CHAPTER 5
SUMMARY AND
CONCLUSION
Colon cancer is a leading cause of cancer deaths worldwide. For a group of individuals, who are at elevated colon cancer risk as well as those diagnosed at initial colon cancer stages, lifestyle changes such as dietary alterations approach alone are not capable of providing rapid improvement in cancer incidence. Therefore, a superior strategy in decreasing colon cancer development as well as its further progression can be achieved through chemoprevention, which may be defined as cancer prevention by intervening with one or more chemical or natural agents. Several advantages of chemoprevention are known, such as reduction in cancer incidence as well as mortality that eventually inhibit the precancerous lesions while prolong the latency period. In the current study we investigated the role of piroxicam, a traditional NSAID (t NSAID) and c-phycocyanin, a selective COX-2 inhibitor from *Spirulina platensis*, individually and in a combination regimen in the chemoprevention of experimental colon cancer induced with 1, 2-dimethylhydrazine (DMH) in rat model.

Results showed that DMH at a dose of 30 mg/kg body wt, seemed to be the optimum dose for creating the colon carcinogenesis model in rats. Also piroxicam at a dose of 4mg/kg body wt as well as c-phycocyanin at a dose of 200mg/kg were significantly able to restrain the inflammatory response as examined by carrageenan induced paw edema test. However, the inhibition of inflammation was achieved to a more significant level with the administration of piroxicam and c-phycocyanin in a combination regimen as compared to the individual treatment of these agents. Thus, the present results of the dual COX-1 and COX-2 inhibitor, piroxicam and selective COX-2 inhibitor, c- phycocyanin on the chemoprevention of the colon cancer are in a greater harmony with the anti-inflammatory dose range of the drugs, as shown in the rat model.

Animals were divided into five groups comprising of control, DMH, DMH+ piroxicam, DMH+ c-phycocyanin and DMH+ piroxicam + c-phycocyanin, respectively.
Summary and conclusion

These groups were sacrificed after 6 weeks (early stage) and 18 week (advanced stage) of the treatment period, respectively.

At the initiation stage of colon cancer (6 week), the occurrence of preneoplastic lesions such as MPLs was detected in the DMH group with a sharp rise in the MPL incidence, burden and multiplicity. DMH+ piroxicam and DMH+ c-phycocyanin, on the other hand, showed considerable reduction in the MPL incidence, burden and multiplicity, which were further decreased with the DMH+ piroxicam+ c-phycocyanin group. DMH treatment showed greater number of aberrant crypts as well as the ACFs. However, DMH+ piroxicam and DMH+ c-phycocyanin groups showed less number of them. A combination of piroxicam and c-phycocyanin further reduced their number considerably. Histopathological outcomes of the epithelia from DMH group showed the occurrence of severe dysplasia with loss of mucous secreting goblet cells, while these features were reduced in DMH+ piroxicam and DMH+ c-phycocyanin groups, respectively. However, DMH+ piroxicam + c-phycocyanin, exhibited a mild hyperplasia with all the other histoarchitectural features nearly intact as in the control.

Macroscopic analysis of the colons in the advance stages (18 week) showed a significant increase in the tumor incidence, burden as well as multiplicity in the DMH group. On the other hand, all of these parameters were found to be lowered with the treatment of piroxicam and c-phycocyanin. Furthermore, the incidence, burden and multiplicity of the tumor was decreased to a more considerable level with the DMH+ piroxicam+ c-phycocyanin, group. Histopathological analysis of colonic epithelia showed the occurrence of great variety of carcinoma cases such as signet ring cell carcinoma, invasive dysplasia as well as the mucinous adenocarcinoma. However, the presence of hyperplasia along with early changes of aberrant crypt foci (intermediate lesions leading to colon cancer, characterized by the distinct aggregation of aberrant/abnormal crypts) was noted in DMH+ piroxicam, DMH+ c-phycocyanin and DMH+ piroxicam + c-phycocyanin, respectively.

Since, there is a strong association between inflammation and cancer progression, we analyzed its role by examining the expression of COX-2 enzyme. Results showed a strikingly raised expression of COX-2 in the DMH group, which clearly indicated the inducible nature of this enzyme in cancer progression. However, treatment of piroxicam and c-phycocyanin...
showed a decrease in expression of COX-2 both at the gene and protein level. Combination of these two agents further reduced its expression. Hence, these findings showed that the over-expression of the inducible enzyme COX-2 is one of the chief characteristics of DMH induced colon cancer. Inflammation was also checked by analyzing the enzyme activity of PGE₂, the metabolic product of arachidonic acid, which was observed to be enhanced with DMH. These findings therefore, implied that an over-expressed COX-2 might contribute to the increased PGE₂ production in DMH treated group while a lowered PGE₂ level in piroxicam and c-phycocyanin treated groups further demonstrated the chemopreventive role of piroxicam and c-phycocyanin in the colon cancer. Piroxicam and c-phycocyanin have also been shown to inhibit the expression of pro-inflammatory mediators such as cytokines (IL-1β, IL-2, TNF-α and IFNγ), chemokine (MCP-1) as well as the transcription factors (Jak3, Stat3 and NFκB) which upregulating MIP-1. NFκB is activated in response to the elevated pro-inflammatory cytokines via the IκB kinase (IKK) complex. These results suggested that cytokines mediate the signaling of Jak3-Stat3 as well as NFκB, which is recognized as an important link between inflammation and cancer and hence, its suppression offers a strategy for treatment of inflammation-induced tumor growth.

Piroxicam and c-phycocyanin were shown to down regulate the PI3-K signaling pathway which was found to be up-regulated with DMH treatment and is recognized for controlling cell metabolism, growth as well as survival. One of the downstream targets of PI3-K is Akt or protein kinase B. Upon activation, Akt restrains its downstream target i.e., GSK3β, a serine/threonine kinase, having an essential role in the glycogen metabolism. PTEN, a tumor suppressor, negatively regulates the PI3-K/Akt signaling by causing conversion of PIP3 into PIP2. Piroxicam as well as c-phycocyanin are seen successfully capable of reducing the over-expressed levels of PI3-K and Akt by raising the expression of GSK-3β as well as that of PTEN. PI3-K/Akt signaling also assists an oncogene Wnt, to enhance the β-catenin levels during inflammation. PI3-K induced and Akt dependent β-catenin signaling is required as biomarker of dysplastic modulations in the colon, therefore, it revealed a positive correlation between PTEN/PI3-K/Akt and Wnt/β-catenin signaling pathway in colon cancer progression. Piroxicam and c-phycocyanin lowered the Wnt /β-catenin expression, thus, these results are in accordance with the above decrease in PI3-K/Akt levels by these agents.
We also examine the alterations in cell cycle machinery, a crucial characteristic of the carcinogenesis process, and for this we studied the role of various cell cycle regulators such as Cyclins (Cyclin D1 and Cyclin E), CDKs (CDK2 and CDK4), p53, p21 and pRb. Piroxicam and c-phycocyanin induced the cell cycle regulations by restricting the Cyclin/CDK activation and further induced cell death by raising the levels of tumor suppressor proteins, pRb, p53 and p21, respectively.

To restrain cancer cell proliferation, piroxicam and c-phycocyanin were shown to induce the apoptotic cell death which is observed from the decrease in ΔΨm and PCNA levels while regulating intracellular ROS levels. Piroxicam and c-phycocyanin brought the stimulation of caspases leading to Apaf-1 activation, increase the pro-apoptotic Bax and Bad while decreasing the anti-apoptotic Bcl-2 expression resulting in cytochrome c release and apoptosome formation, which clearly indicates the mitochondria-dependent intrinsic apoptosis pathway.

A link between angiogenesis and cancer may offer an essential opportunity to hinder the tumor progression via restraining the process of angiogenesis. Cancer cell derived VEGF over expression may stimulate colon cancer development and progression in a rat model which is lowered down by piroxicam and c-phycocyanin. Piroxicam and c-phycocyanin further induced PPARγ activation and inhibition of MMP-2, MMP-9 and HIF-1α, which are symbolizes critical elements in the angiogenic pathway. Also, the suppression of HIF-1α perhaps reduced the potential of the cancer cells to provide an ample vascular supply and acclimatize their cellular metabolism to hypoxia.

Increase in fluidity of plasma membrane in colonocytes may be related to the increase in tumor progression of colon. However, with the piroxicam and c-phycocyanin treatment the regulating effects of these agents toward normalizing the physical conditions in membrane have been seen. Therefore, the evident lipid variations may provide a reliable measure for estimating the modulation of tumor progression in vivo. Our findings also showed the anti-carcinogenic function of enhanced [Ca2+]i from piroxicam and c-phycocyanin. They further elevated the expression of Calpain-9 and raised apoptotic cell count in these groups.
Conclusion: It may be concluded that inhibition of COX-2 remains the essential target in prevention of colon cancer by COX-2 inhibitors. However, a number of alternate and parallel signal transduction pathways are found contributing in the process. PI3-K/Akt/GSK-3β being the most important signaling target in the present study for the colon cancer chemoprevention by piroxicam, a t NSAID and c-phycocyanin, a cyanobacterium derived selective COX-2 inhibitor. Moreover, the enhanced expression of the pro-inflammatory transcription factor NFκB is responsible for COX-2 induction which is again reduced by the treatment with piroxicam and c-phycocyanin. Apoptotic cell death had emerged out to be a crucial molecular mechanism behind the chemopreventive role of piroxicam and c-phycocyanin. Alterations in membrane fluidity as well as lipid phase separation also operate as early events for the induction of apoptotic cell death. Furthermore, angiogenesis suppression presented an additional potential mechanism for the piroxicam and c-phycocyanin induced chemoprevention of colon cancer. Finally, the combination regimen of piroxicam and c-phycocyanin proved out to be more effective as compared to the individual drug treatment.