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The Bioactivity and Toxicological Actions of Carvacrol

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Carvacrol is a monoterpenic phenol produced by an abundant number of aromatic plants, including thyme and oregano. Presently, carvacrol is used in low concentrations as a food flavoring ingredient and preservative, as well as a fragrance ingredient in cosmetic formulations. In recent years, considerable research has been undertaken in an effort to establish the biological actions of carvacrol for its potential use in clinical applications. Results from in vitro and in vivo studies show that carvacrol possess a variety of biological and pharmacological properties including antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, hepatoprotective, spasmolytic, and vasorelaxant. The focus of this review is to evaluate the existing knowledge regarding the biological, pharmacological, and toxicological effects of carvacrol.

Keywords oregano, thyme, herbs, toxicity

INTRODUCTION

Carvacrol (2-methyl-5-(1-methylethyl)-phenol) (Fig. 1) is a monoterpenic phenol, isomeric with thymol, found in many aromatic plants including *Origanum dictammus* (dittany of Crete), *Origanum vulgare* (Greek oregano, wild marjoram), *Origanum majorana* (marjoram), *Thymbra capitata* (Spanish origanum) *Satureja hortensis* (summer savory), *Thymus vulgaris and Thymus zygis* (thyme), *Thymus serpyllum* (white thyme), and *Satureja montana* (winter savory) (Vokou et al., 1993; Ultee et al., 2002; Burt, 2004; De Vincenzi et al., 2004; Monzote et al., 2009; Liolios et al., 2010). Carvacrol can be synthesized by several methods including sulfonation of *para*-cymene followed by alkali fusion (Phillips, 1924), chlorination of alpha-pinene with tert-butyl hypochlorite (Ritter and Ginsburg, 1950) or the aromatization of carvone in the presence of sulfuric acid with an efficient solid acid catalyst (amberlyst 15) (Gozzi et al., 2009). A cleaner and greener process in the chemical synthesis of carvacrol involves alkylation of o-cresol with propylene or isopropyl alcohol over solid acid catalysts (mesoporous silica incorporating persulfated alumina and zirconia [UDCaT-4, UDCaT-5, and UDCaT-6]) where carvacrol can be efficiently obtained with a selectivity up to 82% at an isopropanol conversion of 98% after 2 h over UDCaT-5 at 180°C (Yadav and Kamble, 2009).

Carvacrol is used as a disinfectant, fungicide, and fragrance ingredient in cosmetic formulations (Andersen, 2006). It provides effective control on mosquito populations by reducing the egg hatchability and inducing sterility in mosquitoes (Mansour et al., 2000) as well as it effectively repels mosquitoes in a human forearm assay, with alpha-terpinene and carvacrol showing significantly greater repellency than a commercial formulation, N,N-diethyl-m-methylbenzamide (Park et al., 2005). In dental practice, carvacrol has been used as a substitute for creasote, carbolic acid, and glycerol of thymol in the treatment of odontalgia, sensitive dentine, alveolar abscess, and as an antiseptic in the pulp canals of teeth (Xu et al., 2006).

Carvacrol is generally considered safe for consumption. It has been approved by the Federal Drug Administration for its uses in food and is included by the Council of Europe in the list of chemical flavorings that can be found in alcoholic beverages, baked goods, chewing gum, condiment relish, frozen dairy, gelatin pudding, nonalcoholic beverages, and soft candy (Ultee et al., 1999; De Vincenzi et al., 2004). Carvacrol, either alone or in combination with other naturally occurring organic compounds, is effective in controlling the growth of food spoilage and food-borne pathogenic bacteria and is evaluated as a preservative for a wide variety of food products, including rice, grape tomatoes, grapes, apple juice, semi-skimmed milk, fresh-cut kiwifruit, and honeydew melon (Ultee et al., 1999; Roller and

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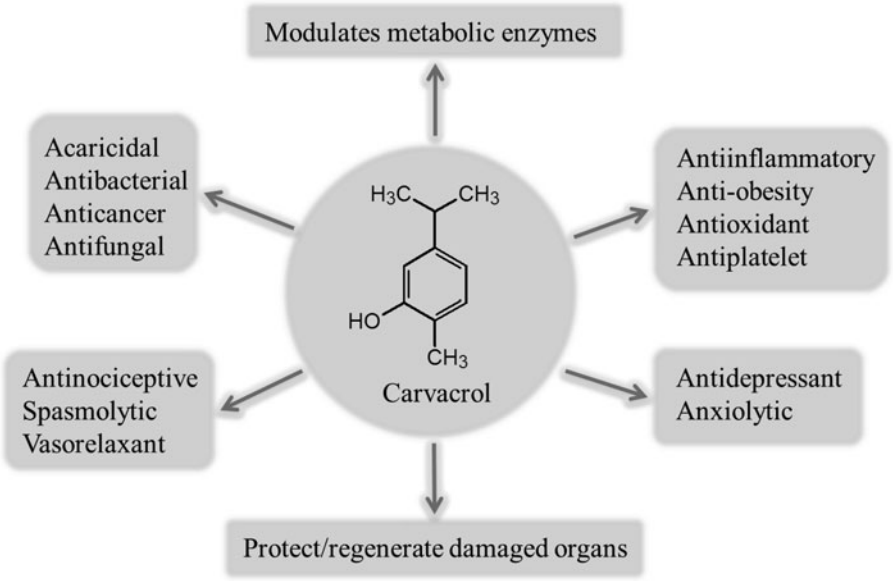

Figure 1 The biological properties of carvacrol. Carvacrol, with the molecular structure inset, and its multiple biological properties.

Seedhar, 2002; Ultee et al., 2002; Ultee et al., 2000b; Olasupo et al., 2004; Kisko and Roller, 2005; Guillen et al., 2007; Lu and Wu, 2010).

Feed supplementation with carvacrol has been shown to improve the quality of poultry meat by inhibiting tissue lipid oxidation known as the major deterioration process affecting both the sensory and nutritional quality of foods (Luna et al., 2010). Feed supplementation may be a simple and convenient strategy to introduce lipid-soluble antioxidants into the phospholipid membrane tissues, where they can effectively inhibit the oxidative reactions at their localized sites (Lauridsen, 1997). Also, concerns regarding the safety of synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisole have encouraged more detailed studies of plant constituents such as carvacrol (Ferguson, 2001).

In addition to the increasing interest of the use of carvacrol as a flavoring constituent and natural preservative for foods, considerable research has been undertaken in the past few years in an effort to establish the biological actions of carvacrol for its potential use in clinical applications. The focus of this review is to evaluate the existing knowledge regarding the biological, pharmacological, and toxicological properties of carvacrol (Table 1).

Absorption, Distribution, Metabolism, and Excretion of Carvacrol

Carvacrol appears to be slowly absorbed from the intestines of rabbits following oral administration of 1.5 g with some 30% remaining in the gastrointestinal tract and about 25% of the dose being excreted in urine 22 h postadministration (Opdyke, 1979). The amount of carvacrol in tissues, blood, urine, and feces measured at 2–24 h after dosing with carvacrol in sesame oil to rats (500 mg) and rabbits (1500 and 5000 mg) by gavage resulted in distribution of carvacrol to the stomach, intestines, and urine with small amounts in lung, liver, and muscle (Schroder and Vollmer, 1932).

Results from a study examining the metabolism of the isomeric phenols, carvacrol and thymol, in male Wistar rats by gas chromatographic-mass spectrometric methods showed that the urinary excretion of metabolites was rapid with only very small amounts being excreted after 24 h and no metabolites detected in the 48- to 72-h posttreatment sample (Austgulen et al., 1987). Although large quantities of carvacrol and, especially thymol, were excreted unchanged (or as their glucuronide and sulfate conjugates), extensive oxidation of the methyl and isopropyl groups also occurred resulting in the formation of derivatives of benzyl alcohol and 2-phenylpropanol and their corresponding carboxylic acids. Ring hydroxylation of the two phenols was a minor reaction (Austgulen et al., 1987). A recent study showed that carvacrol is the substrate of the enzyme UDP-glucuronosyltransferase isoform UGT1A4 (Smith et al., 2003; Dong et al., 2011).

Biologic, Pharmacologic, and Toxic Effects of Carvacrol

Acute Toxicity of Carvacrol

Information on the toxicology of carvacrol is limited. It has been reported that the median lethal dose of carvacrol in rats is 810 mg/kg of body weight when administered by oral gavage (Hagan et al., 1967) while the median lethal dose of carvacrol administered intravenously or intraperitoneally to mice has been estimated at 80.00 and 73.30 mg/kg body weight, respectively (Andersen, 2006). Mice receiving 33.3 mg/kg carvacrol (intraperitoneal) showed no adverse effects, and at a dose

Table 1 Significant findings of carvacrol in *in vitro* and *in vivo* studies and their biological properties

Biological purpose	Significant carvacrol finding(s)	Reference(s)
Antibacterial	- Inhibits *E. coli* and *L. monocytogenes* biofilms	(Perez-Conesa et al., 2011)
	- Inhibits biofilm formation and reduces preformed biofilms of *S. typhimurium* and *S. aureus*	(Rivas et al., 2010)
	- Susceptibility of Verocytotoxigenic *E. coli* increases with decreasing temperature, water activity, and pH	(Ravishankar et al., 2010)
	- Active against *S. enterica* on celery and oysters	(Rattanachaikunsopon and Phumkhachorn, 2010)
	- Active against *V. cholerae* in carrot juice	(Nostro et al., 2009)
	- Eradicates preformed *S. aureus* and *S. epidermidis* biofilms	(Wong et al., 2008)
	- Effective against *M. avium* subsp. *Paratuberculosis*	(Ravishankar et al., 2008)
	- Effective against resistant *C. jejuni*	(Lee and Jin, 2008)
	- Effective against *E. sakazakii*	(Cox and Markham, 2007)
	- Effective against resistant *P. aeruginosa*	(Gill and Holley, 2006)
	- Effective against *E. coli, L. monocytogenes,* and *L. sakei*	(Di Pasqua et al., 2006)
	- Influences long chain unsaturated fatty acids of *S. typhimurium* and *B. thermosphacta*	(Knowles et al., 2005)
Antifungal	- Inhibition of ergosterol biosynthesis and the disruption of membrane integrity	(Ahmad et al., 2011)
	- Eradicates Candida biofilms	(Dalleau et al., 2008)
	- Exerted an anticandidal effect in oral candidiasis model	(Chami et al., 2005)
	- Exerted an anticandidal effect in vaginal candidiasis model	(Chami et al., 2004)
Acaricidal	- Activity against *A. craccivora* and *L. separate*	(Tang et al., 2011)
	- Activity against *C. quinquefasciatus* larvae and *M. domestica*	(Pavela, 2011)
	- Activity against the two-spotted spider mite (*T. urticae* Koch)	(Cavalcanti et al., 2010)
Anticancer	- Anticarcinogenic properties against MDA-MB 231 breast cancer cell lines	(Arunasree, 2010)
	- Anticarcinogenic and antiproliferative properties against Leiomyosarcoma	(Karkabounas et al., 2006)
	- Anticarcinogenic properties against A549 lung cancer cell lines	(Koparal and Zeytinoglu, 2003)
Antioxidant	- Prevents diethylnitrosamine-induced liver cancer in rats	(Jayakumar et al., 2011)
	- Antioxidant effect against Galactosamine-induced hepatotoxicity	(Aristatile et al., 2009b; Aristatile et al., 2011)
	- Protects against DNA damage	(Slamenova et al., 2007; Slamenova et al., 2008; Slamenova et al., 2011)
Antiplatelet	- Induces a reduction in thromboxane A2 production	(Karkabounas et al., 2006)
Anti-inflammatory	- Inhibition of inducible cyclooxygenase-2	(Landa et al., 2009; Hotta et al., 2010)
Antinociceptive	- Reduces pain in mice	(Guimaraes et al., 2010)
Anti-obesity	- Prevents high-fat diet induced obesity	(Cho et al., 2011)
Antidepressant/Anxiolytic	- Contains antidepressant-like and anxiolytic properties on mice	(Melo et al., 2010; Melo et al., 2011)
Organ protection & regeneration	- Increases rat liver regeneration rate after partial hepatectomy	(Uyanoglu et al., 2008)
	- Induces collagen production	(Lee et al., 2008)
	- Protects the liver against ischemia and reperfusion injury	(Canbek et al., 2008)
Metabolic enzyme modulations	- Inhibits UDP-glucuronosyltransferases metabolizing enzymes	(Dong et al., 2011)
	- Increases the activities of xenobiotic-metabolizing enzymes	(Sasaki et al., 2005)
Spasmolytic	- Contains inhibitory effects on muscarinic receptors on guinea pig trachea	(Boskabady et al., 2011)
	- Contains strong acetylcholinesterase inhibitory properties *in vitro*	(Jukic et al., 2007)
Vasorelaxant	- Induces relaxation in rat isolated aorta	(Peixoto-Neves et al., 2010)
	- Induces arterial relaxation	(Earley et al., 2010)
Microcapsule encapsulation for Antibiotic delivery	- Encapsulation in flexible films inhibits *E. coli, S. aureus, L. innocua, Saccharomyces cerevisiae,* and *Aspergillus niger*	(Guarda et al., 2011)
	- Encapsulation in liposomes improves stability and loading	(Coimbra et al., 2011)
	- Carvacrol-loaded chitosan nanoparticles show antimicrobial activity against *S. aureus, Bacillus cereus,* and *E. coli*	(Chen et al., 2009; Keawchaoon and Yoksan, 2011)
	- Encapsulation in calcium-alginate hydrogels inhibits *E. coli*	(Wang et al., 2009)
	- Encapsulation in surfactant micelles inhibits *E. coli* and *L. monocytogenes*	(Gaysinsky et al., 2005; Perez-Conesa et al., 2006)

of 50 mg/kg had nonspecific effects and slight ataxia but at higher doses (110–233.3 mg/kg carvacrol) mice experienced ataxia, decreased spontaneous motor activity, and somnolence prior to death (Andersen, 2006). The LD_{50} in rabbits following dermal application has been estimated at 2700 mg/kg (McOmie et al., 1949). The LD_{50} following subcutaneous administration of carvacrol in mice has been estimated at 680 mg/kg (Andersen, 2006). In dogs, the lethal dose of intravenously administered carvacrol was 0.31 g/kg (Coujolle and Franck, 1944; Andersen, 2006).

Genotoxic Effects

Carvacrol as a flavoring ingredient is usually present in low concentrations in human food. Its potential usefulness in clinical applications will necessitate higher doses of exposure than those used by food industry and this becomes a matter of concern. Toxicity studies are limited and very few have been performed on the mutagenicity, genotoxicity, and clastogenic effects of carvacrol. Preliminarily assessment of the genotoxicity of carvacrol has been carried out by using a number of short-term assays such as SOS chromotest, DNA repair test, and the Ames test. The genotoxic potential of carvacrol at non-toxic doses has been suggested to be weak in the DNA-repair test and the SOS-chromotest (inhibitory concentration IC_{50} of carvacrol in HepG2 cells was 200 μM) (Stammati et al., 1999). The investigators reported that carvacrol increased the number of revertants 1.5- to 1.7-fold regardless of a metabolic activation, but this increase was considered insignificant (Stammati et al., 1999).

Carvacrol at concentrations up of 25 μM in V79 Chinese hamster lung fibroblast cells did not cause any DNA damaging effects as measured by the comet assay (Ündeger et al., 2009). In comet assays with human lymphocytes, major thyme constituents (thymol, carvacrol, and γ-terpinene) did not induce DNA strand breakage at concentrations lower than 50–100 μM, but only at higher concentrations (Aydin et al., 2005b). At non-toxic concentrations, these compounds were also found to protect against DNA damage induced in lymphocytes by peroxide or by proven genotoxins (i.e., 2-amino-3-methylimidazo[4,5-f]-quinoline and mitomycin C) (Ipek et al., 2003; Aydin et al., 2005a, 2005b). Carvacrol showed antigenotoxic activity against H_2O_2-induced genotoxicity in human colonic Caco-2, hepatoma HepG2 and leukemic K562 cell lines (Horvathova et al., 2007; Slamenova et al., 2007). Also, the antigenotoxic effects of carvacrol were demonstrated in *in vivo* and *ex vivo* studies where carvacrol administration alone did not have any effect on DNA damage but protected against DNA damage in the hepatocytes of D-galactosamine (D-GalN)-treated rats (Aristatile et al., 2011) or primary hepatocytes exposed to visible-light excited methylene blue (VL+MB) (Slamenova et al., 2011).

Toxic Effects of Carvacrol on Mitochondria

Mitochondria are recognized as subcellular organelles that are essential for generating the energy that fuels normal cellular function while, at the same time, they monitor cellular health and cellular outcome through the control of apoptosis and autophagy (Moreira and Oliveira, 2011). The electron transport chain is central to the energy metabolism of the cell. In higher organisms, the electron transport chain in the inner mitochondrial membrane is composed of four integral membrane enzyme complexes: NADH:ubiquinone oxidoreductase (Complex I), succinate:ubiquinone oxidoreductase, ubiquinol:cytochrome *c* oxidoreductase (Complex III), and cytochrome *c* oxidase (Complex IV), which reduces oxygen to water. The energy released in this transport of electrons is used to generate a proton gradient across the inner mitochondrial membrane, which is consumed by ATP synthase (Complex V) for ATP production (oxidative phosphorylation). Carvacrol exhibited a complex I inhibition in the low micromolar range (Monzote et al., 2009). Previous studies have shown that mitochondrial respiratory chain complex I inhibition by rotenone resulted in apoptosis, perhaps by increasing the production of reactive oxygen species (ROS) and decreasing mitochondrial membrane potential (Li et al., 2003; Koopman et al., 2010). In studies examining the antiproliferative effects of carvacrol on a human metastatic breast cancer cell line (MDA-MB 231) or a human non-small cell lung cancer (NSCLC) cell line (A549) it was shown that carvacrol induced apoptosis associated with changes such as decreases in mitochondrial potential, release of cytochrome c from mitochondria, caspase activation and cleavage of PARP, cytoplasmic blebbing and irregularity in shape (Koparal and Zeytinoglu, 2003; Arunasree, 2010).

Carvacrol Modulates Intracellular Calcium Homeostasis

Transient receptor potential (TRP) channels are essential components of biological sensors that detect changes in the environment in response to a myriad of stimuli. TRP channels are Ca^{2+} permeable and are critically involved in: photoreception, pheromone sensing, taste perception, thermosensation, pain perception, mechanosensation, perception of pungent compounds, renal Ca^{2+}/Mg^{2+} maintenance, smooth muscle, tone and blood pressure regulation (Freichel and Flockerzi, 2007). Carvacrol has been shown to modulate certain Ca^{2+}-permeable TRP channels (Parnas et al., 2009). In the endothelium of intact arteries, carvacrol activated the TRP cation channels TRPV3 resulting in relaxation (Earley et al., 2010). Elevated Ca^{+2} concentration has also been measured in primary mouse corneal epithelial cells, cultured human corneal epithelial cells (HCE-T cells), and mouse epithelial cells of the distal colon following carvacrol activation of the TRP vanilloid 3 (TRPV3) (Ueda et al., 2009; Yamada et al., 2010), a member of the calcium-permeable thermosensitive TRP subfamily of receptors.

TRPV3 channels are also expressed in skin keratinocytes and activated by innocuous thermal heating, organic chemicals such as 2-aminoethyoxy diphenylborinate and plant-derived compounds such as carvacrol and camphor (Xu et al., 2006; Doerner et al., 2011). TRPV3 in keratinocytes modulates sensory thermotransduction, hair growth, and susceptibility to dermatitis in rodents (Doerner et al., 2011). In organ culture, TRPV3 channel activation by plant-derived or synthetic agonists resulted in a dose-dependent inhibition of hair shaft elongation, suppression of proliferation, and induction of apoptosis and premature human organ-cultured hair follicles regression while stimulation of human outer root sheath keratinocytes induced membrane currents, elevated intracellular calcium concentration, inhibited proliferation, and induced apoptosis suggesting that TRPV3 and the related intracellular signaling mechanism might function as a promising target for pharmacological manipulations of clinically relevant hair growth disorders (Borbiro et al., 2011).

Carvacrol has been shown to inhibit sarcoplasmic reticulum Ca^{2+} ATPase and activated ryanodine receptors in skeletal muscle (Sarkozi et al., 2007). Exposure of isolated canine ventricular myocytes to carvacrol suppressed L-type Ca^{2+} current in a concentration-dependent manner, a treatment effect attributed to the hydrophobic character of the phenol affecting, at least in part, the lipid-protein interface and altering the local environment of ion channels (Magyar et al., 2004).

Effects of Carvacrol on the Detoxification System

Uridine-5′-diphospho-glucuronosyltransferases isoforms (UGTs) are enzymes involved in the metabolism of many drugs [e.g., morphine and paracetamol (acetaminophen)] and also capable of the biotransformation of important endogenous substrates (e.g., bilirubin and ethinylestradiol) and several xenobiotics (de Wildt et al., 1999). Inhibitory effects of compounds on UGTs are clinically important because inhibition of UGT isoforms could not only result in serious drug-drug interactions, but also induce metabolic disorders of endogenous substances (Dong et al., 2011). *In vitro* studies utilizing human liver microsomes and the non-selective substrate 4-methylumbelliferone showed that carvacrol can competitively inhibit the activity of UGT1A9-mediated glucuronidation with negligible effects on the other UGTs (Dong et al., 2011). UGT1A9 is one of the most important UGT isoforms in humans and is involved in the metabolism of many drugs including bulky phenols, flavonoids, and anthraxquinones (Radominska-Pandya et al., 2005). When the specific probe substrate, propofol (anesthetic drug), was employed to determine the carvacrol-induced inhibitory kinetics of UGT1A9, the results demonstrated that the inhibitory type was noncompetitive (Le Guellec et al., 1995; Dong et al., 2011). Although not directly extrapolated to the *in vivo* situation, these preliminary results suggest that such interactions are theoretically possible. To properly address any interaction between carvacrol and other drugs, both *in vitro* and *in vivo* drug-drug interaction studies must be pursued since extrapolations from *in vitro* experiments are encountered with uncertainties leading to an inaccurate prediction of an *in vivo* drug interaction (Wienkers and Heath, 2005).

Antiobesity Effects

Obesity, defined as abnormal or excessive fat accumulation, is a leading cause of preventable illness and death (Cepeda-Valery et al., 2011). In a preliminary study, carvacrol supplemented to a high-fat diet (HFD) at 0.01%, 0.05%, and 0.1% levels for 28 days exhibited a dose-dependent reduction in the body weight of mice. In a follow-up study, after 10 weeks of feeding with 0.1% carvacrol supplemented in the diet (equivalent to 100 mg/kg body weight), the body weight gain, and visceral fat-pad weights of the carvacrol-supplemented diet group were significantly lower than that of HFD mice and this treatment effect was not due to changes in food intake as the daily food intake during the entire feeding period did not differ among groups (Cho et al., 2011). It has been postulated that dietary carvacrol reduced the body weight of mice through modulation of adipogenesis and thermogenesis in visceral adipose tissues probably by suppressing bone morphogenic protein-, fibroblast growth factor 1-, and galanin-mediated signaling and also, by attenuating the production of proinflammatory cytokines in visceral adipose tissues by inhibiting toll like receptor 2 (TLR2)- and TLR4-mediated signaling (Cho et al., 2011). Similarly, resveratrol, a natural polyphenolic stilbene derivative found in a variety of edible fruits, including nuts, berries, and grape skin, inhibited visceral adipogenesis by suppressing the galanin-mediated adipogenesis signaling cascade and attenuating cytokine production in the adipose tissue by repressing the TLR2- and TLR4-mediated proinflammatory signaling cascades in HFD-fed mice (Kim et al., 2011).

Vasorelaxant Effects

Carvacrol induces vasorelaxant effects in rat aortic preparations through its actions on both electromechanical and pharmacomechanical coupling. Carvacrol induced an endothelium-independent relaxation in rat isolated aorta, an effect that seems to be mediated through mechanisms probably involving a transduction pathway between Ca^{2+} release from sarcoplasmic reticulum and/or regulation of the Ca^{2+} sensitivity of the contractile system. Moreover, it is conceivable that carvacrol, at low concentrations, blocked the Ca^{2+} influx through the membrane (Peixoto-Neves et al., 2010).

Diets including a reasonable amount of oregano improve vascular health by promoting endothelium-dependent arterial relaxation and reduction of vascular resistance and systemic blood pressure. Using intact cerebral and cerebellar arteries and freshly isolated endothelial cells from these vessels, it was shown that the vasodilatory actions of carvacrol were mediated through TRPV3 channels present in the endothelium. Increases in endothelial intracellular [Ca^{2+}], through activation of the TRPV3 channels activate intermediate-conductance (IK_{Ca}) basolateral potassium channels and small conductance calcium (SK_{Ca})-activated potassium channels, hyperpolarizing the plasma membrane of endothelial cells and underlying smooth muscle. Inwardly rectifying potassium channels (K_{IR}) channels, specific subset of potassium selective ion channels, in smooth muscle cells amplify this initial hyperpolarization, ultimately resulting in vasodilation. These findings suggest that activation of TRPV3 channels in the endothelium may improve vascular function by promoting arterial relaxation. Furthermore, these results provide a novel mechanistic basis for investigating the cardioprotective benefits of a diet that includes reasonable amounts of oregano (Earley et al., 2010).

The calcium antagonism exerted by the *Origanum compactum* extracts, with thymol and carvacrol being the major components, has been reported by other investigators who showed that the extracts inhibited responses to acetylcholine, histamine, serotonin, 1,1-dimethyl-4-phenylpiperazinium iodide, and nicotine in the guinea-pig ileum with the muscle

relaxant effect being attributed to the a decrease of Ca^{2+} availability for muscle contraction by blocking the release of intracellular bound Ca^{2+} and by the prevention of the extracellular Ca^{2+} influx in the smooth muscle cell (Van Den Broucke and Lemli, 1980; 1982).

Spasmolytic Effects

Carvacrol has been shown to reduce contractions elicited by acetylcholine in guinea pig ileum (Van Den Broucke and Lemli, 1980) and can act as a noncompetitive antagonist against ileum contractions induced by carbachol, histamine, 1,1-dimethyl-4-phenylpiperazinium iodide, and $BaCl_2$ (Van Den Broucke and Lemli, 1982). Also, the l-noradrenaline contractions on the rat vas deferens were reduced by carvacrol (Van Den Broucke and Lemli, 1982). Carvacrol has a potent relaxant effect on tracheal chains of guinea pigs which is not due to beta2-adrenergic stimulatory, histamine H1, and muscarinic blocking effect (Boskabady and Jandaghi, 2003). Inhibition of the nerve action potential in the postganglionic nerve fiber is proposed to be the indirect action of spasmolytic activity (Van Den Broucke and Lemli, 1980).

Hepatoprotective Effects

Carvacrol affords a significant hepatoprotective and hypolipidemic effect against D-GalN-induced-rats. D-GalN is a well-established hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis with oxidative stress playing a vital role in the pathogenesis of D-GalN associated liver injury (Decker and Keppler, 1972). The hepatotoxic and hypolipidemic effects of D-GalN in rats (shown by the elevation in the serum bilirubin level and the serum activities of the hepatic marker enzymes aspartate aminotransferase, alanine aminotransferase; the increased plasma levels of very low density lipoprotein cholesterol and low-density lipoprotein (LDL) cholesterol and decreased high density lipoprotein cholesterol; and, increase in the levels of total cholesterol, phospholipids, triglycerides, and free fatty acids in the plasma and tissues of liver and kidney) were abrogated by a 21-day carvacrol treatment (Aristatile et al., 2009a). The hepatoprotective effects of carvacrol against D-GalN were associated with the capacity of carvacrol to preserve the structural integrity of the hepatocellular membrane and the oxidant-antioxidant balance in the liver (Aristatile et al., 2009b; Aristatile et al., 2011). The protective effects of carvacrol were also demonstrated in a study where carvacrol protected against ischemia/reperfusion-induced liver injury (Canbek et al., 2008).

Anticancer and Antiproliferative Activity

Carvacrol possesses anticarcinogenic and antiproliferative properties. It has been shown that the tumor incidence in Wistar rats treated with the carcinogen 3,4-benzopyrene (B[a]P) that was incubated with carvacrol in comparison with the tumor incidence in animals treated with B[a]P alone was 30% lower with a significant prolongation of animal survival time (Karkabounas et al., 2006). The mechanism(s) for the observed decrease of B[a]P carcinogenic potency is not clear but it was attributed to carvacrol's antioxidant properties responsible for scavenging ROS and B[a]P-diol-epoxides known to be produced during the metabolic activation of B[a]P. These B[a]P metabolites reveal mutagenic effects and function as complete carcinogens (tumor initiators and promoters). It has also been hypothesized that carvacrol incubated with B[a]P may neutralize B[a]P by reducing the double bonds that are responsible for its carcinogenic properties (Karkabounas et al., 2006). In another study, carvacrol supplementation (15 mg/kg body weight) significantly attenuated the diethylnitrosamine (DEN)-induced liver cancer in male Wistar albino rats most likely by protecting the antioxidant defense system and preventing lipid peroxidation and hepatic cell damage (Jayakumar et al., 2011). In an *in vitro* study examining the antiproliferative and proapoptotic effects of carvacrol on the human hepatocellular carcinoma cell line HepG2, it was shown that carvacrol inhibited HepG2 cell growth by inducing apoptosis via the activation of caspase-3, cleavage of PARP, and decreased Bcl-2 gene expression as well as selectively altering the phosphorylation state of members of the MAPK superfamily, decreasing phosphorylation of ERK1/2 in a dose-dependent manner, and activating phosphorylation of p38 but not affecting JNK MAPK phosphorylation (Yin et al., 2012).

Carvacrol, which was obtained by fractional distillation of *Origanum onites L*, inhibited the formation of lung tumors induced by 7,12-dimethylbenze-alpha-anthracene in rats, a treatment effect attributed to disturbances in calcium homeostasis or perhaps inhibition of angiogenesis (Zeytinoglu et al., 1998). Carvacrol has been shown to reduce the growth of murine B16 melanomas (He et al., 1997), human A549 non-small lung cancer (Koparal and Zeytinoglu, 2003) and human erythroleukaemic K562 cell lines (Lampronti et al., 2006). Further investigation of carvacrol as an anticancer and antiproliferative molecule is warranted.

Analgesic and Antiinflammatory Effects

Carvacrol has been shown to possess anti-inflammatory activity due to its ability to suppress the expression of cyclooxygenase (COX)-2, activate the peroxisome proliferator-activated receptors (PPAR) α and γ (Hotta et al., 2010), and inhibit the production and actions of nitric oxide (NO). COX-2, the rate-limiting enzyme in prostaglandin biosynthesis, plays a key role in inflammation, pain, and circulatory homeostasis. PPARs are ligand-dependent transcription factors belonging to the nuclear receptor superfamily that regulate energy homeostasis, lipid and carbohydrate metabolism, cell proliferation and differentiation, and inflammation (Yessoufou and Wahli, 2010). Agonists of PPAR receptors inhibit the expression of mRNA for COX-2 and NO synthase and consequently, the production of prostanoids and NO (Fehrenbacher et al., 2009; Maeda and Kishioka, 2009). It has been shown that carvacrol suppressed lipopolysaccharide

(LPS)-induced COX-2 mRNA and protein expression in differentiated macrophage-like U937 cells and in bovine aortic endothelial cells (BAEC) activated PPARα and γ (Hotta et al., 2010). The production of NO, a mediator of inflammation, by intact murine peritoneal macrophages stimulated by LPS was inhibited by carvacrol probably due its ability to activate PPAR leading to the inhibition of NF-κB transcription and subsequently to a decrease in the iNOS levels (Moraes et al., 2006; Guimaraes et al., 2010; Guimaraes et al., 2011).

Carvacrol has been shown to inhibit the mechanical hypernociception induced by carrageenan, an algae mucopolysaccharide used to induce inflammation and inflammatory pain (Guimaraes et al., 2011). Mice treated with carvacrol (50 or 100 mg/kg; i.p.) or the known analgesic indomethacin (10 mg/kg, ip) 30 min prior to carrageenan administration in the subplantar region of the mouse paw exhibited a significant reduction in mechanical hyper-nociception with levels being comparable to those observed following indomethacin administration. Although the precise mechanism(s) for this effect is not known, it appears to be related to its action on neutrophil recruitment, release of TNF-α and NO, and consequently decrease in production of inflammatory/hypernociceptive metabolites, probably PGE_2 or others (Guimaraes et al., 2011). In separate experiments, it was shown that carvacrol pretreatment conferred protection against mechanical hypernociception induced by TNF-α, but not dopamine and PGE_2, all of which are important mediators of carrageenan signaling cascade. Furthermore, carvacrol reduced the recruitment of leukocytes and TNF-α production in carrageenan-induced pleurisy and inhibited the generation of NO in murine macrophages (Guimaraes et al., 2011). The analgesic properties of carvacrol mediated via inhibition of peripheral mediators (as prostaglandin synthesis) as well as central inhibitory mechanisms (nonopioid central receptors) which could be related to its strong antioxidant activity have been demonstrated in several other *in vivo* nociception models where pretreatment of mice with carvacrol was effective in alleviating pain against acetic acid induced writhing and stretching and the neurogenic and inflammatory phases of formalin-induced pain (Guimaraes et al., 2010).

Antioxidant Effects

It is well known that essential oils, which are rich in carvacrol, possess strong antioxidant properties (Alma et al., 2003; Radonic and Milos, 2003; Sokmen et al., 2004; Tepe et al., 2004; Karioti et al., 2006) equivalent to those of ascorbic acid, butyl hydroxyl toluene and vitamin E (Aeschbach et al., 1994; Miguel et al., 2003; Mastelic et al., 2008). Carvacrol decreased peroxidation of phospholipid liposomes in the presence of iron(III) and ascorbate and were found to be good scavengers of peroxyl radicals (CCl_3O_2) generated by pulse radiolysis (Aeschbach et al., 1994). Carvacrol inhibited LDL oxidation in a concentration dependent manner in an *in vitro* system using human aortic endothelial cells to mediate the oxidation of LDL over a 12-h incubation period (Teissedre and Waterhouse, 2000). NO, a molecule that plays an important role in various types of inflammatory processes, generated from the spontaneous decomposition of sodium nitroprusside was effectively scavenged by carvacrol (Guimaraes et al., 2010). Overall, phenolic antioxidant phytochemicals have been recently implicated for the lower rates of cardiac disease mortality among people consuming a Mediterranean diet (Teissedre and Waterhouse, 2000).

Assessment of the antioxidant capacity of thymol, carvacrol and γ-terpinene in relation to that of the synthetic antioxidant trolox in the trolox equivalent antioxidant capacity assay showed that both carvacrol and thymol were found to have significant antioxidant activity similar to trolox whereas γ-terpinene which lacks a phenolic group showed no antioxidant activity between 1 and 100 μM and at all concentrations tested the antioxidant capacity of carvacrol was found to be significantly higher than the same concentration of its isomer thymol (Ündeger et al., 2009; Guimaraes et al., 2010). However, in incubations where V79 cells exposed to H_2O_2, carvacrol was less efficient in sequestering ROS at lower concentrations (1–25 μM) and even enhanced ROS production at the highest 100 μM concentration (Ündeger et al., 2009). It is not uncommon for phenolic compounds that both an antioxidant and a pro-oxidant activity are observed at different doses (Ferguson, 2001).

The antioxidant properties of carvacrol have also been demonstrated in a limited number of *in vivo* studies. For example, the resistance against hydrogen peroxide-induced DNA damage in hepatic and testicular tissues was higher in rats given carvacrol in drinking water (at 30 and 60 mg/kg for 7 days or 15 and 30 mg/kg for 14 days) (Slamenova et al., 2008). The potent free radical scavenger activity of carvacrol was also demonstrated in a study examining the protective effects of orally administered carvacrol against the *N*-nitroso compound, *N*-Nitrosodiethylamine (DEN) a potent hepatocarcinogen where carvacrol was capable of preventing lipid peroxidation, and significantly increasing the endogenous antioxidant defense mechanisms in DEN-induced hepatocellular carcinogenesis (Jayakumar et al., 2011). In another study, broiler chickens were fed 150 mg/kg of carvacrol and the oxidative deterioration of polyunsaturated fatty acids in the breast and thigh samples were measured at 0, 5, and 10 days at 4°C by the analysis of thiobarbituric acid reactive substances (TBARS) (by-products of lipid peroxidation). Sample storage significantly increased the levels of TBARS, and although feed supplementation with carvacrol did not significantly affect breast sample oxidation, increasingly higher values of TBARS were detected in thigh samples of the control group in comparison to carvacrol supplemented group at 5 and 10 days of storage. These data suggest that the application of carvacrol could be useful to improve poultry meat quality by inhibiting oxidative reactions (Luna et al., 2010).

Antibacterial Effects

Carvacrol exerts a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria

isolated from food and clinical isolates. For example, it has been shown that carvacrol exerts bacteriostatic and bactericidal activities against *Vibrio cholerae*, *Campylobacter jejuni*, *Escherichia coli Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus, Staphylococcus epidermidis, Lactobacillus sakei, Pseudomonas aeruginosa, Pseudomonas putida*, *Streptococcus mutans, and Bacillus subtilis* (Lambert et al., 2001; Friedman et al., 2002; Mathela et al., 2010; Rattanachaikunsopon and Phumkhachorn, 2010; Ravishankar et al., 2010; Rivas et al., 2010). Carvacrol distributes into membranes to permeabilize them thus disrupting ion gradients (Burt, 2004). Indeed, measurement of the average phase transition temperature of the bacterial lipids confirmed that membranes instantaneously became more fluid in the presence of carvacrol (Ultee et al., 2000a). However, hydrophobicity alone does not ensure toxicity, since p-cymene, a precursor of carvacrol, has a higher partition coefficient for lipid membranes but is nontoxic. The presence of a free hydroxyl group and a delocalized electron system is critical for antibacterial activity (Ultee et al., 2002). It has been proposed that the structure of carvacrol would allow the compound to act as a transmembrane carrier of monovalent cations by exchanging its hydroxyl proton for a potassium ion, thereby reducing the gradient across the cytoplasmic membrane. Accordingly, it was shown that in B. cereus, carvacrol reduced cytoplasmic membrane potential, decreased intracellular pH, inhibited ATP synthesis and stimulated leakage of potassium (K^+) (Ultee et al., 2002). K^+ acts as a cytoplasmic-signaling molecule, activating and/or inducing enzymes and transport systems that allow the cell to adapt to elevated osmolarity (Epstein, 2003). The collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death (Ultee et al., 2002).

Carvacrol has been shown to increase the sensitivity of multidrug-resistant *S. enterica* serovar Typhimurium to antibiotics (Johny et al., 2010). It has been suggested that carvacrol, due to its hydrophobic characteristics, it primarily targets the lipid-containing bacterial plasma membrane, making the membrane in *S. enterica* Typhimurium more permeable (Ultee et al., 2000a; Ultee et al., 2002). This change in permeability permits an increased uptake of antibiotics by the bacterial cell (Johny et al., 2010).

Carvacrol has been recently investigated for its effects on bacterial biofilms. A biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and extracellular DNA. Bacterial biofilms are resistant to antibiotics, disinfectant chemicals and to phagocytosis and other components of the innate and adaptive inflammatory defense system of the body (Hoiby et al., 2011). Carvacrol is able to inhibit the growth of preformed biofilm and to interfere with biofilm formation on stainless-steel surfaces (Knowles and Roller, 2001). In a study, it was shown that carvacrol was an effective natural intervention to control dual-species biofilm formation (*S. aureus* and *S. enterica* serovar Typhimurium), although complete eradication of the entire population was not achieved (Knowles et al., 2005). Carvacrol at subinhibitory concentrations attenuated biofilm formation of *S. aureus* and *S. epidermidis* strains on polystyrene microtitre plates (Nostro et al., 2007).

Although carvacrol is a hydrophobic compound, it has been reported to possess relative hydrophilicity (water solubility of 830 ± 10 ppm) (Griffin et al., 1999). Because of this amphipathic nature, it was hypothesized that the relative hydrophilicity may allow the diffusion of carvacrol through the polar polysaccharide matrix of biofilms, whilst the prevalent hydrophobic properties of carvacrol could lead to specific interactions with the bacterial membrane with considerable effects on its structural and functional properties such that it would lose its integrity (Nostro et al., 2009). To improve the effectiveness of carvacrol on bacterial biofilm, surfactant-encapsulated carvacrol, as a new means of a delivery system, was used against *E. coli* and *L. monocytogenes* cells aggregated in a biofilm and was found to be highly effective (Perez-Conesa et al., 2006). Specifically, carvacrol-loaded micelles (0.3–0.7%) inhibited the solid/air biofilm interface within 3 h of exposure (Perez-Conesa et al., 2006). More recently, carvacrol encapsulated in poly(dl-lactide-co-glycolide nanocapsules produced a considerable reduction in the elasticity and mechanical stability of preformed *S. epidermidis* biofilms that could enable the penetration of antimicrobial agents into the deep core of bacterial biofilms (Iannitelli et al., 2011).

Antifungal Effects

Carvacrol has been shown to exert antifungal activity against *Candida albicans, Saccharomyces cerevisiae*, and strawberry anthracnose-causing fungal plant pathogens (*Colletotrichum acutatum, C. fragariae, and C. gloeosporioides*) (Pina-Vaz et al., 2004; Vardar-Unlu et al., 2010). Carvacrol and other terpenoid phenols have been shown to be efficacious not only on planktonic cells but also on biofilms of *C. albicans* that are resistant to many antifungal drugs; carvacrol has the strongest antifungal activity against *C. albicans* biofilms, independent of the age of the biofilm (Dalleau et al., 2008). However, specific mechanisms involved in the antimicrobial action of monoterpene remain poorly characterized. In a recent study, it was demonstrated that carvacrol might exert its antifungal effects by mechanisms resembling Ca^{2+} stress and inhibition of the TOR (Target of Rapamycin) pathway. Exposure of *S. cerevisiae* to carvacrol resulted in dose-dependent Ca^{2+} bursts that correlated with antifungal efficacy, changes in pH that were long lasting and followed the Ca^{2+} transients, with up regulation of genes involved in alternate metabolic and energy pathways, stress response, autophagy, and drug efflux (Rao et al., 2010). TOR, as a central regulator of cell growth, plays a key role at the interface of the pathways that coordinately regulate the balance between cell growth and autophagy in response to nutritional status, growth factor and stress signals (Jung et al., 2010). A *vma* mutant lacking functional vacuolar H^+-ATPase (V-ATPase) and defective in ion homeostasis was hypersensitive to carvacrol toxicity,

consistent with a role for ionic disruptions in mediating cell death (Rao et al., 2010).

Recent studies examining the efficacy of carvacrol and thymol on several sensitive and resistant clinical isolates, besides laboratory strains of *Candida*, including isolates of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei,* and *C. glabrata* from all important infection sites (cutaneous, bronchoalveolar, oropharyngeal, and oesophageal, and from cases including candidaemia, vulvovaginal and urinary tract infections in HIV and non-HIV patients) have shown that carvacrol and thymol affect, in addition to membrane integrity, ergosterol biosynthesis, in a dose-dependent fashion to decrease the ergosterol content (Ahmad et al., 2011). Ergosterol, the main sterol in yeast, is responsible for structural membrane features such as fluidity and permeability in a similar way as cholesterol is in mammalian cells. It plays a crucial role in the viability of all fungi; those unable to synthesize ergosterol because of inhibition, growth conditions or mutation must take it up from the environment (Barrett-Bee and Dixon, 1995).

Fungal growth is influenced by a variety of complex intrinsic and extrinsic factors including temperature, time, moisture, gaseous composition, and antimicrobials agents (Lopez-Malo et al., 2005). The antimicrobial activity of essential oils of herbs or their constituents such as thymol and carvacrol could be the result of damage to enzymatic cell systems, including those associated with energy production and synthesis of structural compounds (Conner and Beuchat, 1984), denaturation of enzymes responsible for spore germination, and interference with amino acids involved in germination (Nychas, 1995; Lopez-Malo et al., 2005). Spores from *Aspergillus flavus* ATCC 16872 strain cultivated on potato dextrose agar slants formulated with selected concentrations of natural and synthetic antimicrobials showed that subinhibitory antimicrobial concentrations of carvacrol delayed mold spore germination time of *Aspergillus flavus* perhaps by inactivating essential enzymes, reacting with the cell membrane, or disturbing genetic material functionality (Lopez-Malo et al., 2005).

Germ tube formation by *Candida albicans* has been identified as a cofactor that promotes adherence and Candida adherence has been implicated as the first step in the pathogenesis of oral candidiasis (Ellepola and Samaranayake, 1998). Germ tube formation by two strains of *Candida albicans* (M1 and ATCC 10231) was inhibited with MIC and subinhibitory concentrations of *Thymus* oils (*Thymus vulgaris, Thymus zygis,* and *Thymus mastichina*) and their major components including carvacrol (Pina-Vaz et al., 2004).

Due to its antifungal and antibacterial properties, carvacrol has been used in the development of an oral mucoadhesive controlled-release delivery system also containing tetracycline (Obaidat et al., 2011). Oral diseases are a health problem in immuno-suppressed patients with most infections mainly due to candidiasis and bacterial infections. Carvacrol has been shown to be very active *in vitro* against oral Candida isolates (Marcos-Arias et al., 2011) and a combination of tetracycline and carvacrol showed excellent activity against *C. albicans* and bacterial strains (Escherichia coli [ATCC 8739], Pseudomonas aeruginosa [ATCC 9027], Staphylococcus aureus [ATCC 6538], Bacillus cereus [ATCC 14579], and Bacillus bronchispti [ATCC 4617]). The synergistic effect was attributed to the enhancement of the permeability of tetracycline through the bacterial cell wall. One of the proposed mechanisms for the development of bacterial resistance against tetracycline might be due to decreased antibiotic influx through the bacterial cell wall (Sompolinsky and Samra, 1981).

Insecticidal Effects

Studies examining the toxicity of several phenols and phenolic acids showed that simple phenols caused mortality within 24 h after application to *Culex quinquefasciatus* larvae and *Musca domestica* adults. Lethal doses for acute toxicity of *C. quinquefasciatus* to thymol, carvacrol, 2-ethylphenol, and salicylaldehyde showed high efficiency with lethal doses LD_{50} being estimated as 30, 36, 38, and 43 μg/mL, respectively. Lethal doses for acute toxicity of *M. domestica* adults for thymol, carvacrol, and 2,6-dimethoxyphenol were estimated at 53, 69, and 87 μg/fly, respectively (Pavela, 2011). Carvacrol isolated from the root powder of *Stellera chamaejasme* L. (family Thymelaeaceae), a toxic perennial herb, showed good insecticidal activity against *Aphis craccivora* and *Leucania separata*, pests in agriculture that have developed resistance to conventional synthetic insecticides, such as pyrethroids (Tang et al., 2011).

Nematicidal Effects

Carvacrol has been shown to be effective in killing nematodes including the pine wilt nematode, *Bursaphelenchus xylophilus* (Kong et al., 2007) in the free-living *C. elegans,* and the pig roundworm, *A. suum* (Lei et al., 2010). The nematicidal activity of carvacrol was mediated through TyrR as it can trigger the signaling cascade downstream from the receptor in cells expressing wild-type ER-2. Carvacrol, as well as its isomer thymol, were demonstrated to interact with TyrR in desensitizing SER-2 for tyramine activation in $[Ca^{+2}]_i$ mobilization assay, and in translocating SER-2 from membrane to cytoplasm in receptor internalization assay. Receptor internalization activity of carvacrol and thymol was significantly blocked in cells expressing mutant SER-2 with the S210A/S214A double mutations, thus confirming specificity of the interactions (Lei et al., 2010). Parasitic nematodes are the causal agents of many diseases in human, animals, and plants.

Fumigant Effects

Comparison of the fumigant toxicity of 12 essential oil components [carvacrol, 1,8-cineole, trans-cinnamaldehyde, citronellic acid, eugenol, geraniol, S-(–)-limonene, (–)-linalool, (–)-menthone, (+)-alpha-pinene, (–)-beta-pinene, and thymol] to adult male, adult female, gravid female, and large, medium,

and small nymphs of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) showed that carvacrol was most toxic to medium and small nymphs, with LC_{50} values of 3.6 mg/L air at 24 h (Phillips and Appel, 2010).

Drug Delivery Strategies to Improve Carvacrol's Biological Effects

Complexation of Carvacrol

Since essential oils and their major components for some of their biological effects are targeted to mammalian cells, a concern has been raised regarding their irritant and cytotoxic effects (Manabe et al., 1987; Bimczok et al., 2008). Recent studies have used drug delivery systems to improve the antioxidant and antibacterial activities of carvacrol and reduce its toxicity by grafting carvacrol to chitosan nanoparticles (Chen et al., 2009; Keawchaoon and Yoksan, 2011). Chitosan (CH), a polysaccharide biopolymer from natural sources, has been proposed for use in a number of applications such as wound healing and food packaging because of its special biocompatible, antimicrobial and antibiofilm properties as well as its versatility to be formulated into nanoparticles, fiber or film and membrane (Ray, 2011). Following the introduction of aldehyde groups to carvacrol, the Schiff base reaction was used to graft carvacrol to chitosan nanoparticles. The antioxidant activity of the carvacrol-grafted chitosan nanoparticles (CHCA NPs) assayed with diphenylpicrylhydrazyl was better than that of the unmodified chitosan nanoparticles. Similarly, antibacterial assays carried out with *E. coli* and *S. aureus* showed that the grafted carvacrol conferred an antibacterial activity equivalent to or better than that of the unmodified chitosan nanoparticles. The cytotoxicity of CHCA NPs in a 3T3 mouse fibroblast cell line (minimal concentration giving rise to 50% [MC_{50}] of deaths value 1 mg/mL) was significant lower than the pure carvacrol (MC_{50} value 0.28 mg/mL) (Chen et al., 2009).

Carvacrol shows promise as acaricides against the *V. destructor*, but the delivery of this compound remains a challenge due to the low water solubility and uncontrolled release into the colony. β-cyclodextrins (β-CD) complexation has become an effective method for increasing the solubility of carvacrol and for providing a delivery shuttle to cross-biological membranes. In a study, carvacrol complexed with β-cyclodextrins (β-CD) in order to improve the delivery of the monoterpenoid into the hemolymph of honey bees showed promise to deter Varroa mites (*Varroa destructor*) from feeding from them. *V. destructor* has become ubiquitous worldwide and is a serious threat to honey bees. Currently, the most noted acaricides against *V. destructor* are the pyrethroid class of insecticide fluvaliniate and with the organophosphate coumaphos, but the mites have shown resistance. Also, since these insecticides are used in the proximity of honey, it is desirable to use natural alternatives (Stella and He, 2008). High levels of carvacrol (in the mM range) have been detected in bee tissues without any imposed toxicity to the bees (LeBlanc et al., 2008).

Liposomal and Micellar Formulations of Carvacrol

Most natural compounds have limited stability as they are prone to degradation or are highly metabolized to inactive derivatives in circulation. Furthermore, they generally have a poor solubility and bioavailability (Shoji and Nakashima, 2004). The use of delivery systems may help overcome these issues and liposomes have been extensively studied for such purposes and offer the potential to formulate both hydrophilic and hydrophobic molecules (Suntres, 2011). Furthermore, liposomal encapsulation of natural compounds can increase drug solubility and stability, may allow systemic drug administration, and can enhance drug bioavailability and offer control over the absorption and distribution profiles (Shoji and Nakashima, 2004; Suntres, 2011).

Liposomal formulations of carvacrol could circumvent difficulties related to the lipophilicity to improve its biological activity. High concentrations of carvacrol can disrupt the lipid membrane, and only liposomes with lower carvacrol content (final molar ratio of 0.125, 0.063, and 0.31 drug:total lipid) should be used (Cristani et al., 2007). This restricts the maximum amount to be encapsulated, with a final drug/lipid ratio of 0.002 (<0.1 mg/mL). Similar results were reported in another study, which show that only 4.16% (0.045 mg/mL) of the starting amount of carvacrol could be incorporated in liposomes (Liolios et al., 2009). Thus, the solubility of carvacrol cannot be increased by liposomes prepared from phosphatidylcholine lipids. However, the development of liposomal formulations from different lipids might allow higher levels of carvacrol incorporated in the liposomal membranes. To obtain optimum encapsulation of a drug into a liposomal preparation, parameters influencing both the liposome and the drug need to be carefully considered. Factors that affect encapsulation of drugs in liposomes include liposome size and type, charge on the liposome surface, bilayer rigidity, method of preparation, and characteristics of the drug to be encapsulated (Kulkarni et al., 1995).

In an attempt to increase its solubility and thereby entrapment in the aqueous core of long-circulating liposomes, a phosphate derivative of carvacrol was synthesized. Carvacrol disodium phosphate liposomes were prepared by lipid film rehydration followed by extrusion and were found to be 0.12 μm in diameter and monodisperse (PDI<0.1). The increase in hydrophilicity resulted in successful encapsulation in the liposomal core with a final carvacrol phosphate concentration of 6 mg/mL. Furthermore, a stability study at 37°C for 100 h indicated that the carvacrol derivative was stably entrapped with particle size remaining constant. The derivatization of carvacrol did not change its activity since cells incubated for 2 h with 0.1 mM of the same compounds, followed by heat shock at 42.5°C, carvacrol phosphate co-induced the expression of Hsp70 in BMDC to the same extent as carvacrol. It was concluded that the high drug levels reached in the final formulation are suitable for further

therapeutic *in vivo* evaluation and the long-circulating property of liposomes will likely increase local drug concentrations which may translate in improved therapeutic effect (Coimbra et al., 2011).

In addition to the use of liposomes, micelles have also been employed as drug delivery systems to deliver drugs that are poorly soluble in water. Micelles are small (5–100 nm in diameter) colloidal dispersions that are constructed from amphiphilic molecules such as lipids, which contain a hydrophobic core and a hydrophilic head oriented outwardly. The solubilization of hydrophobic drugs in the hydrophobic core not only ensures its delivery at higher concentrations, but also reduces the common risk of potential drug aggregation following intravenous administration, which can lead to severe adverse side effects arising from complications such as the formation of an embolism (Cukierman and Khan, 2010). In a recent study, carvacrol and eugenol were encapsulated in micellar nonionic surfactant solutions to increase active component concentrations in the aqueous phase which were used to treat *L. monocytogenes* and *E. coli* biofilms on stainless steel coupons. In general, *L. monocytogenes* strains were more resistant to both micelle-encapsulated antimicrobials than *E. coli* strains. For both bacteria, most of the bactericidal activity took place in the first 10 min of antimicrobial exposure. Biofilm morphology and viability revealed an increasing number of dead cells when biofilms were treated with sufficiently high concentrations of carvacrol-loaded micelles. The results from this study demonstrated the effectiveness of the application of surfactant-encapsulated essential oil components on two pathogen biofilm formers such as *E. coli* and *L. monocytogenes* grown on stainless steel coupons (Perez-Conesa et al., 2011).

Carvacrol as a Skin Penetration Enhancer

In general, the transdermal route of administration has been shown to be capable of avoiding the hepatic first pass effect, thus achieving the required systemic bioavailability of a drug. For many drugs, the desired effect may not be possible without the use of penetration enhancers, primarily because of the barrier function of the stratum corneum (Nino et al., 2010). In a study examining the use of carvacrol as a skin penetration enhancer for the transdermal delivery of propranolol hydrochloride, it was shown that carvacrol, at concentrations of 5% and 10%, enhanced the permeation of propranolol through the excised hairless mouse skin at a better rate than the other terpenes (menthol, limonene, and linalool). At lower concentrations (1%), carvacrol was not as effective as menthol for the first 8 h, but by 10–12 h carvacrol was a more effective penetration enhancer. The authors attributed the skin transport enhancement of propranolol by carvacrol to be a result of its hydrogen-binding ability through its hydroxyl group and its aromaticity (Kunta et al., 1997). A significant enhancement in permeation of luteinizing hormone-releasing hormone by carvacrol was demonstrated in studies with newborn pig skin (Songkro et al., 2009).

In another study, haloperidol, an antipsychotic drug that needs to be available in a long-acting formulation for maintenance therapy to prevent the relapse of psychosis, was reported to penetrate only subtherapeutically through the human skin in vitro. Carvacrol enhanced the permeation of haloperidol by extracting and disrupting the lipids by aligning within the bilayer and increasing the solubility of the drug in stratum corneum lipids. However, carvacrol increased the lag time perhaps because it takes a longer time to distribute within the stratum corneum (Vaddi et al., 2002).

The transdermal delivery of the anti-AIDS drug, 3′-azido-3′-deoxythymidine (zidovudine or AZT) was studied *in vitro* and *in vivo* using the enhancers *t*-anethole, carvacrol, thymol and linalool. *In vitro* studies were carried out in Franz Cells using excised full thickness CD-1 nude mouse and Sprague–Dawley rat skin while the *in vivo* transdermal bioavailability of AZT was determined in rats using enhancer containing gel formulations. In general, the amount and rate of AZT transport in the *in vitro* studies were higher than the corresponding values in the *in vivo* studies demonstrating the potential of t-anethole, carvacrol, thymol and linalool as effective transdermal enhancers (Kararli et al., 1995).

CONCLUSION

Carvacrol is used in low concentrations as a flavor ingredient in foods and as a preservative. In recent years, significant research efforts have demonstrated that carvacrol possess a variety of properties including antioxidant, antibacterial, antifungal, anticancer, hepatoprotective, spasmolytic, and vasorelaxant. Although most of these studies have been carried out in *in vitro* systems, its potential usefulness in clinical applications necessitates extensive *in vivo* research to establish the disposition of and confirm the biological and pharmacological properties of carvacrol. The poor solubility and bioavailability of carvacrol may overcome by the use of drug delivery systems that can increase drug solubility and stability, may allow systemic drug administration, and can enhance drug bioavailability and offer control over the absorption and distribution profiles.

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