SUMMARY AND CONCLUSION
Cancer is the second largest single cause of death in children and adults. The international union against cancer has defined cancer as a disturbance of growth characterized by excessive proliferation of cells without apparent relation to the physiological demands of the organs involved. All cancers are multifactorial in origin. They include genetic, hormonal, metabolic, physical, chemical and environmental factors. Major advances in the treatment of cancer have resulted from the recent evolution on medical interventions. However significant heterogeneity in the efficacy and toxicity of the chemotherapeutic agent is consistently observed across the human population (Evans and Relling, 1999). Administration of the same dose of an anticancer drug to a population of patients results in a range of toxicity, from unaffected to lethal events (Sargent et al., 2001). An ideal drug is one, which selectively affect the cancer cells without being injurious to healthy tissue.

In recent years many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals, cephalopods, sea slugs, etc. (Donia et al., 2003; Haefner, 2003). Squid and cuttlefish come under the class Cephalopoda (phylum -Mollusca), which eject ink from their ink sac to escape from their predators. Crude ink of cuttlefish, Sepia pharaonis was evaluated for its toxicity on chick embryo. The crude ink of cuttlefish showed significant inhibitory effect on the development of chick embryo. The size of the ink treated chick embryo was considerably reduced compared to the control. Most of the chemotherapeutic drugs are having cytotoxic effect on growing cells. The ink also induced DNA fragmentation in treated embryos. The
results of this preliminary investigation showed the inhibitory effect of the crude ink of cuttlefish which warranted further studies on the therapeutic potential of the ink of cuttlefish, *Sepia pharaonis*.

Antiproliferative effect of the Tris-HCl extracted ink of cuttlefish and squid was investigated on chick embryo fibroblast cells. Three different species of cuttlefish (*Sepia pharaonis, Sepia aculeata, Sepiella inermis*) and one species of squid (*Loligo duvauceli*) were used for the study. The results revealed that among different species of cuttlefish and squid ink extract, the extract from *Sepia pharaonis* ink showed highest antiproliferative effect on chick embryo fibroblasts. So further studies were carried out using the ink of *Sepia pharaonis*. The significant reduction in the number of viable cells in chick embryo fibroblasts was a clear indication of the antiproliferative effect of cuttlefish ink extract. The ink extract also induces nuclear condensation and DNA fragmentation, both hallmarks of apoptosis. Hence the significant antiproliferative and apoptosis inducing activity might be one of the major contributing factors for the anticancer activity of the ink extracts.

The ink from cuttlefish, *Sepia pharaonis* was first extracted using Tris-HCl and fractionated using ion exchange and gel filtration chromatography. The molecular mass of the purified fraction was determined to be 10 kDa. The chemical analysis of the different fractions revealed that the protein content is decreased with increasing purification. Further analysis showed that the fraction C2 was a peptidoglycan made up of 84.4% uronic acid rich polysaccharide, 5.6% peptide and 10% pigments (carotenoids). HPLC analysis of the peptide part showed that it is made up of five aminoacids namely aspartic acid, serine, threonine, glutamic acid and alanine. Studies carried by different
researchers revealed that the polysaccharide from different biological sources have antitumour activity. So the antitumour and anticancer properties of the purified peptidoglycan fraction from cuttlefish ink were further studied in vivo and in vitro.

The antitumour activity of peptidoglycan fraction C₂ of the cuttlefish, *Sepia pharaonis* was studied using Dalton’s lymphoma ascites in BALB/c mice. The cytotoxic activity of the fraction C₂ was studied in vitro using Trypan blue dye exclusion assay. The results showed that the fraction had no direct cytotoxic activity. The results revealed that there was a 70% increase in the life span of DLA bearing mice treated with the purified peptidoglycan (C₂ fraction). The results also revealed that the antitumour activity is increased with increasing the purification of fractions. The polysaccharide fraction showed 66% increase in the life span of DLA bearing mice. The results revealed that the active component responsible for the antitumour activity of purified fraction C₂ might be the peptide polysaccharide complex. The protein bound polysaccharides isolated from the mushrooms have been used as an immune therapy agent in the treatment of cancer in Asia for over 30 years (Wasser and Weis, 1999).

The fraction C₂ which showed highest antitumour activity was studied for its effects on tissue antioxidant enzymes, antioxidants, lipid peroxidation products and neurotransmitters. It is increasingly realized that majority of diseases/disorders are mainly due to imbalance between pro-oxidant and anti-oxidant homeostatic phenomenon in the body. There was a significant decrease in the activities of the antioxidant enzymes and antioxidants in DLA bearing mice compared to normal mice. These values were brought to almost normal levels in the DLA bearing mice treated with the purified peptidoglycan (C₂ fraction). Lipid peroxidation products were found to be increased
significantly in DLA bearing mice, but they were lowered to the normal values in mice treated with C₂ fraction. The Reactive oxygen species (ROS) play a major role in tumour progression by cellular damage. The present study indicated that the disease promotion might have taken place through ROS. As the tumour progresses, the antioxidant enzymes and the antioxidants are affected and this might be affecting the normal cell resulting in cell death. Treatment of the lymphoma with the peptidoglycan could control the changes in lipid peroxidation and antioxidant efficiency, resulting in the re-establishment of cellular metabolism and control of cellular damage. Thus, peptidoglycan isolated from the cuttlefish ink showed antitumour activity by stimulating the antioxidant status of the experimental animal, thus arresting the tumour progression. Hence the significant antioxidant activity might be one of the major contributing factors for the antitumour activity of the fraction. This indicated the therapeutic potential of the purified fraction C₂.

Although chemotherapy is a widely accepted method for cancer treatment, chemotherapeutic agents show several toxic side effects. Activities of serum SGPT, total protein and albumin levels were measured to evaluate the hepatotoxicity of the fraction C₂ of cuttlefish ink. S-GPT was found to increase significantly in DLA bearing mice. But this value was decreased to the normal value in mice treated with the C₂ fraction. However, the level of total protein and albumin did not show any significant variation among the three groups. The results revealed that peptidoglycan treatment of the DLA bearing mice imparted a hepatoprotective effect. Effects of C₂ fraction on different neurotransmitters (5-HT, HIAA, DA, HVA, NE, and EPI) were studied. The results revealed that the neurotransmitters in the two regions of the brain were significantly lower in the mice bearing DLA when compared to the normal mice. On the other hand,
the treatment with C₂ fraction resulted in the elevation of neurotransmitter levels, may be
due to the increase in the level of vitamin C required for the biosynthesis of
neurotransmitters.

Cancer of the uterine cervix is the most common female malignancy in the world. In India, nearly one lakh women develop this cancer every year, constituting about 16% of the world’s annual incidence (Das et al., 2000). Hence compounds, which are useful in the therapeutic intervention of cervical cancer, have a major role in delimiting the incidence of human cancers. Purified fraction C₂ of cuttlefish, Sepia pharaonis showed a significant dose dependent anticancer activity on cervical cancer cells in vitro. The fraction also induced typical morphological characters of apoptosis like chromatin condensation, membrane blebbing and DNA damage. The findings suggest the profound anticaricinogenic activity of purified peptidoglycan fraction on cervical cancer cells and thus renders itself as a potential chemotherapeutic drug for the treatment of cervical cancer.