1. INTRODUCTION

Cancer is a major worldwide public health problem. It is the uncontrolled growth of abnormal cells in the body. Currently surgery radiotherapy and chemotherapy are all used to treat different forms of cancer. They can each be used alone or together depending on type, location and spread of the cancer.

Cervical cancer is the most commonly diagnosed malignancy in women ranks second among women population. It is the cancer that forms in tissues of the cervix and usually a slow growing cancer. Approximately 80% of cervical cancer occurs in developing countries. It affects about 16 among 100,000 women per year and kills about 9 among 100,000 per year (Kent, 2010).

Human Papillomavirus (HPV) Infection is a necessary factor in the development of almost all cases of cervical cancer. There are many different types of HPV, some strains lead to cervical cancer and other strains may cause genital warts. HPV infection is limited to the basal cells of stratified epithelium, the only tissue in which they replicate. The HPV life cycle strictly follows the differentiation program of the host keratinocyte. It infects the tissue through micro-abrasions and process of infection is slow takes 12-24 hours for initiation of transcription.

Upon infection, the viral genome is transported to the nucleus and establishes itself at a copy number between 10-200 viral genomes per cell. Integration of HPV genome with the host cell results in the expression of E6 and E7 genes present in HPV genome. The function of E6 and E7 proteins alters host cell metabolism to favor neoplastic development by inactivating two tumor suppressor proteins p53 (inactivated by E6) and pRb (inactivated by E7) which results in development of oncogenic property to host keratinocyte to form cancerous lesion (Chaturvedi et al., 2010).
Most HPV infections in young females are temporary and have little long-term significance. 90% infections are resolved in 1 or 2 years (Goldstein et al., 2009). The immune system helps to clear HPV infection by antibodies that play a major neutralizing role naturally, but in certain cases the Virions still reside on the basement membrane and cell surfaces (Schiller et al., 2010). When the infection persists longer, about 5-10% of infected women have high risk of developing precancerous lesions of the cervix, which can progress to invasive cervical cancer. This process takes 10-15 years, but this can be prevented by regular screening and treatment of the pre-cancerous lesion. The early stages of cervical cancer are completely asymptomatic, but vaginal bleeding indicates the presence of malignancy.

Generally pap smear screening every 3-5 years with appropriate follow-up can reduce cervical cancer incidence up to 80%, the presence of pre-cancerous changes results in abnormal cervical cells. If precancerous lesion or cervical cancer is detected in early stage it can be monitored and treated relatively and prevent invasive cancer. In developed countries like USA cervical cancer incidence were low due to regular screening of pap smear test which reduces the cancer cases when treated earlier.

Treatment of cervical cancer varies according to the stage of the cancer. Systemic treatment for cervical cancer with chemotherapy initially consisted of single drug, later combination therapy had employed. Advanced stage tumors (IIB-IVA) are treated with radiation therapy and cisplatin-based chemotherapy. On June 15, 2006, the US Food and Drug Administration (FDA) approved the use of a combination of two chemotherapy drugs hycamtin and cisplatin for women in late-stage (IVB) cervical cancer treatment (FDA, 2006). Combination therapy shows more effect in treatment of cancer. A vaccine to prevent cervical cancer is available. There are two types of HPV vaccine (Gardasil and Ceravix) are used in developed countries to reduce the risk of cancerous or precancerous changes of the cervix. There is no effect of chemotherapy were observed in some cases of cervical cancer in certain patients due to one of the reason is cancer recurrence.
When cancer comes back after treatment it is called cancer recurrence, in such cases patients should get regular treatments with chemotherapy, radiation therapy throughout their life time. One of the reasons for cancer recurrence may be development of drug resistance to common chemotherapeutic drugs used in cancer treatment.

One of the major causes of chemotherapy failure in cancer treatment is multidrug resistance (MDR). It shows resistance to general anticancer drugs used and shows cross resistant to many different structurally unrelated drugs causing multidrug resistance. Human increasingly employs chemicals as chemotherapeutic agents to treat not only to cancer but also bacterial, viral, parasitic infection. The repeated use of these chemicals often leads to becoming ineffective due to other onset of resistance by the target cells.

Drug resistance can arise as a consequence of various biochemical mechanisms in cancer including reduced drug delivery, decreased drug uptakes, reduced metabolic activation of drug, increased deactivation of drugs, and sequestration of drug to prevent interaction with target sites.

MDR is over expression of the ATP-binding cassette (ABC) transporters such as p-glycoprotein (ABCB1 / p-gp), multidrug resistance proteins (ABCC / MRP) and breast cancer resistant protein (ABCG2 / BCRP). These transporters actively efflux a variety of structurally and functionally diverse chemotherapeutic drugs out of cancer cells, thereby reducing the intracellular cytotoxicity (Cserepes et al., 2004). Among these transporters the ABCB1 transporter is the most important mediator of MDR and is responsible for chemotherapeutic drug resistance to a variety of drugs including vinca alkaloids, anthracyclins, epipodophyllotoxins and taxanes (Szakacs et al., 2006).

MDR in cancer cells may appear upon prolonged treatment with anticancer drugs. MDR may result from enhanced drug efflux resulting from a membrane glycoprotein of 170 kDa (p-gp) encoded by the mdr1 gene in human cancer cells. This protein appears to cause MDR via an ATP-dependent drug efflux mechanism, which prevents the intracellular accumulation of drugs to an effective cytotoxic concentration.
Reversal of MDR has been accomplished *in vitro* by a series of agents like verapamil, trifluoperazine, reserpine, quinidine, vinca alkaloid analogs and cyclosporines. In general these agents used to antagonize MDR are called chemosensitizers. The mechanism responsible for this reversal of resistance is believed to be competition between the modulator and cytotoxic drug for binding to the ATP-dependent efflux pump p-gp.

For clinical utility of any modulator, depends not only on its ability to reverse drug resistance at low concentration but also on whether it has a low toxicity *in vivo*. Although these chemosensitizing agents are effective p-gp inhibiting agents, one of the major problem with most these inhibitors is that the *in-vivo* plasma concentration required to obtain inhibiting on p-gp are too high resulting severe toxic side effects. The cardiac toxicity seen during the clinical evaluation of verapamil as a chemosensitizing agent pointed out the need for less toxic modulator (Rossi *et al.*, 1994). A number of pharmacological agents have been shown to reverse MDR *in vitro* but there needs to identify more potent, more specific and less toxic chemosensitizers for clinical use.

Recently many efforts have been made on a global scale to discover new drugs using plant extracts as screening libraries. These phytochemicals have the advantage of being dietary compounds that are less toxic to animals, plentiful and inexpensive (Govindarajan, 1980). Dietary phytochemicals have been found to be very promising in reversing the resistance to anticancer.

Turmeric powder has been used for centuries as a spices, coloring and therapeutic agents. Curcuminoids - a natural phenolic coloring compound from rhizomes of turmeric (*Curcuma longa* L.), Curcuminoids is the major yellow pigment and contains about 1-5% in rhizome of turmeric. The rhizomes contain three major pigments of curcuminoids such as Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin (Ammon and Wahl, 1991).
Curcuminoids has a wide range of biological and pharmacological activities including, anti-oxidant, anti-cancer, anti-inflammatory, anti-fungal, anti-parasitic, anti-venom, anti-mutagenic activity in vitro, anti-carcinogenic effects, hypocholesterolemic effects in rats, hypoglycemic effects in human, antiviral effective against warts caused by human papillomavirus.

Since curcuminoids pigments vary in chemical structures, it is possible that the physico-chemical characteristics as well as functional properties would vary among them. It could be important to obtain these pigments in high purity for detailed study of their biological properties.

A number of studies are undertaken to separate curcuminoids pigments by Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) and Column Chromatography (CC) using silica gel as stationary phase. The detailed analytical techniques like HPLC, NMR, IR, and GC-MS have been employed for characterization of individual pigments. The safety of curcuminoids and its derivatives has been studied in various animal models. It showed commercial grade curcuminoids down-regulated both MDR1 gene expression and p-gp function (Anuchapreeda et al., 2002).

Multidrug resistance in human and rodent cell lines is associated with increased p-glycoprotein expression in many multidrug resistant cell lines results from amplification of the MDR1 gene. Failure to respond to chemotherapy due to drug resistance represents a critical problem in the treatment of cervical cancer. Akiyama et al., demonstrated that among many cell line studied KB cell line showed high efficiency of cloning and sensitivity to common MDR drugs. HeLa origin of KB cells reveals the presence of HPV-18 specific sequences without major structural reorganization (Akiyama et al., 1985). Therefore drug resistance phenotype for cervical cancer is studied in vitro in KB cell line containing HPV-18 DNA.
To investigate the basis of drug resistance in vitro, drug resistance cell lines have been isolated by exposing various cancer cells to increasing amounts of chemotherapeutic agents such as adriamycin, daunomycin, colchicine, doxorubicin, actinomycin-D, taxol, vinca alkaloids etc (Gottesman and Ling, 2006).

In colchicine series selection for drug resistance KBChR8-5 cell lines were commonly used to examine anti-MDR activity which was derived from stepwise increase concentration of colchicine treated KB cells. It is also important to compare parental cell line with resistance cell line to study anti-MDR activity because parental cell line doesn’t express the MDR phenotype.

The development of anti-MDR agents has become a major focus on overcoming cancer drug resistance. These agents could effectively inhibit the proliferation of cancer cells with MDR phenotype. Thus, looking for novel natural compounds and its derivatives with anti-MDR effect may be a useful strategy to circumvent MDR.

We demonstrate curcuminoids is found to be effective in circumventing MDR in cancer cells. The experiments were carried out on colchicine selective cell line (KBChR8-5) as resistant cell line in comparison with KB cell line (HeLa derivative) as parental cell line.

The first line of study for anti-MDR activity was assessment of MDR phenotype by cytotoxic effect of that drug. The effect of curcuminoids on cell expressing MDR phenotype was assessed by cytotoxic assays, the collateral sensitivity value indicates that curcuminoids can kill MDR cells more effectively than parental cells.

Since MDR expressing cells have over-expression of P-gp in drug resistance phenomenon, this increase in p-glycoprotein expression in many multidrug resistant cell lines results from amplification of the MDR1 gene. The effect of curcuminoids for cytotoxicity on MDR cells may reduce the MDR1 gene expression either directly or indirectly were analysed by gene expression studies of MDR1 gene by real time PCR.
This indicates reduced p-gp produced in MDR cells and results in decrease in efflux of anticancer drugs from MDR cells and may increase in the uptake of drugs into cells.

Curcuminoids may act as MDR modulator may either block the induction of MDR gene expression or inhibit its promoter and down-regulate p-gp expression or it can block p-gp transporter which inhibits efflux of anticancer drugs which are active against tumor resulting in cytotoxic to MDR cells at lower concentration.

Further to study the reversal effect of MDR, the curcuminoids which reduce the expression of MDR1 gene are involved in reversal effect and it can enhance the cytotoxicity of general drugs used in cancer chemotherapy. But in order to overcome drug resistance the most obvious response that widely employed is to use combination drug therapy. The general rationale for choosing drugs to combine is to use one drug which is active against the tumor when used individually and to combine another drug that have different mode and site of action to produce synergistic additive effect of reversing multidrug resistance. In our study this was employed by co-incubation of curcumin and doxorubicin. Both the compounds may induce cytotoxic effect either individually or in combination. Effective cytotoxicity was observed in combination drug which leads to cell apoptosis.

Apoptosis has been observed through confocal microscopy using AO / EtBr staining. The level of cells undergoing apoptosis were stained and visualized as early and late apoptotic cells. The cell that undergoes apoptosis leads to the cell cycle arrest at certain stages was analyzed by flow cytometry. The ability of curcumin to modulate cytotoxicity of doxorubicin in MDR cells results in increased number of apoptotic cells and cell cycle arrest which concluded combination drug shows anti-MDR activity.

In our study we examined three types of curcuminoids for effective cytotoxicity and effect of co-incubation with doxorubicin followed by modulatory effect of MDR1 gene expression. The cytotoxic effect of curcumin in combination with doxorubicin which results in decrease in efflux of anticancer drug (doxorubicin) and sensitize MDR
cells at low cytotoxic level to enhance the susceptibility of tumor cells to apoptosis induced by anticancer drugs.

These emerging results clearly suggest that specific targeting of MDR gene by natural agents may open new avenues for the complete eradication of tumors by killing the drug-resistant cells and improve survival outcomes in patients diagnosed with malignancies.