Summary & conclusion
SUMMARY AND CONCLUSION

Chronic inflammation mediated cancer is a common ailment throughout the world, in which the GI tract mucosa becomes ruptured and perforations leading to bleeding. Ulcerative colitis related tumoriogenesis is prevented through using non-toxic chemical entities and it is considered to be an alternative, but more realistic and fundamental strategy for the dread disease management. A wide variety of preclinical studies demonstrate the success of chemoprevention in reducing the burden of cancer. Results from in vitro and in vivo studies show that carvacrol possess a variety of biological and pharmacological properties including antioxidant, anticancer, anti-inflammatory and anti-proliferative. Due to its broad-spectrum health beneficial effects carvacrol is used as the state-of-the-art nature’s medicine.

- Preliminary studies with carvacrol using various concentrations showed that carvacrol at a dose of 50 mg/kg b.w was significantly more effective in suppressing DMH/DSS induced colitis associated colon cancer. Hence the dose 50mg/kg b.w was fixed as the optimum dose for further experiments.

- Carvacrol supplementation to DMH/DSS exposed rats significantly reduced the colon cancer burden, tumor incidence, tumor multiplicity in the colon of induced rats.

- Carvacrol significantly reduced the formation of aberrant crypt foci upon DMH/DSS exposure. Since there is a close link between aberrant crypt foci formation and colon carcinogenesis, the observation imply that carvacrol
reduces the preneoplastic lesions in the colon indicates its anti-tumorigenic potential.

- In this study, carvacrol restored the levels of xenobiotic metabolizing enzymes such as Phase-I & II enzymes in the inflamed colonic tissues of rats administrated with DMH/DSS.

- A noticeable elevation in the activities of the antioxidant enzymes and improvement of non-enzymatic antioxidants and lipid peroxidation product were altered upon carvacrol administration in DMH/DSS induced animals.

- The levels of lysosomal enzymes were increased in the colonic tissues of DMH/DSS induced animals. Carvacrol supplementation to DMH/DSS exposed rats significantly reduced the activities of these enzymes and thus reduced the risk of gastrointestional genotoxicity.

- In this study, DMH/DSS exposed rats showed elevated level of Pathophysiological enzymes such as AST, ALP, ALT and LDH. Carvacrol treatment to the all of these tumor developed rats, the levels of these enzymes was decreased.

- In this study, a remarkable increase in the colon cancer marker carcino embryonic antigen, gamma glutamyl transferase, 5’- nucleotidase and lactate dehydrogenase was observed in DMH/DSS induced tumor bearing rats. The activities of these enzymes were effectively prevented by carvacrol treatment suggesting its protective action.

- The levels of mitochondrial TCA cycle enzymes and loss of membrane integrity was observed by changes in membrane bound ATPase such as
Na/K\(^+\)ATPase, Ca\(^+\) ATPase and Mg\(^+\)ATPase levels were significantly restored upon carvacrol administration in DMH/DSS induced rats.

- Carvacrol treatment effectively restored the levels of glycoproteins (Sialic acid, hexose, hexosamine, fucose and mucoprotein) in the colon of DMH/DSS induced animals. This indicates that carvacrol has the capacity of retrieving normal structure, rigidity and function of damaged cell membrane.

- Histopathological analysis revealed the inhibition of mast cell infiltration, collagen deposition, mucus depletion and proliferative and occurrence of crypt upon carvacrol treatment to DMH/DSS induced rats.

- Carvacrol effects on the Ultra structural analysis (SEM and TEM) of colitis associated colon cancer tissues showed the morphological changes such as nucleus condensation and mitochondrial cristae degradation in DMH/DSS induced rats during apoptosis.

- Carvacrol administration significantly inhibited the levels of pro-inflammatory mediators such as IL-1\(\beta\), iNOS, COX-2, TNF-\(\alpha\) and NF-\(\kappa\)B during experimental colon carcinogenesis.

- In this study, oxidative stress mediated DNA damage was mostly observed in the rats exposed to DMH/DSS which leads to increased amount of DNA adduct in tumor bearing animals. Oral administration of carvacrol gradually reduced the levels these DNA adducts in treated rats.

- Carvacrol supplementation to DMH/DSS induced rats showed release of cytochrome-c, upregulation of Bax, down regulation of Bcl-2, activation of
Caspase-3 and p53. The results reveal that carvacrol could interfere with the early event of carcinogenesis.

➢ In this study, a remarkable increase of extra cellular matrix degrading enzymes (MMP2 & MMP9) was observed in DMH/DSS induced animals. Its role is tumor cell migration and invasion was abrogated by carvacrol treatment.

➢ In this study, colitis associated colon cancer rats the levels of proliferative markers such as PCNA, Ki67 was very high. Carvacrol administration were effectively reduced the rate of cell proliferation in DMH/DSS exposed tumor bearing rats.

➢ Oral administration of carvacrol was effectively ameliorates SUMO mediated conjugation enzymes (UBC9) and deconjugating enzymes SENP1 through which carvacrol regulates the SUMOylation pathway during colon carcinogenesis.

➢ Anti-cancer efficacy of carvacrol was assessed using HCT-116 cell line. Cell viability was performed using MTT assay in a dose and time dependent manner.

➢ The level of ROS was estimated using DCFH-DA in carvacrol treated HCT-116 Cells in time dependent manner.

➢ The morphological change of apoptosis was observed in carvacrol treated HCT-116 cells using AO/EtBr staining.
Carvacrol treated HCT-ll6 cells showed decreased level of expression to SUMOylation responsible elements such as SUMO-1, UBC9 and SENP1 which are all these components was confirmed by qPCR and immunobloting method.

SUMO-inhibiting potential of carvacrol was clearly studied by bioinformatics tools-molecular docking. In this study, the UBC9 inhibitory role of carvacrol was cross checked with the small molecule SUMO-Inhibiting positive control ginkgolic acid. These results reveal that carvacrol also a remarkable bioactive compound and its effect on UBC9 inhibition were similar to that of ginkgolic acid role on SUMOylation pathway

**CONCLUSION**

The biochemical, histopathological, ultra structural and molecular markers were evaluated in a reliable animal model to demonstrates the chemopreventive potential of carvacrol against colitis associated colon carcinogenesis, which provides the effective chemopreventive approach to disease management. In this study, carvacrol was shown to possess anti-oxidant, anti-proliferative, anti-inflammatory, anti-mutagenic role in an experimentally induced ulcerative colitis related carcinogenicity of Fischer 344 rats. This study provides a strong base for future mechanistic studies in chemoprevention and development of novel drug target for inflammation associated cancers through regulating cell signaling pathways. Further clinical trials are warranted before carvacrol could be consider as a drug for the treatment to ulcerative colitis related carcinogenesis.
Fig. 5.1 Schematic representation of chemopreventive role of carvacrol on colitis associated colon carcinogenesis through regulating SUMOylation Pathway