TRPM7 of HEK293 cells in vitro and mouse hippocampal neurons in vivo respectively. HEK293 treated with carvacrol in the concentration range of [200-800], were protected against anoxic insult (OGD). Carvacrol pre-treatment 30 minutes before hypoxic-ischemic in mouse neonatal brain at the 30 and 50 mg/kg doses similarly protected against the hypoxic-ischemic brain injury, decreasing pro-apoptotic signalling while increasing pro-survival signalling [84].

Carvacrol pre-treatment was reported to inhibit the Akt (p-Akt) dephosphorylation usually observed 8 and 24 hours post-hypoxic-ischemic in rats [90], via significantly increasing expression of p-Akt proteins, consequently activating PI3K/Akt pathway, which is a major pro-survival signalling pathway, and thus contributes to neuroprotection in cerebral ischemia injury and neonatal stroke [48]. In inhibiting pro-apoptotic signals during hypoxic-ischemic, carvacrol was further seen to increase the ratio of Bcl-2/Bax protein while decreasing caspase-3 activation [49].

Carvacrol has been observed to modulate the release of tumour necrosis factor- α [TNF-α], transforming growth factor (TGF- α) and interleukin 1β (IL-1β) throughout the process of tissue repair. Such mechanism of pharmacological action [anti-inflammatory action] may well be associated with the modulation of numerous important molecular targets, such as NF-κB, COX-2 and other pro-inflammatory cytokines [91, 92, 93]. For instance, an increase in levels of TNF-α and IL-6 in the liver activates the IL-6/STAT3 signalling pathway, which is quite important in initiating regeneration after partial hepatectomy (PHx) [94].

CONCLUSION

Carvacrol in different pathophysiological conditions can be observed to effect mitochondria-mediated cell death [95, 96] signalling via increased Ca2+ influx into mitochondria [70, 71], ROS formation [53, 54], change in Bcl-2/Bax ratio [48;49], complex I inhibition [52] and p38/JNK MAPK phosphorylation [39, 40, 41]. In a simultaneous fashion, cell survival mechanisms are rather inhibited by carvacrol. Phosphorylation of the ERK1/2 MAPK [38] and PI3K/Akt [30] signalling pathways were reportedly decreased. Expressions of cyclins and CDKs such as D1, B1; CDK4, CDK6, and other several proteins including pRb as key cell cycle proteins were either up-/down-regulated. MMP-2, MMP-9, ZEB-126, EMT-related transcription factor protein β-catenin, P70S6K, and PDK1, GSK3B are critical proteins involved in cell migration and invasion as a function of metastatic cell survival, and these protein factors are also inhibited by carvacrol

In the case of I/R models involving carvacrol treatment, antioxidant enzymes

such as CAT and SOD were markedly increased in most of the cases [renal, liver, cardial, etc] and affected various pro-survival signalling pathways, to promote cytoprotection and tissue repair. Carvacrol in modulation of TNF-α, TGF- α and IL-1β factors caused the activation of IL6/STAT3 signalling pathways and in other instances MAPK pathways, which are quite critical to initiation of regeneration. Carvacrol also reportedly reduced mitochondrial injury and as such promoted anti-apoptotic signalling via phosphorylation of MAPK ERK and Akt-eNOS pathways, in a characteristic fashion typical of aspirin.

These biological activities of carvacrol in activating several cell signalling pathways to effect cell survival and death, and its ability to mimic other important pharmacological agents [aspirin] can only be a small indication of potentialities and promises this agent hold. More molecular and cellular level studies involving its possible complexity in cellular signalling and future usability are needed to ascertain its wide course of molecular activity.

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