ANTICANCER ACTIVITY OF HERBO-MINERAL FORMULATION IN VITRO: A PRELIMINARY STUDY

Anita Mehta, Dipti Patel, Kuntal Shah, Bhavini Shah, Jayesh Beladiya and Kiranj Chaudagar

1Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad-380009, India.

ABSTRACT
Cancer is characterized by uncontrolled proliferation of cells with the potential to invade and metastasize. It is one of the leading causes of morbidity and mortality which affects the human population globally. Scientific and research interest is drawing its attention towards naturally-derived compounds as they are considered to have less toxic side effects compared to current treatments such as chemotherapy. The plant kingdom produces naturally occurring secondary metabolites which has been reported for its anticancer activities. In our study we have evaluated the anticancer activity of CYTOCRUEL a herbo minerals formulation which contain different plant extracts and minerals from the nature. The concentration dependent cell viability (MTT) assay of CYTOCRUEL was performed on the two different cancer cell lines that is MDA-MB-231(Breast cancer) and A549 (Lung cancer). The results of MTT assay CYTOCRUEL show dose dependent reduction in % of cell viability at 48 hour of treatment in both the cell lines as compared with normal control which suggests its effective use in treatment of cancer.

KEYWORDS: Cancer, Herbo-mineral, Cytocruel.

1. INTRODUCTION
Cancer is one of the most dreadful and fastest growing disease which is characterized by uncontrolled and rapid proliferation of cells. This cells may unite together to form ‘tumor’. These cells also has tendency of invasion and metastasis in adjacent tissues from site of origin, leading to death of the host if remain untreated. Chemotherapy is highly successful
in producing fruitful outcome in many cancers. An ideal anticancer drug should kill or incapacitate cancer cells without causing excessive damage to normal cells, which is difficult, or perhaps impossible, to attain.\(^2\) Moreover, cancer patients have to suffer frequently from side effects of chemotherapy which are more severe in numerous patients. Chemotherapy can make patient more likely to get infections because most anticancer drugs produce myelosuppression, making bone marrow harder to produce white blood cells (WBCs).\(^3\) Other side effects are alopecia, weakness, loss of weight, fever, peripheral neuropathy and other gastrointestinal side effects like Diarrhea, Loss of appetite, Anorexia, Vomiting, Constipation, Stomatitis, Burning sensation.\(^4, 5\) Expenses and associated side effects of anticancer drugs have provoked the research for drugs with minimum side effects and low in cost.\(^6\)

Plants have been used as an age old remedy for treatment of cancer.\(^7\) In fact, the medicinal plants are easily available, cheaper and possess less toxicity as compared to the modern allopathic drugs.\(^8,9\) Phytoconstituents promotes and strengthens digestion. It also rejuvenates the body tissues & controls the metabolic activities of the body.\(^6\) The antioxidants present in medicinal plants has been reported for remarkable anticancer activity. Rational use of this plant combination may definitely proves its efficacy in cancer management.\(^10\)

Breast cancer is now the most common cancer in Indian women accounting occurrence of 25.1 % and have mortality rate of 27.3 % of all cancers.\(^11\) Lung cancer is leading cause of cancer related death among men in India. It is the commonest in men accounting for 11.3% of all new cancers and also is the most common cause of cancer death (13.7%).\(^12\) After breast cancer, lung cancer is the second leading cause of cancer related death among women worldwide.\(^13\)

Herbo-mineral formulation is the combination of herbs and minerals designed for specific disease. This combination imparts edge for targeting multiple mechanisms in a disease to improve treatment.\(^14\) CYTOCRUEL, a herbo-mineral formulation was prepared to achieve the beneficial effect of a combinatorial regimen. It contains eight different genera of plants which include Curcuma spp., Cinnamomum spp., Boerhavia spp., Capsicum spp., Moringa spp., Glycyrrhiza spp., Tecomella Spp. and Syzygium spp. It also contains metals like Sulphur, Mercury and Copper. The herbs and minerals are individually recognized for its anticancer activity along with antioxidant, anti-inflammatory, anti-tubercular, immuno-stimulant, antidiarrhoeal, analgesic, hepatoprotective
and powerful rejuvenating activity. The species and contents of formulation are not revealed for intellectual property protection. Consequently, this work aimed to investigate the anticancer potential of a herbo-mineral formulation CYTOCRUEL in cancer cell line in breast and lung cancer.

2. MATERIALS AND METHODS

2.1 Reagents/chemicals: Modified Eagle’s Medium (MEM), Roswell Park Memorial Institute medium (RPMI 1640), Antibiotic-antimycotic solution and Fetal Bovine Serum (FBS) purchased from Gibco Invitrogen, USA. Doxorubicin was purchased from Sigma-Aldrich, St Louis, USA. Trypsin, 3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and sodium bicarbonate were obtained from Himedia, Mumbai, India.

2.2 Cell lines
Human lung carcinoma A549 and Human breast adenocarcinoma MDA-MB-231 were purchased from National center for cell science, Pune university campus, Pune, India. MDA-MB-231 cells were maintained in MEM and A549 was maintained in RPMI-1640 medium supplemented with 10% FBS and 1% antibiotic antymycotic solution. The cells were maintained in an incubator at 37±1˚C supplemented with 5% CO₂ atmosphere. The cells were harvested and subcultured when it reaches ~80% of confluency.

2.3 Cell proliferation assay: Cell proliferation assay was performed by using MTT. A549 and MDA-MB-231 cells (approx. 1x10⁴) were plated in 100 µl of RPMI-1640 and MEM respectively in a 96 well plate. After the 24 h of plating, 100 µl of CYTOCRUEL solution at concentration 0.125, 0.25, 0.5, 1 and 2 mg/ml was added to 96 well plate. Wells designated as normal control were supplemented with 100µl of drug free medium. The 96 well plate was incubated for 48 hour at 37°C in incubator supplied with 5% CO₂. 20µl of MTT (5mg/ml) was added to each well & plate was incubated for 3 hour. Absorbance was measured at 570 nm using ELISA plate reader (EL800 BioTek Instruments, Inc.). Experiments were carried out in triplicates.

2.4 Statistical analysis
Data were expressed as Mean±SD. Prism statistical analysis software (Graph pad software, version 5.0) was used for statistical analysis. Statistical analysis was performed by One-way ANOVA followed by Bonferroni’s multiple comparison test was used to evaluate significant
treatment effects. A P value of 0.05 and 0.001 or less was considered to be statistically significant.

3. RESULTS
3.1 Cell proliferation assay: MTT assay of CYTOCRUEL at concentration of 0.125, 0.25, 0.5, 1 and 2 mg/ml shows significant reduction in % of cell viability of MDA-MB-231 and A549 cells as compared to untreated cells at 48 h of treatment. The IC\textsubscript{50} value of CYTOCRUEL was 0.722 and 0.889 mg/ml in MDA-MB-231 and A549 cell lines respectively (Figure 1 and 2).

4. DISCUSSION
Herbal plants has gained scientific attraction for its therapeutic principles in cancer treatment over the years.\textsuperscript{[15]} Antioxidant agents from plants have gained special interest for scavenging the free radicals, one of the major risk factor for cancer development.\textsuperscript{[16]}

The herbo-mineral formulation CYTOCRUEL contains drugs from different herbs viz., Cinnamomum spp., Boerhavia spp., Capsicum spp., Moringa spp., Curcuma spp.,
Glycyrrhiza spp., Tecomella Spp. and Syzygium spp. It also contains metals like Sulphur, Mercury and Copper.

The leaves of *Cinnamomum kotoense* exhibited selectively antiproliferative effects in breast cancer (MCF-7 and MDA-MB-231) cell lines without showing any toxicity in normal mammary epithelial cells.\(^{[17]}\) *Cinnamomum osmophloeum* bark extract has also inhibited the growth of colonic (Colon 205) and breast(MCF-7) cancer cell lines.\(^{[18]}\)

Oxidative stress by reactive oxygen species has been known in the pathology of various diseases including cancer.\(^{[19, 20]}\) It is reported that antioxidant from plants protects the cells against oxidative stress by scavenging free radicals. *Boerhaavia diffusa* is a known traditional medicinal plant for improving health benefits because of its antioxidant property.\(^{[21]}\)

Capsaicin from capsicum spp. has been reported for potent inhibition of the growth of ER-positive (MCF-7, T47D, BT-474) and ER-negative (SKBR-3, MDA-MB231) breast cancer cell lines. The inhibition was associated with G0/G1 cell-cycle arrest, increased levels of apoptosis and reduced protein expression of human epidermal growth factor receptor (EGFR), HER-2, activated extracellular-regulated kinase (ERK) and cyclin D1.\(^{[22]}\) Capsaicin has also induced apoptosis in human small cell lung cancer cells via the TRPV6 receptor and the calpain pathway.\(^{[23]}\)

Previous studies suggests, the leaf extract of *Moringa oleifera* greatly induced apoptosis, inhibited tumor cell growth, and lowered the level of reactive oxygen species (ROS) in human lung cancer cells A549 and MCF-7 cells.\(^{[24]}\) Aqueous leaf extract of *Moringa oleifera* increases the sensitivity of chemotherapy in pancreatic panc-1 cancer cells by down-regulation of nuclear factor-kappaB.\(^{[25]}\)

Curcumin from curcuma spp .induced cell cycle arrest by DNA damage and increased apoptosis of A549 cells through the activation of caspases cascade and mitochondrial dependent pathway.\(^{[26]}\) Demethoxycurcumin (DMC), one of the main active compounds of curcuminoids found in curcuma spp. suppresses migration and invasion of MDA-MB-231 human breast cancer cell line.\(^{[27]}\)

Licorice Root extract (*Glycyrrhiza uralensis*) has been reported for inhibition of breast cancer cell (MCF-7) proliferation by modulating the pathways of Bcl-2/Bax apoptosis G1 cell cycle
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arrest.\textsuperscript{28, 29} *Tecomella undulata* bark extract shows antiproliferative activity on K562 cells.\textsuperscript{30}

In our study, we have evaluated the anticancer activity of herbo-mineral formulation CYTOCRUEL on breast cancer cells (MDA-MB-231) and lung cancer cells (A549). Cell viability assay of CYTOCRUEL has shown significant reduction in % of cell viability of both the cell lines which shows its cytotoxic action on MDA-MB-231 and A549 cells. The reduction in cell viability was in concentration dependent manner in both the cell lines. Further studies need to elucidate the mechanism of CYTOCRUEL before its clinical application.

5. CONCLUSION
The data of in-vitro study suggests CYTOCRUEL reduces the % of cell viability of two cancer cell lines MDA-MB-221 and A549 cell lines. It could render prospective candidate for the therapy of cancer. Further clinical trial will be of use in establishing it as a potential anticancer formulation.

6. REFERENCES


