ABSTRACT
Carvacrol (2-methyl-5-isopropylphenol), a phytochemical derived from aromatic plants of the genera Oregano, Thyme and Satureja. Carvacrol is known to have potent anti-inflammatory and anti-oxidant effects and to inhibit platelet aggregation and the growth of a variety of cancer cells. Its potential hepatoprotective and neuroprotective activities have been demonstrated in various animal models. Evidence from numerous in vitro and in vivo studies has confirmed its ability to modulate various targets and signaling pathways involved in obesity, inflammation and cell proliferation. This article discusses the current data available on pharmacological effects of carvacrol, which may serve to exploit carvacrol for the pharmacotherapy of various diseased conditions.

Key words: Carvacrol; antioxidant; anticancer; obesity; meat quality.

INTRODUCTION
Carvacrol (2-methyl-5-isopropylphenol) is a monoterpane phenolic constituent of essential oils of various aromatic plants. The essential oils obtained from the genera origanum, Thymbra, Cordiothymus, Satureja, Thymbra and Lippia are rich sources of carvacrol (CRL). It is synthesized by the specialized secretory cells in the aerial parts of plants and is stored in a subcuticular storage cavity. The essential oils containing the active substances are then extracted from the plant materials by mechanical pressing or steam distillation or by using organic solvents.\(^1\)
Physical and chemical properties of Carvacrol

Carvacrol (C_{10} H_{14}O, Fig.1), is represented by the synonyms: isopropyl-o-cresol, p-cymen-2-ol, 2-hydroxy-p-cymene, 5-isopropyl-2-methylphenol and iso-thymol. CRL has a molecular weight of 150.21.

Carvacrol is a liquid with thymol taste. It’s density varies; 0.976g/cm³ at 20°C and 0.975g/cm³ at 25°C. The boiling point of CRL is 237~238°C and the Refractive index is 1.52295. With steam it can be volatile. Carvacrol is almost insoluble in water and soluble in alcohol or ether. Alkylation of o-cresol with propylene or isopropyl alcohol (IPA) over solid acid catalysts results in the formation of carvacrol.\(^4\)

Metabolism of carvacrol

In male albino rats a major portion of administered CRL (1mmol/kg) was excreted in urine, as such or as its glucuronide and sulphate conjugates. Extensive oxidation was observed mainly at the methyl groups and this resulted in derivatives of benzyl alcohol and 2-phenylpropanol with their corresponding carboxylic acids. Besides these metabolites, a minor metabolite produced by ring hydroxylation was also detected.\(^5\) Pig gastric fermentation simulation experiments indicated 29% degradation of CRL in cecum with no such changes in jejunum. But oral feeding of 13.0, 13.2, 12.5 and 12.7 mg kg\(^{-1}\) body weight of carvacrol to piglets, showed half-life between 1.84 and 2.05 h in total digestive tract. It was mainly and almost completely absorbed in the stomach and the proximal small intestine. Plasma concentrations (sum of free and conjugated compound) peaked at 1.39 h and this was accompanied by high concentrations in urine.\(^6\)
Anticancer effects of CRL
The anticancer effect has been demonstrated in vivo during pulmonary tumors, chemical induced carcinogenesis and hepatocellular carcinoma.[6-10] At nontoxic concentrations (<0.05mM), CRL exhibits antigenotoxic effect and it needs further investigation.[11-13]

Free radical scavenging and antioxidant effects of Carvacrol
During the normal metabolism, the cells generate reactive oxygen intermediates (ROI) such as superoxide radical (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$). As excessive accumulation of ROI leads to tissue damage and loss of cellular functions, they are kept in check by the antioxidants. Besides protecting the cells against ROI, antioxidants possess a variety of biological activities, including induction of drug-metabolizing enzymes, inhibition of prostaglandin synthesis, etc. An alteration in the balance with an increase in the ROS over the capacity of the antioxidant is defined as oxidative stress and this leads to oxidative damage of macromolecules (proteins, nucleic acids and lipids).[14].

CRL efficiently scavenges free radicals including peroxyl radicals, superoxide radicals, hydrogen peroxide and nitric oxide.[15,16]. CRL exhibits antioxidant activity both in vitro and in vivo and its antioxidant activity is attributed to the presence of hydroxyl group (OH) linked to aromatic ring. [17-19] The weak acid character of CRL facilitates its reaction with a free radical, thereby donating hydrogen atoms to an unpaired electron, producing another radical that is stabilized by electron scattering generated at a molecule resonance structure.[16]

Hepatoprotective effect of CRL
CRL is hepatoprotective both ex vivo and in vivo in a D- galactosamine induced rat model. The increased concentrations of plasma, liver, kidney lipids, lipid peroxidation products, liver marker enzymes and the decreased concentrations of enzymic and nonenzymic antioxidants induced by D- galactosamine in rat was restored to normalcy by CRL (80mg/kg bw) and this effect was comparable to that of the standard drug silymarin (25mg/kg bw). In addition, the D-galactosamine induced reductions in mitochondrial enzymes and DNA damage were restored and controlled by CRL. The antioxidant effect of CRL on mitochondrial and hepatocellular membranes and its genoprotective nature were demonstrated to contribute to the hepatoprotective property of CRL.[17,19-21]
Insufficiency or stopping of blood flow to an organ causes a shortage of oxygen and glucose needed for cellular metabolism and results in Ischemia. Restoration of blood flow to the tissue by eliminating the factor, which causes ischemia is termed Reperfusion. Reperfusion following ischemia results in the passage of toxic products to the systemic circulation. Hepatic ischemia is a frequently encountered problem during liver transplantations, liver surgery and renal I/R injury. By decreasing lipid peroxidation products and improving liver antioxidant defence, CRL protects liver during hepatic I/R injury and renal I/R injury.\cite{22-24}

**Neuroprotective effect of CRL**

Alzheimers disease is a complex, late-onset disorder leading to mental illness characterized by lossof memory and loss of multiple cognitive functions. CRL is considered as a potential drug for Alzheimer's disease due to its inhibitory effect on acetylcholinesterase (AChE) activity. In the central nervous system, the phenolic hydroxyl group of CRL binds to AChE and thereby leads to a loss of function of AChE.\cite{25-27} Recently antidepressant-like and anxiolytic like effects of CRL in mice is reported to occur through modulation of GABAergic transmission and dopaminergic system. The inhibitory effect of CAR on muscarinic receptors in guinea-pig tracheal chains and the anticonvulsant effect of CRL in rodents suggests the neuroprotective effect of CRL. It is also suggested to cross the blood brain barrier due to its low molecular weight and lipophilic nature and exert its neuroprotective effects, however further studies are warranted.\cite{28-31}

**Anti-inflammatory and anti-hypernociceptive effects of CRL**

Inflammatory hyperalgesia, also called hypernociception is caused by hypersensitivity of nociceptive pathways. During inflammation, the mediators (including cytokines, interleukins, tumor necrosis factor-α) are released by cells of the immune system. This produces sustained activation and sensitization of both primary nociceptors and higher order neurons involved in the transmission of the nociceptive input. This hypersensitivity of nociceptive pathways contributes to hypernociception, in laboratory animals increased pain sensitivity.\cite{32}

CRL (50 and 100mg/kg) increased the threshold sensitivity of mice exposed to carrageenan and this property was comparable to indomethacin, a COX inhibitor and standard drug. In hypernociception induced by carrageenan in mice, the cytokine TNF-α triggers secretion of interleukin-1β (IL-1β) which in turn induces the expression of COX-2 responsible for the
biosynthesis of various prostanoids, such as PGE2. CRL is proposed to inhibit hypernociception by inhibiting migration of neutrophils and mononuclear cells involved in production of proinflammatory cytokines, including TNF-α, nitric oxide, with consequent decrease in prostaglandins. But CRL did not affect the morphology of the cells except that it decreased the TNF-α levels in pleural lavage. In an earlier study, it was reported activation of PPARα and γ by CRL which then inhibited COX-2 expression and prostaglandin synthesis during inflammation. Further, the stimulatory action of CRL on PPAR, inhibits the transcription of NF-kB. This decreases the inducible nitric oxide synthase enzyme levels and in turns the nitric oxide in macrophages, contributing to anti-hypernociceptive effect of CRL. Contradictory statements are proposed on the antioxidant potential of CRL in contributing to the antihypernociceptive role of CRL. The involvement of the antioxidant effect of CRL in controlling lipid peroxidation and nitric oxide production during hypernociception is debated with supporting and contradicting reports.

Stress proteins and CRL

Stress proteins, such as members of the heat-shock protein (HSP) family, are up-regulated by cells in inflamed tissue and can be viewed functionally as “biomarkers” for the immune system to monitor inflammation. CRL was shown to have a notable capacity to coinduce cellular Hsp70 expression in vitro in various mammalian cell types: bone marrow-derived dendritic cells (BMDCs) and RAW 264.7 cells from BLB/c mice and human peripheral blood mononuclear cells (PBMCs). During stress, CRL activated Hsp70-specific T cell hybridoma in vitro and amplified T cell responses to Hsp70 in vivo. But it did not induce Hsp expression in the absence of stress, indicating that CRL could act by acting as a co-inducer and amplify the natural stress response only in the endangered cells and tissues that require Hsp expression. The authors further evaluated the fact, that increasing endogenous HSP expression could boost protective T cell responses to a self stress protein and downregulate inflammatory disease. Intragastric administration of CRL to mice enhanced cellular expression of Hsp70 in Peyer’s patches, specifically promoted T cell recognition of endogenous Hsp70 and almost completely suppressed proteoglycan-induced experimental arthritis. The novel finding is that CRL can boost protective T cell responses to a self stress protein and down-regulate inflammatory disease.
Anti-obese effects of CRL

Obesity is one of the risk factor for chronic diseases such as diabetes, coronary heart disease, hyperlipidemia and cancers. In the current scenario, anti-obese agents are of utmost importance and. In humans and animal models, consumption of high levels of dietary fat is considered a major factor in promoting obesity and associated metabolic diseases. Carvacrol inhibited intracellular fat accumulation and adipocyte differentiation in mouse embryo 3T3-L1 cells. Recent study on the possible effect of CRL on obesity indicated interesting results. They fed mice with a normal diet, high fat diet (HFD) or a HFD with CRL (0.1 w/w) for 10 weeks. Higher body weight, body weight gain, total visceral fat pad weight and liver weight, plasma and liver total cholesterol, HDL-cholesterol, triglyceride and free fatty acids were shown in mice fed HFD diet and all such effects were decreased by CRL. In visceral adipose tissues, CRL decreased the expression of adipogenesis related genes- fibroblast growth factor receptor 1 (FGFR1) and bone morphogenetic protein receptor 1 (BMPR1). A decrease in expression of galanin receptor1 (GalR1) and galanin receptor 2 (Gal R2) the neuropeptide galanin that stimulates the intake of fat rich diet were observed. The expression of nuclear receptor PPARγ, coactivator CCAAT- enhancer binding protein (C-EBPα) and their target genes adipocyte protein 2 (AP2), lipoprotein lipase (LPL) and leptin were reduced and thereby adipogenesis induced by feeding high fat diet was inhibited by CRL. However, carvacrol did not show any affect on thermogenesis regulation. In obese animals, high levels of free fatty acid levels are reported and this is due to their release either from adipose tissues or from high fat diet. These free fatty acids increase expression of several pro-inflammatory cytokines such as tumour necrosis alpha (TNF-α), interleukin1 (IL-1β) and interleukin 6 (IL-6) (Itany et al., 2002). In this same study, CRL reduced free fatty acid levels and the mRNA and protein levels of toll-like receptors (TLR) TLR2, TLR 4, their downstream molecules-myeloid differentiation primary response gene 88 (MyD88), TIR-domain- containing adapter inducing interferon (TRIF), TNF receptor- associated factor 6 (TRAF6), interferon regulatory factor 5 and 3 (IRF5, and IRF3) and the pro inflammatory cytokines, tumour necrosis factor -α (TNF-α), and interferon-α in mouse HFD. All the findings indicated the involvement of novel pathways in inhibition of visceral adipogenesis and attenuation of production of pro-inflammatory cytokines in the visceral adipose tissues. These results illustrate the need for further detailed studies to recommend CRL as a anti-obese drug. [38,39]
CRL and Vascular health:
The Transient Receptor Potential (TRP) superfamily channels are located mostly on the plasma membrane of numerous human and animal cell types. They are essential components of biological sensors that detect changes in the environment in response to a myriad of stimuli including cold or hot temperatures, natural chemical compounds and mechanical stimuli and CRL was suggested to modulate their action. The inhibitory role of CRL on *Drosophila* TRPL channels and mammalian TRPM7 (Melastatin) channels that were heterologously expressed in HEK cells and ectopically expressed in a primary culture of CA3-CA1 hippocampal brain neurons has been established. Later, the role of CRL in the regulation of vascular tone was revealed. CRL, by acting as an agonist to TRPV3 channels in the arterial endothelium, increased Ca$^{2+}$ influx via Transient Receptor Potential Vanilloid 3 (TRPV3) channels. This in turn caused hyperpolarization of plasma membrane of endothelial cells underlying smooth muscle, ultimately resulting in vasodilation of cerebral arteries, confirming the role of CRL in improving vascular health. Additionally, the involvement of CRL in blocking cardiac L-type calcium channel too adds up to its hypotensive effect. Further, CRL exhibited antiplatelet activity and inhibited platelet aggregation by decreasing the production thromboxane A$_2$ in platelets.

Gastroprotective effect
CRL exhibited gastroprotective effect in absolute ethanol-, acidified ethanol-, ischemia and reperfusion-, and nonsteroidal anti-inflammatory drug-induced models of gastric lesions in mice and rats. A recent indicated that the gastroprotective effect of CRL is mediated by endogenous prostaglandins, increase of mucus production, K$_{ATP}$ channels opening, NO synthase activation, and its antioxidant properties.

Cytotoxic and antiproliferative effects
The cytotoxic antiproliferative effects of CRL are given in Table 1.

Antimicrobial effects of Carvacrol
CRL exhibits antimicrobial effects against a wide range of microbial species including *Aspergillus, Fusarium, Bacillus, Salmonella, Listeria, Streptococci, Pseudomonas* etc.
Effect of CRL on poultry

Antibiotic growth promoters, due to their undesired effect on livestock and environment have been banned by various countries and essential oils are studied for their efficacy to replace antibiotic growth promoters. Female broilers fed 200ppm CRL from one day to four 4 weeks of age with cholesterol-free or cholesterol-rich diets reduced feed intake and weight gain but improved feed-to-gain ratio. Further, CRL lowered plasma triglyceride concentrations without affecting plasma cholesterol. However the same levels of CRL did not produce any effect on feed efficiency or plasma cholesterol when it was supplemented with corn-soybean meal based basal diet enriched with carboxymethyl cellulose. On the other hand, feeding CRL with cinnamaldehyde at 200 ppm dose (each) lead to a negative interaction and resulted in body weight reduction. On the contrary, a recent study indicated no significant effect on body weight, feed intake, feed conversion ratio and ileal microbiota by feeding CRL along with thymol and organic acids in broilers. These studies present an inconclusive effect of CRL on feed efficiency in broilers and further studies are warranted to obtain confirmed results.

Though the effect of CRL on was inconclusive, a very recent study has highlighted the protective role of CRL against live coccidiosis. Dietary CRL (5.0ppm) from one day to seven days of age, improved weight gain, reduced gut lesions, oocyte shedding, lowered pro-inflammatory cytokine gene expression during coccidiosis in birds challenged with *Eimeria acervulina*. Further, CRL regulated lipid metabolism, linoleic acid, androgen and estrogen metabolism in intestinal intra epithelial lymphocytes of broilers. In another study an altered expression of 74 genes (26 up regulated, 48 down regulated) in intestinal intra epithelial lymphocytes was observed in chickens fed 5.0ppm CRL. Microarray data revealed upregualtion of several genes related with the endocrine and metabolic system, eg. selenoprotein X, 1 (SEPX1) and protease serine 3 (PRSS3). Future studies based on these results could contribute to comprehensive understanding of the molecular mechanism of CRL in the chicken digestive tract and facilitate the development of novel dietary strategies to immunomodulate host response in normal and disease states.

During the past decades, consumer concern on the quality of meat and meat products has greatly increased. As poultry meat has high concentration of polyunsaturated fatty acids, it is prone to oxidative deterioration, that affects the quality of meat. Feeding 150 ppm of
carvacrol to broilers from 1 to 42 days of age resulted in reduced production of thiobarbituric acid (an indicator of lipid peroxidation) in thigh samples stored for 5 to 10 d. Similarly, CRL reduced lipid oxidation, improved shelf life of poultry meat and reduced microbial load in chicken patties stored at low temperature (0-3°C). \(^{[51,58,59]}\) Hence, application of the natural antioxidant CRL could be useful to improve poultry meat quality.

**Table 1. Cytotoxic and antiproliferative effects of carvacrol**

<table>
<thead>
<tr>
<th>Cell lines used</th>
<th>IC(_{50})</th>
<th>Effects of carvacrol</th>
<th>References</th>
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<tr>
<td>Murine B16 (F10) melanoma cells</td>
<td>-</td>
<td>Cytotoxic effect</td>
<td>44</td>
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<tr>
<td>Human non-small cell lung cancer (NSCLC) cell line, A549</td>
<td>250 µM of CRL</td>
<td>Cytotoxic and anticancer effect: Degeneration of cell morphology and induction of apoptosis (cytoplasmic shrinkage, loss of cell-cell contacts, fragmentation of cytoplasm nuclear chromatin)</td>
<td>45</td>
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<tr>
<td>Mouse muscle cell line (CO25) bearing a glucocorticoid inducible mutated human N-ras oncogene</td>
<td>60 µg 10 µg/ml</td>
<td>Cytotoxic effect Anticancer effect-Inhibition of DNA synthesis in N-ras transformed cells</td>
<td>46</td>
</tr>
<tr>
<td>H-RAS transformed 5RP7 cells</td>
<td>0.04mg/ml</td>
<td>Cytotoxic effect-Apoptotic morphological changes, internucleosomal DNA fragmentation</td>
<td>47</td>
</tr>
<tr>
<td>N-RAS transformed CO25 cell line</td>
<td>0.1mg/ml</td>
<td>Cytotoxic effect -Apoptotic morphological changes, no internucleosomal DNA fragmentation</td>
<td>47</td>
</tr>
<tr>
<td>Human cervical cancer HeLa and SiHa cells</td>
<td>IC(_{50}) 50mg/L</td>
<td>Increase in apoptosis</td>
<td>48</td>
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Conflict of Interest
Authors declare no conflicts of interest.

References


58. Kim DK, Lillehoj HS, Lee SH, Jang SI, Bravo D. High-throughput gene expression analysis of intestinal intraepithelial lymphocytes after oral feeding of carvacrol,


