C-Phycocyanin (C-PC) is a water-soluble phycobiliprotein pigment isolated from *Spirulina platensis*. This water-soluble protein pigment is of greater importance because of its various biological and pharmacological properties. C-PC is reported to have anti-inflammatory, antioxidant, and antiarthritic properties. Recent studies have shown that C-PC selectively inhibits cyclooxygenase-2 (COX-2) and also induces apoptosis in RAW 264.7 cells stimulated with LPS, and AK5 rat histiocyte cell line. Celecoxib was the first COX-2 inhibitor approved for use in U.S.A. In addition to the analgesic, antipyretic and anti-inflammatory activity, it has chemopreventive properties against colon cancer. Celecoxib significantly reduces the number of colon polyps in patients with familial adenomatous polyposis (FAP) and was recently approved by FDA for the treatment of FAP patients. Celecoxib was recently shown to induce apoptosis in COX-2 expressing androgen responsive (LNCap) and non-responsive (PC3) prostate cancer cells.

Prostaglandins (PGs) are a family of intercellular and intracellular messengers derived from arachidonic acid. These mediators exert a wide range of effects on processes such as smooth muscle tone, vascular permeability, cellular proliferation and inflammatory/immune function. The initial step in the synthesis of PGs from arachidonic acid is mediated by cyclooxygenases (COX also known as prostaglandin H synthase or prostaglandin endoperoxide synthase), of which two isoforms are recognized. COX-1 is expressed constitutively in most cell types, and prostanoids derived from COX-1 are thought to be important in gastric and renal homeostasis. COX-2 is the product of immediate early gene and is rapidly expressed only after exposure of cells to
hormones, mitogenic stimuli, bacterial lipopolysaccharides and inflammatory mediators. The induction of COX-2, with the resultant production of prostanoids can contribute to parturition, inflammation, pain, fever and certain types of cancer.

NSAIDs are used to treat acute and chronic inflammatory disorders. This anti-inflammatory mechanism of NSAIDs is due to a reduction of PG synthesis by the direct inhibition of cyclooxygenase. Since COX-2 is responsible for the production of prostaglandins at the site of inflammation, the selective inhibition of COX-2 results in potent anti-inflammatory effect. Unlike the non-specific NSAIDs, the selective COX-2 inhibitors have lesser gastrointestinal and other side effects. In addition to the role of COX-2 in inflammation, a number of studies have shown its role in cancer. A strong correlation has been established between the use of NSAIDs and the decreased incidence of colorectal, breast and lung cancers.

In view of involvement of cyclooxygenases in the mediation of cancers, particularly COX-2 which is over expressed in many cancers and inhibition of COX-2 leads to a markedly reduced tumor growth & block angiogenesis, the therapeutic role of COX-2 inhibitors towards a variety of cancers appear promising. Hence in the present study it is proposed to test the effect of COX-2 inhibitors on the growth and multiplication of CML cell line in K562. For this study C-PC a natural COX-2 inhibitor, and Celecoxib, a synthetic COX-2 inhibitor in the market, were employed. An attempt also was made to understand the mechanism(s) of C-PC induced cell death and to correlate the same with Celecoxib.
Summary

The present study demonstrates that C-PC (natural COX-2 inhibitor) and Celecoxib (synthetic COX-2 inhibitor) induce apoptosis in chronic myelogenous leukemia cells (K562 cells). The results indicate that C-PC and Celecoxib reduced the growth and multiplication of K562 cells. Phase contrast microscopic studies also revealed the presence of cells with web like activated membrane structure and also decrease in cell number. Ultrastructural changes, like membrane blebbing and nuclear condensation, typical of apoptosis, were observed by Scanning and Transmission electron microscopy.

In the present study the effect of C-PC and Celecoxib was monitored on COX-1 and COX-2 expression in K562 cell line. Both C-PC and Celecoxib showed significant reduction in growth only at very high concentrations i.e. 50 uM, while their COX-2 IC_{50} values are in 0.18 and 0.26 uM range. It is to be noted at this juncture, that both C-PC and Celecoxib inhibit COX-1 also at high concentrations employed in the present study. COX-1 IC_{50} for C-PC and Celecoxib are 4.5 and 16.3 uM respectively (Reddy et al., 2000). The concentrations of C-PC and Celecoxib required for inducing apoptosis in K562 cells is much higher compared to the IC50 values obtained for in vitro inhibition of enzyme. The present data indicate that the effects of C-PC and Celecoxib on K562 cells may not necessarily be related to inhibition of prostaglandin synthesis. However, the effective concentrations of C-PC and Celecoxib available in the cells for inhibition of COX-1 and COX-2 is to be evaluated in the present study.

The enzymatic cleavage at the DNA linker region renders a "classical laddering" of DNA, regarded as a marker of apoptosis, was clearly detected in cells treated with C-PC and Celecoxib. Flow cytometric analysis of the K562
cells treated with C-PC and Celecoxib showed 14-21 % of cells in sub G0/G1 phase. Flow cytometric analysis of treated cells showed the increase of hypodiploid apoptotic cells in a concentration-dependent manner and the decrease of the cells at S and G2 phase of cell cycle. This result suggested a possibility that C-PC and Celecoxib induced apoptosis occurs at S and G2 phase of the cell cycle.

Cytochrome c is known to be an essential factor in the mitochondrial respiratory chain and is released in response to various stimuli that elicit apoptosis. The precise mechanism for cytochrome c translocation is still unclear. Western blot analysis showed that cytochrome c is released significantly in the treated cells as early as 6 h compared to the control cells. Poly-ADP-Ribose-Polymerase (PARP), a 116-kDa protein that binds specifically at DNA strand breaks, is also a substrate for certain caspases (for example, caspase 3 and 7) activated during early stages of apoptosis. These proteases cleave PARP to fragments of approximately 85 kDa and 23 kDa. Detection of the 23 kDa PARP fragment with anti-PARP antibodies thus serves as an early marker of apoptosis. In the present study, a dose dependent increase in PARP cleavage was observed in cells treated with C-PC as well as Celecoxib.

Bcl-2 prevents the release of apoptosis inducing factor and cytochrome c from mitochondria, which is considered to be a key event during apoptosis. The present studies revealed a down regulation of Bcl-2 in K562 cells treated with C-PC and Celecoxib. Bcl-2 can form heterodimers with Bax protein, a Bcl-2 associated protein, which antagonizes Bcl-2 action and induces apoptosis. In this study no influence was seen on Bax protein levels in K562 cells treated with C-
Summary

PC and Celecoxib. The resulting net effect could thus lead to a lowered ratio of Bcl-2/Bax, which might be responsible for the C-PC and Celecoxib induced apoptosis in K562 cells. The interaction between the proto-oncogene c-myc and members of the Bcl-2 family may play an important role in the control of cell apoptosis. Among various proapoptotic factors, Myc oncoprotein has been reported to promote apoptotic responses. The present studies have demonstrated an increase in the expression of c-Myc in response to C-PC and Celecoxib treatment. C-PC and Celecoxib inhibited PKC activity in K562 cells, suggesting that the antiproliferative signal from C-PC and Celecoxib may be partially mediated through PKC. Also the present data demonstrating the induction of apoptosis is preceded by arrest of cells in the S and G2 phase of the cell cycle engine such as cyclin-dependent kinases and phosphorylation events regulated by other kinases.

Heat shock proteins are known to modulate apoptotic cell death induced by various stimuli and would modulate the balance between cell death and survival. The present study also demonstrated an increase in mitochondrial Hsp60 levels during C-PC and Celecoxib induced apoptosis. It was reported that telomerase activation and telomerase catalytic subunit gene (hTERT) are correlated with the deregulation of apoptosis. The telomere hypothesis postulates stabilization of telomere length and telomerase activation as key events in cellular immortalization and carcinogenesis. Accordingly, telomerase could be a novel and highly selective target for antitumor drug design. The present studies indicate an apparent decrease in telomerase activity in K562 cells with C-PC treatment for up to 5 days and hence this biliprotein from Spirulina platensis could
be a candidate for cancer therapy. **Immunological** studies employing C-PC polyclonal antibodies and fluorescent *in situ* hybridisation on confocal microscope suggest that C-PC enters into the K562 cells and is concentrated in the cytosol. However, it is not clear whether the entry of C-PC into the cells is mediated by any cell surface receptors or by other mechanisms.

In conclusion our studies reveal that C-PC, a naturally occurring biliprotein from *Spirulina platensis* and a known COX-2 inhibitor, induces apoptosis in chronic myeloid leukemia K562 cells and is comparable with Celecoxib, a known COX-2 inhibitor and a chemopreventive agent. This is confirmed by MTT assay, nuclear condensation assay (DAPI staining), electron microscopic studies (SEM & TEM), DNA fragmentation, PARP cleavage and FACS analysis. The induced apoptosis is accompanied by the release of cytochrome c into the cytoplasm and downregulation of Bcl-2 expression, c-Myc overexpression, and downregulation of PKC activity. The overall signal transduction mechanism of C-PC induced apoptosis in K562 cells is presented in Fig.36. Being a natural compound and having characteristic stability and solubility in aqueous solution, C-PC could form a more acceptable chemopreventive and/or chemotherapeutic agent for CML patients. Further in depth studies on CML patients, however, are required to test this possibility.