



Determination of cytotoxicity of a poly herbal 'Meta-block 9' tablet powder and its phytosomes on MCF-7 and MCF-10A cell lines, *In-vitro*

Jayni J Maru*, Swati S Patil

Department of Pharmacognosy, Prin K M Kundnani college of Pharmacy, Cuffe Parade, Mumbai, Maharashtra, India

Abstract

The primary healthcare of majority of population is dependent on natural herbs. The concept of combining different herbs has been highlighted in the ayurvedic literature '*Sarangdhar samhita*'. Phytoconstituents like alkaloids, saponins, tannins, flavonoids alkenyl phenols, terpenoids, sesquiterpene lactones and phorbol esters contribute to the therapeutic effect of the natural herbs. Combination of different herbs with varying potency produces greater therapeutic effectiveness compared to the single herb. Poly herbal combination of drug mainly acts due to synergistic effect of the phytoconstituents present in the individual herbs. Meta-block 9 tablet consists of hydro-alcoholic extracts of herbs like haridra, amla, arjuna, ashwagandha, tulsi, marich, gudduchi, guggulu, and yashtimadhu. These herbs are used in ayurvedic rasayana therapy. Since the active phytoconstituents present in the poly herbal mixture have poly-phenolic multi ring structure and poor lipid, water solubility their absorption in the cell is significantly less. So the tablet mixture is formulated into a novel drug delivery system i.e a Phytosome. Active phytoconstituents present in the tablet have been reported to show significant cytotoxic potential. Thus *In vitro* cytotoxicity studies of the tablet powder and the phytosomal formulation is carried out on MCF-7 (Human breast adenocarcinoma cell lines) and MCF-10A (human breast epithelial cell lines). The tablet powder showed lesser IC50 values and greater cytotoxic potential compared to the phytosomal formulation on MCF-7 cell lines. Tablet powder showed lesser cytotoxicity on MCF-10A cell lines, whereas the phytosomes showed no cytotoxicity on normal breast epithelial cells by MTT assay.

Keywords: poly-phenolic, IC50, MTT, Synergistic, cytotoxic, ayurvedic

Introduction

Ayurveda an ancient system of medicine includes herbals as one of its most powerful healing ingredients. It focuses on eliminating the root cause of the disease in contrast to modern system of medicine which focuses only on the symptomatic relief. It is estimated by WHO that 80% of the population still relies on natural herbs for their primary health care.

Ayurvedic literature '*Sarangdhar Samhita*' has highlighted the concept of poly herbalism in ancient medicinal system. Most of the dosage forms prepared from ayurvedic herbals are poly herbal formulations as the concentration of the active phytoconstituent present in the individual plant is very less and has always proven to be insufficient to attain desired therapeutic effect. Thus combination of different plants of varying potency produces greater therapeutic activity as compared to the use of individual plant only, mainly due to positive interaction between the herbs present in different medicinal plants. Such an interaction between the herbs is known as synergism. Sometimes certain pharmacological actions of the active herbal constituents are significant only when they are potentiated by the phytoconstituents of some other plants, but are not prominent when used alone [1].

Combination of the herbs into poly herbal formulation might simultaneously act on numerous targets to attain desired action efficiently. The phenomenon of synergism in a poly herbal formulation confers extra benefits as compared to the single herbal formulation. Poly herbal formulation might lead to a number of benefits like [2].

- Reduced dose of herbal preparation to attain desired pharmacological action.
- Decreased risk of deleterious side effects due to large doses.
- Increased convenience for the patients as poly herbal formulation eliminate the need of administration of more than one herbal formulation simultaneously.
- Increasing the overall therapeutic potential and patient compliance [3].

These benefits have enabled the popularity of the poly herbal formulation in the market.

The natural herbs which aim to improve longevity, intelligence, youth complexion, memory, health, voice and motar strength are used in rasayana therapy. They are also used to elicit immune modulatory, antiageing and adaptogenic action. A poly herbal tablet namely Metablock-9 tablet is formulated from the hydro alcoholic extracts of haridra, amla, arjuna, gudduchi, guggulu, marich, tulsi, ashwagandha, and yashtimadhu [6].

The major active phytoconstituents present in the herbs exhibit significant *In-vitro* pharmacological action. However oral administration of the phytoconstituents extracted from the plant source leads to their decreased absorption in the human body. The main reason for poor absorption of phytoconstituents is multi-ring structure of polyphenols and poor lipid and water solubility. Thus Meta-block 9 tablet powder was formulated into a novel drug delivery system i.e a phytosome by combining the herbal extracts with Soy-phospholipids and cholesterol in organic

and aqueous solvents. In-vitro cytotoxic potential of the formulated phytosome and the Meta-block 9 tablet powder was determined by MTT assay ^[16].

MTT assay is a colorimetric assay used for determination of cell proliferation and cytotoxicity, based on reduction of the yellow colored water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by liver cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured at the wavelength of 570 nm by a spectrophotometer ^[17]. Description of various herbs present in the poly herbal mixture is as follows.

Table 1

Drug	Major active constituents
Haridra (<i>Curcuma longa</i> ; Fam: Zingiberaceae).	Curcuminoids like Curcumin, demethoxy curcumin, bisdemethoxy curcumin ^[3] .
Amalaki (<i>Embelica officinalis</i> ; Fam: Euphorbiaceae).	Derivatives of gallic acid and ellagic acid. Alkaloids, glycosides, resins, saponins, sterols and vitamin C ^[4] .
Arjuna (<i>Terminalia arjuna</i> ; Fam: Combretaceae).	Arjunic acid, arjunin, arjunolic acid, arjungenin, terminic acid, arjunolone, arjunone and arjunosides ^[5] .
Guduchi (<i>Tinospora cordifolia</i> ; Fam: Menispermaceae).	Triterpenoids, alkaloids, lignans and steroids. Miscellaneous chemical constituents like Giloin.
Yashtimadhu (<i>Glycyrrhiza glabra</i> ; Fam: Fabaceae).	Saponins like Glycyrrhizin and flavonoids like liquiritigenin, isoliquiritigenin, liquiritin ^[6] .
Marich (<i>Piper nigrum</i> ; Fam: piperaceae).	Alkaloids like piperine, lignans, flavonoids and essential oils containing pinene, linalool and limonene ^[7]
Guggul (<i>Commiphora weightii</i> ; Fam: Bruseraceae).	Guggulosterone, guggulipids and triterpene myrrhanol ^[8]
Tulsi (<i>Ocimum scantum</i> ; Fam Lamiaceae).	Volatile oils containing eugenol, methyl eugenol, carvacrol and sesquiterpene hydrocarbon caryophyllene ^[9]
Ashwagandha (<i>Withania somnifera</i> ; Fam solanaceae).	Withanolides, Withaferin A and Withaferin D and a few alkaloids and saponins are also present ^[10] .

Materials and Methods

Materials used are ^[17]

- Cell lines
 - MCF7- Human breast adenocarcinoma cell lines (From NCCS, Pune)
 - MCF-10A-Human breast epithelial cell line (From ATCC, USA)
- Cell culture medium: DMEM-High glucose media - (Cat No:2120785, Gibco)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- MTT Reagent (5 mg/ml) (# 4060 Himedia)

- DMSO (#PHR1309, Sigma)
- Cisplatin (#PHR1624, Sigma)
- D-PBS (#TL1006, Himedia)
- 96-well plate for culturing the cells (From Corning, USA)
- T25 flask (# 12556009, Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 ul tips (TORSON)(17)

Equipments

- Centrifuge (Remi: R-8°C).
- Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
- Inverted microscope (Biolink)
- 37°C incubator with humidified atmosphere of 5% CO₂ (Healforce, China)

Assay controls

- Medium control (medium without cells)
- Negative control (medium consisting of cells but not the experimental drug/compound)
- Positive control (medium with cells and 11uM of Cisplatin)

Steps followed

- Seeded 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Cells were allowed to grow for 24 hrs.
- Added appropriate concentrations of the test agent
- Incubated the plate for 24 hrs at 37°C in a 5% CO₂ atmosphere.
- After the incubation period, took out the plates from incubator, and removed spent media and added MTT reagent to a final concentration of 0.5mg/mL of total volume
- Wrapped the plate with aluminium foil to avoid exposure to light.
- Returned the plates to the incubator and incubated for 3 hours.
- Removed the MTT reagent and then added 100 µl of solubilisation solution (DMSO).
- Gentle stirring in a gyratory shaker, enhanced dissolution. Occasionally, pipette was moved up and down to ensure complete dissolution of the MTT formazan crystals especially in dense cultures.
- Recorded the absorbance on a spectrophotometer or an ELISA reader at 570nm and 630nm used as reference wavelength.
- The IC₅₀ value was determined by using linear regression equation i.e. $Y = Mx + C$.

Here, Y = 50, M and C values were obtained from the viability graph.

Results of MTT Assay

Effect of Meta-block 9 tablet powder on MCF-7 cell lines.

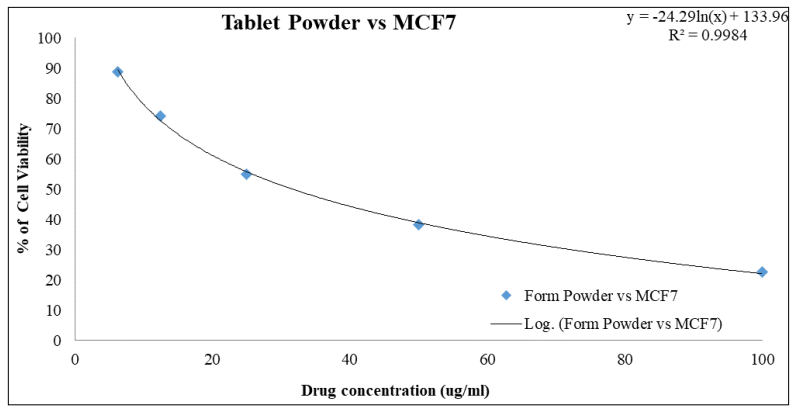


Fig 1

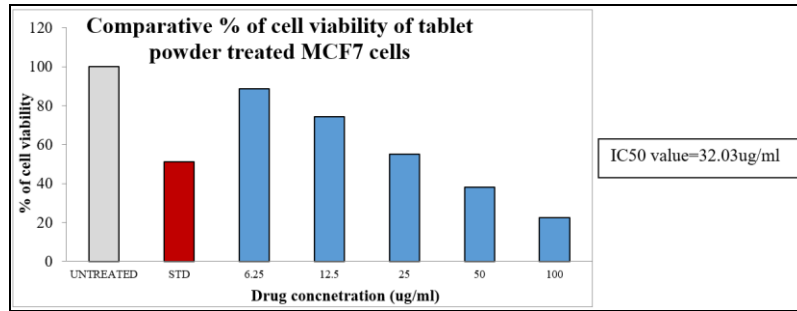


Fig 2

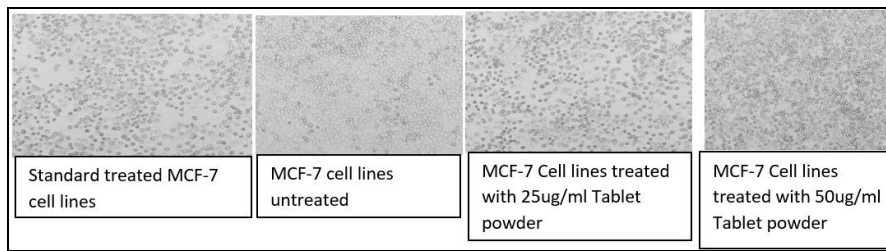


Fig 3

Effect of optimized phytosomes on MCF-7 cell lines

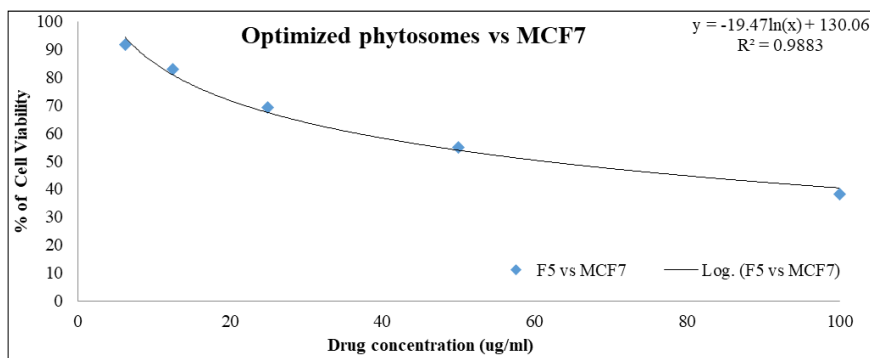


Fig 4

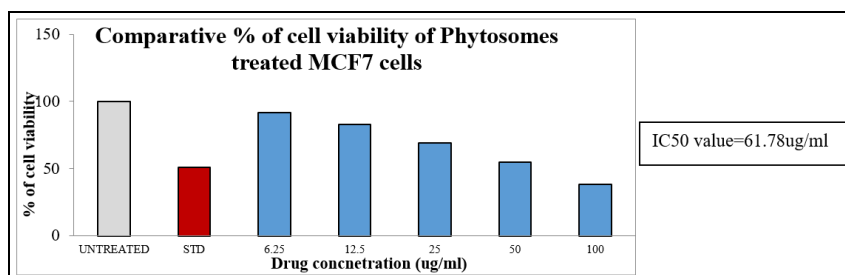


Fig 5

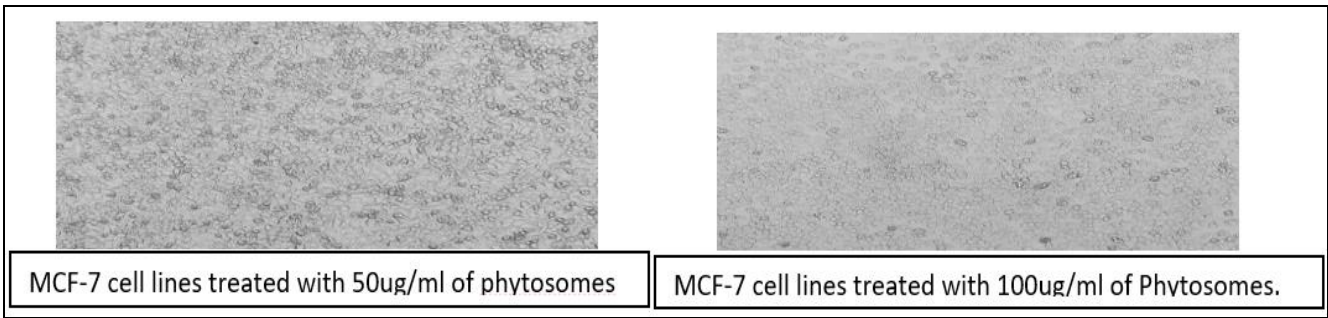


Fig 6

Effect of Meta-block 9 Tablet on MCF-10A cell lines

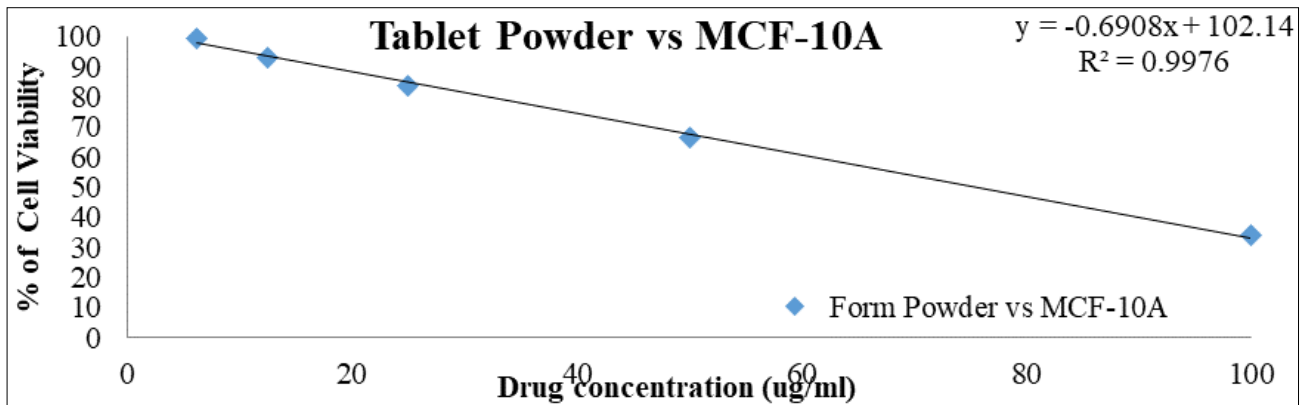


Fig 7

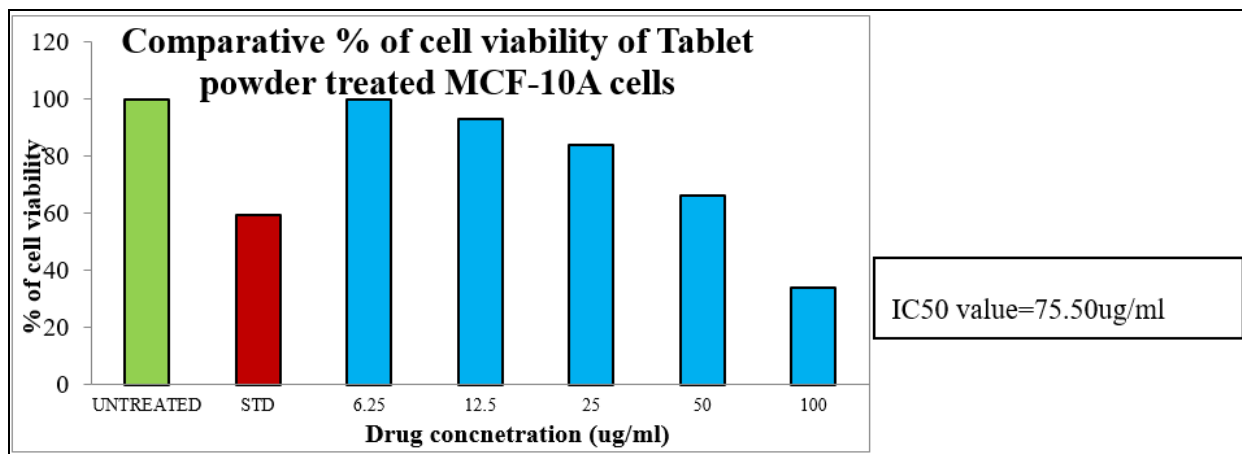


Fig 8

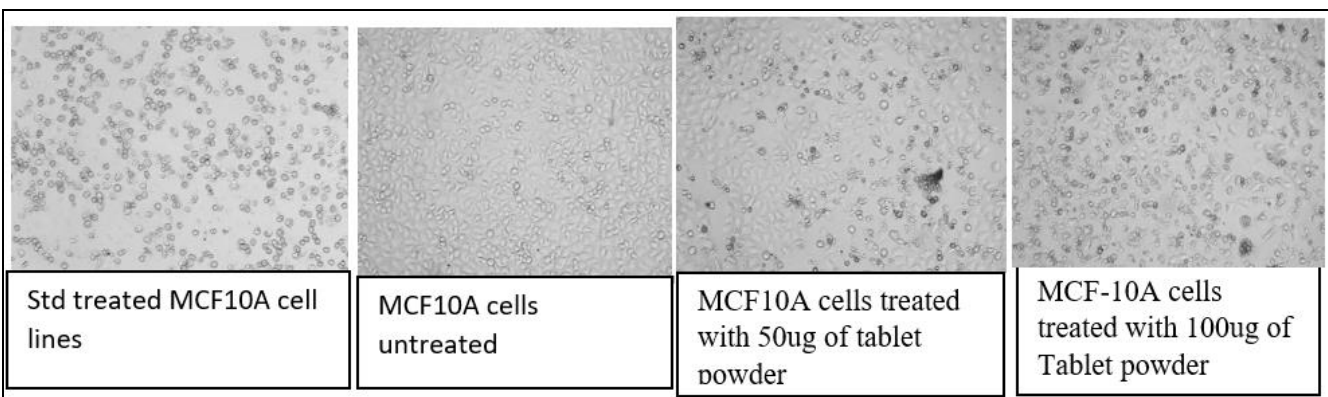


Fig 9

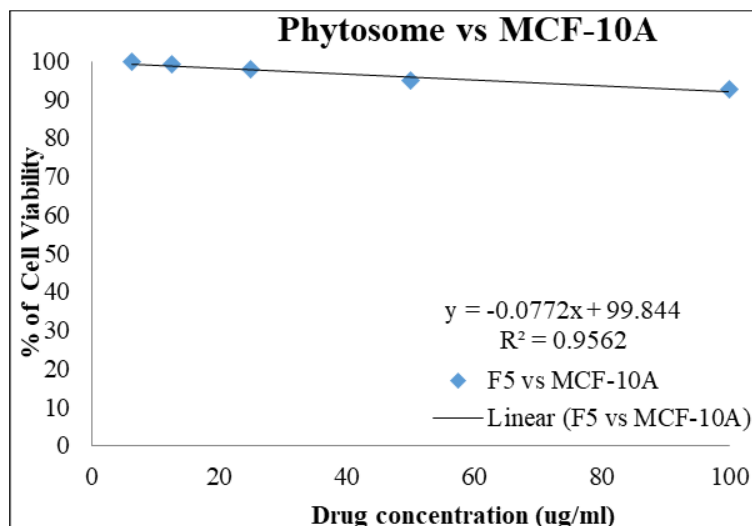


Fig 10

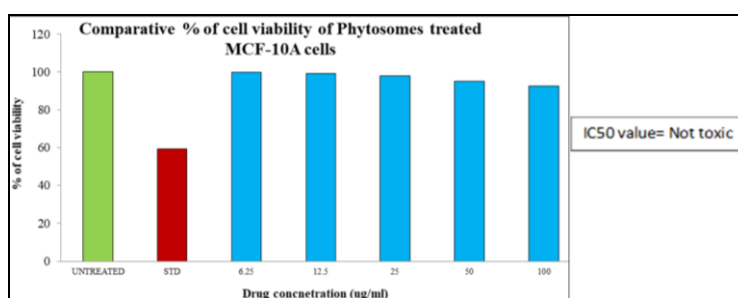


Fig 11

Conclusion

MTT assay is used for determination of cell proliferation and cytotoxicity. The observations and statistical data of cell toxicity study on MCF-7 (human breast adeno carcinoma) cell lines indicates that test compounds namely Meta-block 9 tablet powder and optimized Phytosomal batch prepared with the poly herbal extracts show significant cytotoxic potential with IC₅₀ concentrations at 32.03ug/ml and 61.78ug/ml respectively in comparison with the standard used for the study. However observations and statistical data of cell toxicity study on MCF-10A (human breast epithelial) cell lines indicates that Meta-block 9 tablet powder shows low cytotoxicity on normal breast cell lines with IC₅₀ value of 75.5ug/ml and the phytosomal batch showed no cytotoxicity on human breast epithelial cell lines. The Phytosomes can therefore be considered as the potent cytotoxic agent due to its low IC₅₀ value and non-cytotoxic action on normal breast cell lines. Poly herbal mixture of extracts has moderate cytotoxicity on MCF-7 cell lines and also shows minimum cytotoxicity on normal breast cell lines.

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