INTRODUCTION

Cancer of the cervix is the commonest genital tract malignancy in the female and it has been ranked second to breast cancer. About half a million new cases are seen worldwide each year, most occurring in developing countries (Awodele et al., 2011 and Ertem, 2009). In 2008, approximately 5,30,000 women were diagnosed with invasive cervical cancer worldwide and 2,75,000 women died from it. Cervical cancer is the top cancer site for women in most East African and South Asian countries both in terms of incidence and mortality (Ferlay et al., 2010 and Arbyn et al., 2011).

India accounted for a quarter of both the world’s estimated cervical cancer burden of 5,29,000 cases and 2,75,000 deaths in 2008 (Ferlay et al., 2010). Cervical cancer is the most frequent primary site of cancer among Indian women with an estimated incidence and mortality rates of 27 and 15 per 100,000 women respectively in 2008 (Ferlay et al., 2010). Furthermore, India shows the highest rates of cervical cancer worldwide, especially among rural populations (Rajkumar et al., 2000 and Swaminathan et al., 2009a).

A risk factor is something that increases chances of developing a disease or condition. Epidemiological studies have identified a number of risk factors such as infection with certain oncogenic types of human papilloma viruses (HPV), sexual intercourse at an early age, multiple sexual partners, multiparity, long-term oral contraceptive use, tobacco smoking, low socio economic status, infection with Chlamydia trachomatis, micronutrient deficiency and a diet deficient in vegetables and fruits, that contribute to the development of cervical cancer (IARC Working Group, 1995 ; Walboomers et al., 1999 and Ferenczy and France, 2002). However, the primary underlying cause for cervical cancer is human papilloma virus (HPV) (Marrazzo et al., 2001).
Although there are several strains of HPV infection, two strains: HPV 16 and 18, account for more than 70% of all cervical cancer cases; five other strains; HPV 31, 33, 35, 52 and 58 account for an additional 20% of cases (Bosch and Desanjose, 2003). However, there is considerable regional and between country variation in this association, with HPV 16/18 prevalence in invasive cervical cancer cases ranging from 65% in South/Central America to 76% in North America (Smith et al., 2007). In India, prevalence of HPV 16/18 in invasive cervical cancer cases is 82.5% (National Cancer Registry Programme and World Health Organization, 2002). HPV is largely asymptomatic, making it difficult to recognize and detect among the general population, which will limit any behaviour modification (Singh, 2005). Vaccinations may thus provide a solution for prevention. Two different vaccines that have been developed to prevent infection from HPV 16 and 18 and one of these offers added protection against HPV 6 and 11.

Cervical cancer is preventable and curable if detected at an early stage (WHO, 2006). Since early detection predicts better prognosis, one of the most effective ways of preventing and controlling cervical cancer is regular screening and early diagnosis. The most effective method of screening employed in the developed world has been cytology based using pap smears, which has contributed considerably to reducing incidence of, and mortality from, cervical cancer (Miller et al., 1990). However this method of screening requires excessive resources in terms of laboratories, equipment, trained personnel and transport of specimens (Miller et al., 2000). Lack of adequate financial and human resources in developing country settings has prevented the quick uptake of such cytology based screening programmes at the population level.

However, there are socio-cultural barriers to cervical cancer screening in India. Lack of privacy and confidentiality during screening, cultural norms encouraging modesty among women and insufficient importance given to women’s health issues to be significant barriers to cervical cancer screening (Dabash et al., 2005). It is advocated only for women, at least to begin with,
and the target age group is adolescent girls. The parents may not give consent and the young adult women may not be willing to go for such a vaccine. The health care providers may also be reluctant to recommend the vaccine to general population due to their personal beliefs and anxieties about the parental reactions (Basu and Chowdhury, 2009).

Epidemiological studies in present and past have significantly contributed in our understanding about cancer associated risk factors and thus prevention of cancer. This study is an initiative to assess the awareness among women about screening procedures (Pap test) and vaccination programmes against cervical cancer. Apart from major risk factors, this study attempts to find out some of the minor risk factors of cervical cancer among patients in Western Tamil Nadu covering Coimbatore, Erode, Karur and Salem attending Valavadi Narayanasamy Cancer Centre, G.Kuppusamy Naidu Memorial Hospital, Coimbatore.

Identification and development of natural products used for cancer prevention have attracted a lots of attention globally. Herbal extracts with their proven potential and less side effects in therapeutics have replaced the synthetically derived drugs in modern allopathic medication system (Sakthivel and Guruvayoorappan, 2012). Evidence has been provided that dietary phytochemicals may play important roles as chemopreventive or chemotherapeutic agents in the prevention of many diseases, possesses antimutagenic effects and indeed modulating and stimulating the immune system (Raskin et al., 2002 and Rates, 2001) that in turn results in normal functioning of the whole body.

The plant of tea (Camellia sinensis) has been grown in South East Asia for thousands of years and now is cultivated in more than 30 countries around the world. Its consumption has reached a point where it has become the second most commonly consumed beverage worldwide. This popularity was due to its characteristic aroma, flavor and most influencing health benefits (Ahmad et al., 1998 and Harbowy and Balentine, 1997). The term ‘green tea’
refers to the product manufactured from fresh tea leaves by steaming or drying at elevated temperature with the precaution to avoid oxidation of polyphenolic components (Chow and Kramer, 1990). Like most herbs, the precise composition of green tea varies with the geographic origin of the leaf, the time of harvest and processing techniques.

Green tea contains several antioxidant compounds, known as polyphenols which plays a key role in diseases. Epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallo catechin (EGC) and epicatechin (EC) are some of the polyphenol compounds found in green tea. EGCG is a valuable scavenger of reactive oxygen species and has strong antioxidant activity (Norwood et al., 2006 and Bagchi, 1999). It protects cellular damage by inhibiting DNA damage and oxidation of LDL and many putative health benefits of tea are presumed to be caused by its antioxidant effects. The protective effects of EGCG are due to its ability to decrease lipid peroxidation, oxidative stress, and the production of nitric oxide (NO) radicals by inhibiting the expression of inorganic nitric oxides. It also ameliorates the over production of pro-inflammatory cytokines and mediators, reduces the activity of NF-kappa-B and AP-1, and the subsequent formation of peroxynitrite with NO and reactive oxygen species (Tipoe et al., 2007).

Polyphenols account for the pungency and the unique flavor of green tea. Catechins and other polyphenols present in green tea are antioxidant and anti-inflammatory in nature and have been shown to possess anticarcinogenic activity (Baliga and Katiyar, 2006). The biological activity of green tea is due to different catechins and EGCG is identified as the principal antioxidant contributing approximately 30% of the total antioxidant capacity of green tea and has been recognized as the major and potentially effective chemo preventive agent present in green tea leaves (Stewart et al., 2005; Ahmad et al., 1998 and Katiyar and Mukhtar, 1996). Antioxidants protect the body by neutralizing the free radicals and donating one of their electrons, thus ending the scavenger reaction. They have been found to be quite successful
in the prevention of certain diseases for years especially cancer (Thomas, 1995 and Frankel, 1984).

Oxidative damage to DNA promotes mutation and thus enhancing the risk of carcinogenesis. In modern times, man is exposed to a multitude of environmental poisons (Renner, 1990). Numerous environment and host factors, some of which are known and many are unknown, contribute to cancer development. Repeated exposures of small doses of natural toxicants may lead to hazardous situations (Iverson, 1991). It is difficult to eliminate mutagenic / carcinogenic factors present in our environment but is possible to eliminate with the risk of cancer through simple dietary and herbal recommendations (Block et al., 1992).

Reports on potent antimutagenicity of green tea reveals that EGCG is perhaps the most potent antimutagenic agent protecting DNA scissions and non-enzymatic interception of superoxide anions. This leads to the general conclusion that development of cancer is prevented by tea consumption through antimutagenic protection paralleling to their antioxidant efficacy. In the present investigation an attempt was made to evaluate the antimutagenic effect of green tea extracted using four different solvents viz., petroleum ether, chloroform, ethanol and water in *Salmonella* microsome assay.

Experimental tumor models have a wide role in anticancer drug discovery. A Dalton’s Ascites Lymphoma (DAL) tumorigenesis model in Balb/C/Swiss albino mice provides a convenient model system to study antitumor activity within a short time (Shanker et al., 2000). Dalton’s Ascites Lymphoma is referred as an undifferentiated carcinoma and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumour specific transplantation antigen (TSTA) (Ozaslan et al., 2011). Following transplantation of DAL cells into the abdominal cavity of healthy recipient mice, tumorigenesis begins immediately and aggressively.
Recently many of the beneficial effects of green tea including the prevention of cancer were attributed to its most abundant catechin, EGCG (Moyers and Kumar, 2004 ; Mandel et al., 2004 and Higdon and Frel, 2003). However the preparation methods influence the catechins both qualitatively and quantitatively. Since the preparation of fresh green tea cannot totally extract catechins from the leaves, the concentration found differs from the absolute values determined through the complete extraction of leaves (Fernandez et al., 2000). The amount of catechins also varies in the original tea leaves due to differences in variety, origin and growing conditions (Khokhar and Magnudottir, 2002). Thus comparison of ingested doses in animal studies is not possible because the catechin quantification before administration is often not known. In light of the above observations, in the present study, the crude methanolic extract of green tea was evaluated for its in vitro cytotoxicity and in vivo antitumor properties along with its antioxidant potential in DAL model.

Green tea has been shown to inhibit the occurrence of many different types of cancers (Zou et al., 2002 ; Mao et al., 2011 ; Khan et al., 2012 and Nguyen et al., 2012). In many animal studies, the polyphenolic fraction isolated from green tea, the water extract of green tea, or individual polyphenolic antioxidants present in green tea have been shown to afford protection against chemically induced carcinogenesis in lung, liver, oesophagus, forestomach, duodenum, pancreas, colon and breast (Ahmad et al., 1998 and Katiyar and Mukhtar, 1996).

In the developing world cervical cancer remains a common malignancy impacting the lives of women during their period highest productivity. In a low resource country like India, an inexpensive dietary chemopreventive intervention would be an attractive adjunct to existing cervical cancer prevention programmes. Being an easily affordable drink, green tea can be consumed by women as a daily beverage.
Recent epidemiological study conducted by Jia et al. (2012) suggested that women drinking green tea have a lower risk of developing cervical cancer. However, studies of the relationship between cervical cancer and green tea are limited, and the observational data are very rare. In this study, an attempt was made to find out the effect of green tea on the antiproliferation of cervical cancer cells (HeLa).

Human lymphocytes in culture constitute an ideal test system to evaluate the cytogenetic damage induced by environmental factors (Rossner et al., 2005). The use of chromosomal alterations as markers of early biological effects is well established in genotoxicity studies. A relationship between chromosomal damage and cancer development has been suggested since the beginning of the 20th century, but only since 1960 have extensive data been gathered on the frequency of chromosomal alterations (CAs) in Peripheral Blood Lymphocyte Culture (PBLC) of humans exposed to known or suspected genotoxic carcinogens. The idea of causal association between chromosomal alterations and cancer risks based on the concept that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis.

For carcinogenic processes in the target tissues, structural chromosomal aberrations in peripheral blood lymphocytes have been applied for over 30 years in occupational and environmental settings (including radiation dosimetry) as a biomarker of early effects of genotoxic carcinogens. Chromosomal aberrations (CA) include chromosomal breaks and exchanges visible in arrested metaphase stage cells and are usually divided into Chromosome Type Aberrations (CSAs) and Chromatid Type Aberrations (CTAs), which differ from each other morphologically. Chromosome type aberrations involve the same locus on both sister chromatids on one or multiple chromosomes whereas CTAs affect one or several sister chromatids of a chromosome or several chromosomes. The frequency of cells with structural chromosomal aberrations in peripheral blood lymphocytes is the first genotoxicity biomarker that has actually shown an association with overall
cancer risk. In the present study, the reduction of chromosomal damage by the addition of green tea extracts to peripheral blood lymphocyte culture is taken as a biomarker tool for DNA repair capacity of green tea. Therefore, the DNA repair capacity of green tea extract in cultures of human peripheral blood lymphocytes of cervical cancer patients was examined.

In view of the above, the present investigation was undertaken with the following objectives.

- To study the epidemiology of cervical cancer in Western Tamil Nadu
- To estimate the antimutagenic potential of green tea extracts using Ames reverse mutation assay.
- To evaluate the antitumor activity of green tea extract against Dalton’s Ascites Lymphoma (DAL) cell lines.
- To estimate the antiproliferation effect of green tea extract in cervical cancer cell line (HeLa)
- To study DNA repair capacity of green tea extracts on cultured lymphocytes of cervical cancer patients.