Recent times have seen an increase in the incidence of cancer. It is a serious clinical problem that possesses significant social and economic challenges to the healthcare system. Despite improved imaging and molecular diagnostic techniques, cancer continues to affect millions of people globally. In many countries, it is the second leading cause of death after cardiovascular diseases.

Cancer is a disease of misguided cells which have high potential of excess proliferation without apparent relation to the physiological demand of the process. These cells have the ability to spread into adjacent tissues by direct growth or are capable of implantation into distant sites producing either a benign or a malignant tumor.

Cancer affects people at all ages with the risk for most types increasing with age. According to the American Cancer Society, deaths arising from cancer constitute 2-3% of the annual deaths recorded worldwide (American Cancer Society, 2006). It is the second most common cause of death in the developed world and a similar trend has emerged in the developing countries too (Stewart et al, 2003). In the United States, cancer is the second leading cause of death and accounts for one in four deaths whereas in developing countries, it accounts for one-tenth of all deaths. In India, it is estimated that there is about 1.5 million cases of cancer in the country at any given point of time with about 0.5 million new cases being added every year. Cancer prevalence in India is estimated to be around 2.5 million, with over 8,00,000 new cases and 5,50,000 deaths occurring each year due to this disease (Nandakumar et al, 1996).

The World Health Organization estimates that only 4% of all cancers are inherited and that the majority of cancers are preventable. Even though the biochemical aspects of malignant diseases relating the molecular biology of DNA and RNA synthesis, protein and polypeptide biosynthesis, enzyme activity and membrane receptor to ultrastructural and cellular compounds have been discussed in a number of reviews, the exact causes of cancer are only partly understood. It is believed that cancers arise from both genetic and environmental factors that
lead to aberrant growth regulation of a stem cell population or by the dedifferentiation of more mature cell types.

The causes of cancer are diverse, but the common thread in all known cancers is the acquisition of abnormalities in the genetic material of the cancer cell and its progeny. Cancers are primarily an environmental disease with 90-95% of cases due to lifestyle and environmental factors and 5-10% due to genetic predisposition. Among the common environmental factors leading to cancer are tobacco (25-30%), diet and obesity (30-35%), infections (15-20%), radiation, stress, lack of physical activity and environmental pollutants.

All cancers are different and require different treatment. Treatment for cancer depends on the type of cancer, the size, location and stage of the tumor and the person's general health. Conventional cancer treatment methods include surgery, radiation, chemotherapy, hormone therapy and biological therapy. Newer and experimental approaches include hematopoietic stem cell transplant, use of nanoparticles, minicells and gene therapy.

Although curative treatments are available for some cancers including childhood leukemia and testicular cancer, many metastatic solid tumours are not sensitive to current therapies. Thus the need to find a safe and highly effective cure for neoplastic diseases remains a major challenge for modern science.

Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. WHO estimates that approximately 80% of the world’s inhabitants rely on traditional medicine for their primary health care (Farnsworth et al, 1985) and that plants have long been used in the treatment of cancer (Hartwell, 1982). It is anticipated that plants can provide potential bioactive compounds for the development of new leads to combat cancer. The Vinca alkaloids vincristine and vinblastine derived from Catharanthus roseus are the two most important cancer chemotherapeutic agents in current use followed by the semi-synthetic podophyllotoxin derivative, etoposide. Colchicine derivatives are also used to treat cancer in some countries of the world and the pyrrolizidine alkaloid monocrotaline is used topically in China to treat skin
cancers. A number of other plant derived antitumour agents have been subjected
to clinical evaluation including taxol, harringtonine, teniposide, indirubin and
ellipticine derivatives, of which taxol is a compound of exceptional chemical,
biological and clinical interest.

Even though natural products or natural product derivatives comprised 14
of the top 35 drugs in 2000 based on worldwide sales (Butlet, 2004), it has been
estimated that only 5-15% of the approximately 250,000 species of higher plants
have been systemically investigated for the presence of bioactive compounds
(Cragg et al, 2005). Thus the investigations in this field are highly promising and
have to be continued.

The present work is an attempt to study the effect of Clitoria ternatea
Linn. (Family: Fabaceae) as an anticancer agent. Clitoria ternatea is a perennial
twining herb seen widely distributed in India. The roots, seeds and leaves of this
plant are of medicinal importance. The plant is reputed for its folkloric use in
various ailments (Evans WC, 2002). Different parts of Clitoria ternatea have
been used in traditional Ayurvedic medicine for several diseases. The roots are
bitter, ophthalmic, laxative, intellect promoting, diuretic, anthelmintic,
depurative, sedative, aphrodisiac and tonic. It is used in ophthalmology,
helminthiasis, leprosy, leucoderma, elephantiasis, bronchitis, asthma, ascites and
fever (Warrier, 1995). The seeds are cathartic and are useful in visceralgia. Leaves
are useful in otalgia, hepatopathy and eruptions. The plant was used by Indian
traditional healers for treating ulcer, eye infections, bronchitis and tuberculosis.

Different parts of Clitoria ternatea have also been used in traditional
Ayurvedic medicine for the treatment of several diseases. The plant extract is
reported to have significant effects on the central nervous system (Jain et al,2003).
The roots of Clitoria ternatea have a reputation for promoting intellect
(Misra, 1998). The methanolic extract was found to possess antipyretic and anti
inflammatory activities (Devi, 2003). The crude extract from seeds of the plant
showed strong antifungal activity on the test fungus Rhizoctonia Solani Kühn.
(Kelemu et al, 2004). The protein was also found to be useful as a biopesticide.
Chloroform and methanol extracts of Clitoria ternatea showed significant
antibacterial activity against enteric and urinary pathogens (Babu et al, 2009). Studies also suggest that Clitoria ternatea leaf and flower extracts exhibit antihyperglycemic effect in alloxan-induced diabetes (Daisy et al, 2009). The leaves and flower extracts of the plant also showed antihyperlipidemic effects and may be useful in the alleviation of liver and renal damage associated with alloxan-induced diabetes in rats (Daisy et al, 2009). Crude methanol extract of leaves, seeds and stem-bark of Clitoria ternatea demonstrated a significant antioxidant (Kamkaen et al, 2009) and cytotoxic activity (Shahidur et al, 2006). The plant also exhibited anthelmintic (Khadatkar et al, 2008) sedative and larvicidal effects (Nisha et al, 2009). Though studies have been conducted on the anticancer activity of the plant, very little data is available in this context. The present investigation is an attempt to assess the efficacy of Clitoria ternatea as an anticancer agent. The objectives of the study include

1. In vitro screening of cytotoxicity of the extracts of selected medicinal plants using murine cancer cell lines.
2. To study the antiproliferative effect of the methanolic extract of Clitoria ternatea by MTT assay.
3. To evaluate the in vitro antioxidant potential of the methanolic extract of Clitoria ternatea.
4. To study the antitumour effect of the extract in EAC and DLA ascites and transplanted solid tumours in Balb/c mice.
5. To study the anticarcinogenic effect of methanolic extract of Clitoria ternatea in murine two stage skin chemical carcinogenesis model.
6. To study the apoptotic effect of the methanolic extract in cultured human cancer cell lines.
7. To identify the phytochemical components of the methanolic extract of Clitoria ternatea and to analyse these components by HPTLC and LCMS.
ORGANISATION OF THE THESIS

The thesis is divided into 9 chapters.

Chapter I  Introduction
Chapter II  Review of literature
Chapter III Materials and methods
Chapter IV  In vitro cytotoxic and antioxidant potential of Clitoria ternatea L.

Screening of certain selected medicinal plants including Clitoria ternatea for cytotoxicity was done by the trypan blue dye exclusion method. The murine cancer cell lines DLA and EAC were used for the in vitro screening studies.

The study reveals the cytotoxic potential of Clitoria ternatea. In vitro screening studies by the trypan blue method demonstrated that of the selected plants, Clitoria ternatea possess maximum cytotoxic activity against DLA and EAC cell lines. Further studies showed that the methanolic extract was more effective than the other organic extracts.

The antiproliferative effect of the methanolic extract of Clitoria ternatea was further confirmed by MTT assay using human cancer cell lines. The cell lines used for the study include MCF7, SW480, HeLa and HCT116.

The results of MTT assay is a reliable indicator of the efficacy of methanol extract of Clitoria ternatea against various human cancer cell lines. Different concentrations of MECT was studied for its cytotoxic effects for a period of 24 and 48hrs using MTT assay and it was demonstrated that the extract possess considerable cytotoxic effects.

The antioxidant potential of the methanolic extract of Clitoria ternatea (MECT) was evaluated in vitro using reducing power assay, DPPH radical scavenging assay, hydroxyl and superoxide anion radical scavenging activity and lipid peroxidation assay. It was observed that MECT exhibited a dose dependent antioxidant activity in vitro. This proves that the methanol extract of Clitoria ternatea could act as radical scavengers against different free radicals under conditions of oxidative stress.
CHAPTER V Antitumour activity of *Clitoria ternatea* in DLA, EAC and DMBA induced chemical carcinogenesis

The cytotoxic effect of methanolic extract of *Clitoria ternatea* on Dalton’s lymphoma (DLA) and Ehrlich ascites carcinoma (EAC) were studied. The studies were conducted using Balb/c mice. The effect of MECT on ascitic models was evaluated by measuring parameters like tumour volume, total cell count, viable/non-viable tumor cell count and percentage increase in life span. Various haematological parameters (RBC, WBC, DC, Hb) were also evaluated.

To study the effect of MECT on transplanted solid tumours, reduction in tumour volume and percentage increase in life span were determined. The assay of the antioxidant enzymes superoxide dismutase, catalase, glutathione-S-transferase and glutathione peroxidase were done to determine the *in vivo* antioxidant effects of the plant. The levels of thiobarbituric acid reactive substances, conjugated dienes and reduced glutathione content were also estimated as a measure of antioxidant efficacy of the plant.

The levels of the transaminases, alanine transaminase (ALT) and aspartate transaminase (AST) and the phosphatases alkaline phosphatase (ALP) and acid phosphatase (ACP) were also determined. Assay of the enzymes lactate dehydrogenase and gamma glutamyl transferase were also performed and total protein was estimated by Lowry’s method. The levels of blood urea and creatinine were also evaluated to assess nephroprotective effect of the extract. MECT was found to be effective against EAC and DLA ascites and transplanted tumours. The administration of the methanol extract to the DLA and EAC ascitic models led to a decrease in the tumour volume, total cell count and viable/non-viable tumor cell count. The life span of the treated group was also increased when compared to the control. The haematological parameters (RBC, WBC, DC) decreased in the treated group, whereas the haemoglobin level was found to be elevated when compared to normal.

The effect of the plant extract on transplanted solid tumours was demonstrated by a reduction in tumour volume and an increase in the life span.
of the treated group as compared to control. The levels of the antioxidant enzymes superoxide dismutase, catalase, glutathione-S-transferase and glutathione peroxidase were estimated in the tissues of DLA and EAC bearing mice. The levels of the antioxidant enzymes were found to be lower in the treated group when compared to the control. The levels of thiobarbituric acid reactive substances, conjugated dienes and reduced glutathione content were also found to be brought to near normal levels in the treated group.

The methanol extract of *Clitoria ternatea* was also found to be able to lower the levels of the transaminases and the phosphatases in the treated group, which were brought to almost normal when compared with the control group. The levels of blood urea and creatinine were also lowered to near normal levels. The levels of the enzymes lactate dehydrogenase and gamma glutamyl transferase were also found to be lower in the treated group compared to the normal. The total protein content of the treated group was found to be elevated when compared with the control group.

The chemopreventive effect of methanolic extract of *Clitoria ternatea* was studied in murine two stage skin chemical carcinogenesis model. In this study, skin papillomas were produced on dorsal shaven skin of Balb/c mice. Dimethylbenzantracene (DMBA) was used to induce papillomas in mice and croton oil was used as promoter.

The methanolic extract of *Clitoria ternatea* exhibited considerable anticarcinogenic effects. The number and the percentage of mice with papillomas during different stages of the study were noted as a measure of the chemopreventive effect of MECT. It was observed that the extract reduced the extent of papilloma formation and its latency period in MECT administered groups.

**CHAPTER VI Apoptotic effects of Clitoria ternatea**

The plant extract was also subjected to apoptotic studies. Hoescht staining and TMRM (tetramethyl rhodamine methyl ester) assay were done to study the apoptotic potential of the plant extract.
The plant extract exhibited remarkable apoptotic potential. Hoescht staining was done to assess the extent of nuclear condensation. TMRM assay was also performed using the human cancer cell lines HCT116 and HeLa. Various concentrations of MECT were tested for their effect on membrane permeability for 12, 24, 48 and 72 hours. From the results, it was observed that the methanol extract of *Clitoria ternatea* could induce apoptosis in various cancer cell lines.

CHAPTER VII  Phytochemical analysis of *Clitoria ternatea*

Qualitative analysis of the plant extract was conducted and the phytochemical components present in the methanolic extract of *Clitoria ternatea* were identified. The components of the methanol extract were further analysed by HPTLC and LCMS.

The results of phytochemical analysis indicated the presence of alkaloids, saponins and flavonoids in *Clitoria ternatea*. The total phenolic content and flavonoid content were estimated as a measure of the antioxidant activity of the plant. The presence of anticancer agents like quercetin was demonstrated in the methanol extract of *Clitoria ternatea* by LCMS analysis.

CHAPTER VIII  General Discussion

CHAPTER IX  Summary and conclusion

*Clitoria ternatea*, a plant belonging to the Fabaceae family was investigated for its anticancer activity. The cytotoxic effects of the methanol extract of the plant was evaluated using DLA and EAC cell lines. Chemopreventive ability of the plant was also assessed in murine two stage chemical carcinogenesis models. An effort was also made to evaluate the antioxidant and apoptotic potential of *Clitoria ternatea* and to study its phytochemical constituents. The results demonstrated that the plant was effective against DLA, EAC and DMBA induced chemical carcinogenesis. It also exhibited remarkable antioxidant and apoptotic effects. So it is concluded that *Clitoria ternatea* possess significant anticancer and antioxidant activity.