

Evaluation of Anti-Cancer Potential of Homeopathic Medicines by In-Vitro Study on Human Breast, Lung and Colorectal Cancer Cells

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ABSTRACT

The purpose of this study is to assess the cytotoxic effects of homeopathic drugs on cancer cell lines by In-vitro experiment.

Background: Homeopathy is a renowned therapeutic approach in many nations across the world. However, the approach has sparked much scientific discussion concerning its efficacy and methods of action. This is particularly true when it comes to the application of homeopathy in cancer treatment.

Objective: The purpose of this study was to evaluate the cytotoxic effect of homeopathic remedies on cancer cells and to emphasise the therapeutic effects of 50 Millesimal potencies.

Materials & Methods: Carcinosinum, Conium maculatum, and Asterias rubens were the drugs used in 0/1 potency against the MCF 7 breast cancer cell line. Carcinosinum, Nitric acid, and Ruta were the drugs in 0/1 potency used against the HCT 116 colorectal cancer cell line and the NCI-H460 lung cancer cell line. 90% alcohol medicated on poppy-sized globules, dried fully and used as a control for all cell lines. The cytotoxicity has been assessed using the standard MTT test.

Results: The LC50 values of the medicines tested on breast and lung cancer cells were not obtained up to 40000 ppm concentrations, as a result, cytotoxicity evaluation was not possible. The LC50 values of the medicines tested on Colo-rectal cancer cells were obtained around 20,000 ppm concentration and showed a gradual increase in Cell Death with increasing Concentration of exposure of the drugs. As a result, these drugs revealed cytotoxic qualities as well as anti-cancer actions.

Conclusion: Despite the objective facts, we cannot reach a definite conclusion about the anti-cancer effectiveness of these drugs in vitro because several aspects of homeopathy's action are unlikely to be replicated in In-vitro study. Determination of reasons for the same or understanding the molecular mechanism of resistance of the cell lines to the cytotoxic effects of the drugs were not within the purview of the study and these can be addressed in future studies.

Keywords: Homeopathy, Cancer, MCF-7, HCT 116, NCI-H 460, In-Vitro, Carcinosinum, Conium maculatum, Asteria rubens, Ruta graveolens, Nitric acid, 50 Millesimal Potency, MTT Assay.

INTRODUCTION

As the average life expectancy increases, the incidence of cancer, one of the primary causes of mortality, keeps rising. Breast, Lung and colon cancer are the most common types worldwide.^[1]

Breast cancer is the most frequent invasive cancer in women worldwide.^[2] Breast cancer, along with lung cancer, is the most often diagnosed malignancy, with 2.09 million cases each in 2018. Breast cancer affects one in every seven (14%) women worldwide. Breast cancer is a disorder in which the cells of the breast proliferate uncontrollably. There are various types of breast cancer. The type of breast cancer is determined by which cells in the breast develop into cancer. The majority of breast cancers start in the ducts or lobules. Breast cancer can spread outside the breast through blood vessels and lymph vessels. Breast cancer is said to have metastasized when it spreads to other regions of the body.^[3]

Lung cancer is the most common and deadly cancer in the world, with 2.2 million cases expected in 2020 and 1.8 million deaths. Lung cancer is uncommon in people under the age of 40; the average age at diagnosis is 70, and the average age at death is 72.^[4] Incidence and consequences vary greatly over the world, depending on tobacco usage patterns. Lung cancer pathogenesis is extremely complex and poorly understood. It is hypothesised that repeated exposure to carcinogens, such as cigarette smoke, causes lung epithelial dysplasia. If the exposure is prolonged, it causes genetic alterations and interferes with protein production. As a result, the cell cycle is disrupted and carcinogenesis is promoted.^[5]

The incidence of colorectal cancer (CRC), which is now the third most common cancer fatality globally, is rapidly increasing in developing nations. CRC, also known as colorectal adenocarcinoma, typically develops from the glandular, large intestine epithelial cells. When specific epithelial cells get a number of genetic or epigenetic alterations that provide them a selective advantage, cancer develops.^[6]

Homeopathy has many drugs that are used for the successful treatment of cancer. Studies on the effectiveness of homeopathic medications in animal models exist, but there has been little research on the in vitro actions of these drugs. To comprehend how homeopathic drugs work based on scientific facts, in vitro models with specific biomarkers are used.^[7] In this study 5 Homeopathic Medicines were selected from Clinical Materia medica and Repertories. Many in-vitro studies using Mother Tinctures, Decimal and Centesimal Potencies were available, however nothing was found in 50 millesimal potencies. As a result, the primary objective of this research is to provide scientific evidence as well as the action of 50 millesimal potencies in homeopathy by In-vitro study.

METHODS

CELL PROLIFERATION ASSAY- MTT ASSAY

Grown the cell line until it reaches 80% to 90% confluency before performing the assay. Two passages have done prior to using the cells for the experiment.

1. Seeded 2×10^5 cells/well in the 96-well plate in 100 μ L of complete growth medium. After seeding, swirled the plate incubated at 37°C in a humidified 5% CO₂ incubator and let the cells to grow.
2. Once it reaches above 70% confluency, aspirated the media and treated the cells by adding the medicines at various concentrations and the total volume was made up to 100 μ L using plain media. Proper control and blank have maintained for the experiment.
3. Cells along with media and no medicine (alcohol) would be considered as control and only plain Media would be used as a blank. Incubated at 37°C in a humidified 5% CO₂ for 24 hours.
4. Aspirated the media, added 10 μ L of MTT with 100 μ L of plain media. Incubated at 37°C in a humidified 5% CO₂ for 4 hours. Live cells take up MTT dye and form purple insoluble crystals called formazan.

5. After incubation, added 100µL of solubilization buffer, mixed well. Incubated at 37°C in a humidified 5% CO₂ overnight. This will ensure that the insoluble purple crystals get solubilised.

6. Checked absorbance at 570nm. Using the above protocol the LC₅₀ of the compound was be estimated. [8][9]

Calculation:

$$\text{Percentage cell viability} = \frac{\text{Absorbance treated} - \text{Absorbance blank}}{\text{Absorbance control} - \text{Absorbance blank}} \times 100$$

Cell percentage viability is essentially used for screening the response of the cells against a drug or a chemical agent. In particular, efficacy targeted studies widely use viability assays to evaluate the influence of developed agents on the cells. Here MTT assay is applied in order to screen the outcome of Homeopathic drugs targeted on cancer cells

Place of study: The entire experiment was carried out in NITTE University Centre for Science Education and Research

BREAST CANCER

Cell Line

MCF-7 (Human Breast Adenocarcinoma Cells)

Cell Line History

The breast adenocarcinoma-stricken pleural effusion of a 69-year-old woman was used to create MCF7. It is the most researched human breast cancer cell line in the world and was named after the Michigan Cancer Foundation (MCF). [10]

Key Characteristics

MCF7 cells are noteworthy since they retain certain traits that are similar to breast epithelium. Due to fluid build-up between

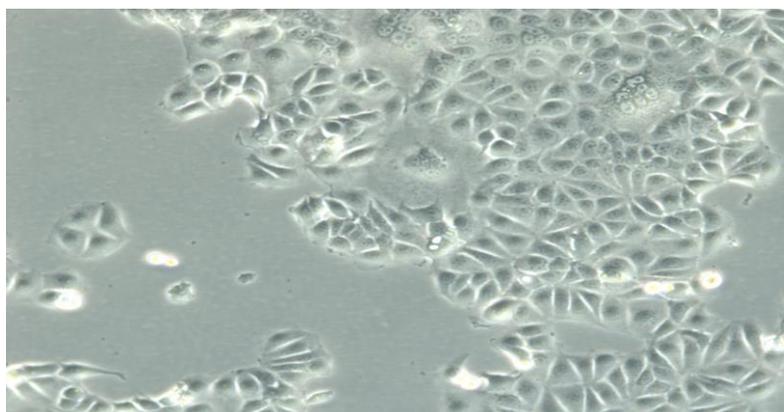
the cell monolayer and the culture dish, the cell line has an epithelial-like shape, and monolayers form dome formations. One of the few breast tumours that express oestrogen receptor alpha (ER-alpha) is this one. Additionally, the cells express androgen, progesterone, and glucocorticoid receptors, making them useful study tools in medical research. [11][12]

Homeopathic Medicine

- Interventions for breast cancer cell lines include Asterias Rubens, Carcinosin, and Conium.
- These are the homoeopathic cancer drugs that have been proven to be effective in clinical studies. [13]
- Potency: 0/1
- Poppy sized globules were medicated with 90 percent Alcohol, dried and then used as a Control

RESULTS

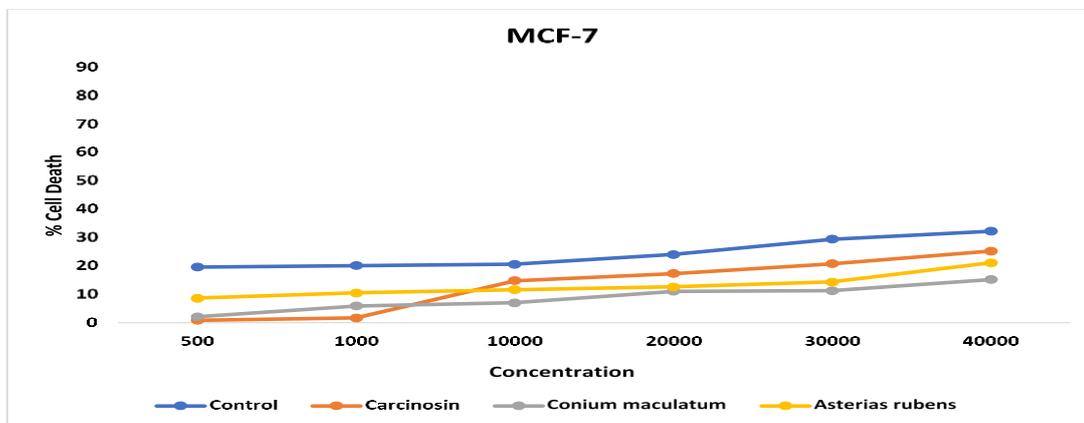
- The Drugs tested on MCF7 Cell line did not show a gradual increase in Cell Death and hence estimation of LC₅₀ was not possible.



Picture – 1: MCF 7 Cell Line under Microscope

LC50 Values

S.NO	HOMEOPATHIC MEDICINES	LC50 VALUE (IN PPM)
1	Carsinosinum 0/1	>40000
2	Asteria rubens 0/1	>40000
3	Conium maculatum 0/1	>40000
4	Control – Globules medicated with 90% alcohol, dried and then used	>40000



Graph - 1: Cell viability results showing minimal increase in cell death with increasing concentration of tested drugs in MCF7 cell lines

LUNG CANCER

Cell Line

NCI-H460 (Human Small Cell Lung Cancer)

Cell Line History

Developed in 1982 from a patient's pleural fluid with lung cell cancer; commonly used and extensively characterised cell line.^[14]

Key Characteristics

NCI-H460 cells are distinguished by their rapid proliferation and resistance to apoptosis, which is a type of programmed cell death that aids in the control of cell growth and division. A loss in the TP53 gene, which encodes for the p53 tumour suppressor protein, and a mutation in the RB1 gene, which encodes for the pRB tumour suppressor protein, are also present in these cells. NCI-H460 cells have been used to study several aspects of lung cancer biology, including as the signalling pathways that drive tumour growth and metastasis, drug resistance mechanisms, and the discovery of

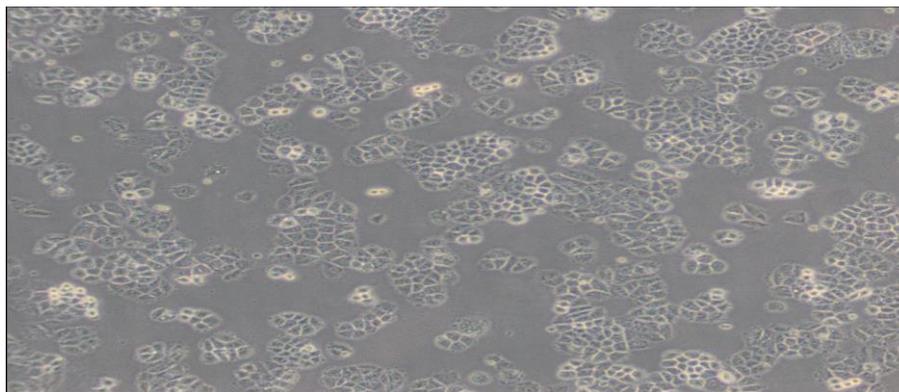
new therapeutic drugs. This cell line has also been used to investigate the roles of p53 and pRB in cancer development, as well as to evaluate the efficacy of novel medicines that target these pathways.^[14]

Homeopathic Medicine

- Interventions for breast cancer cell lines include Carcinosinum, Ruta graveolens and Nitric acid
- These are the homeopathic cancer drugs that have been proven to be effective in clinical studies.^[13]
- Potency: 0/1
- Poppy sized globules were medicated with 90 percent Alcohol, dried and then used as a Control

RESULTS

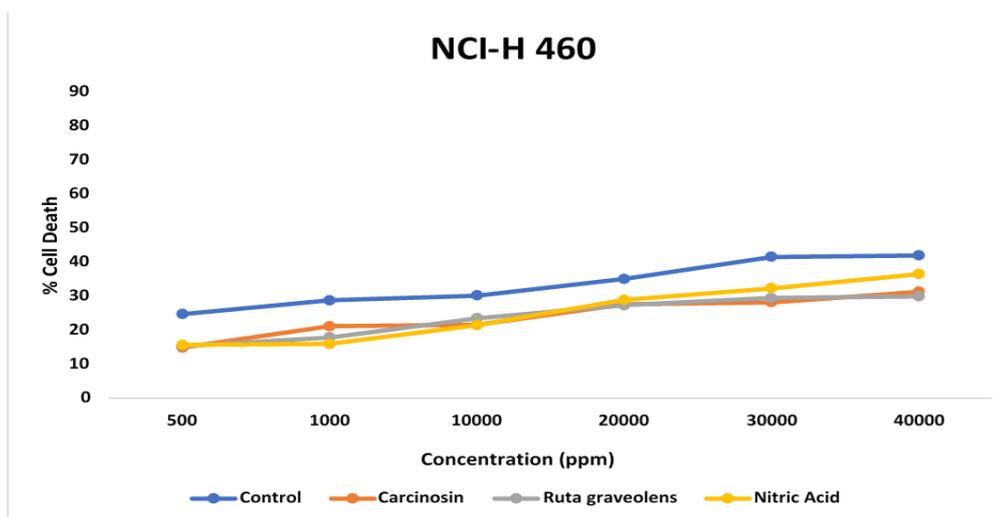
- The Drugs tested on NCI-H460 Cell line did not show a gradual increase in Cell Death and hence estimation of LC50 was not possible.



Picture – 2: NCI-H460 Cell Line under Microscope

LC50 Values

S.NO	HOMEOPATHIC MEDICINES	LC50 VALUE (IN PPM)
1	Carsinosinum 0/1	>40000
2	Ruta graveolens 0/1	>40000
3	Nitric acid 0/1	>40000
4	Control – Globules medicated with 90% alcohol, dried and then used	>40000



Graph - 2: Cell viability results showing minimal increase in cell death with increasing concentration of tested drugs in NCI-H 460 cell lines

COLORECTAL CANCER

Cell Line

HCT 116 (Human Colon Cancer Cell)

Cell Line History

HCT 116 is a Duke's type-A human colorectal cancer cell line derived from a 48-year-old male.^[15]

Key Characteristics

HCT116 cells grow in vitro with a doubling time of roughly 18 hours. They can be used for both in vivo and in vitro experiments. HCT116 cells have a mutation in KRAS

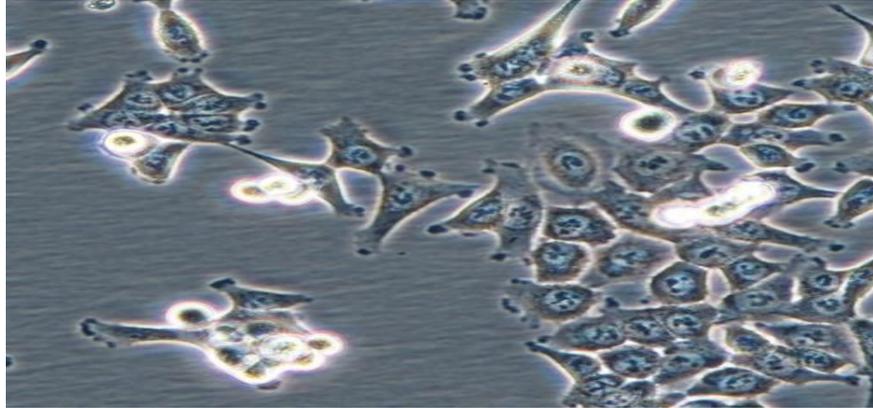
proto-oncogene codon 13 and are good transfection targets for gene therapy research.^[16]

Homeopathic Medicine

- Interventions for breast cancer cell lines include Carcinosinum, Ruta and Nitric acid
- These are the homoeopathic cancer drugs that have been proven to be effective in clinical studies.^[13]
- Potency: 0/1

- Poppy sized globules were medicated with 90 percent Alcohol, dried and then used as a Control
- The Drugs tested on HCT 116 Cell line showed a gradual increase in Cell Death with increasing concentration and hence LC50 values of each drug was estimated

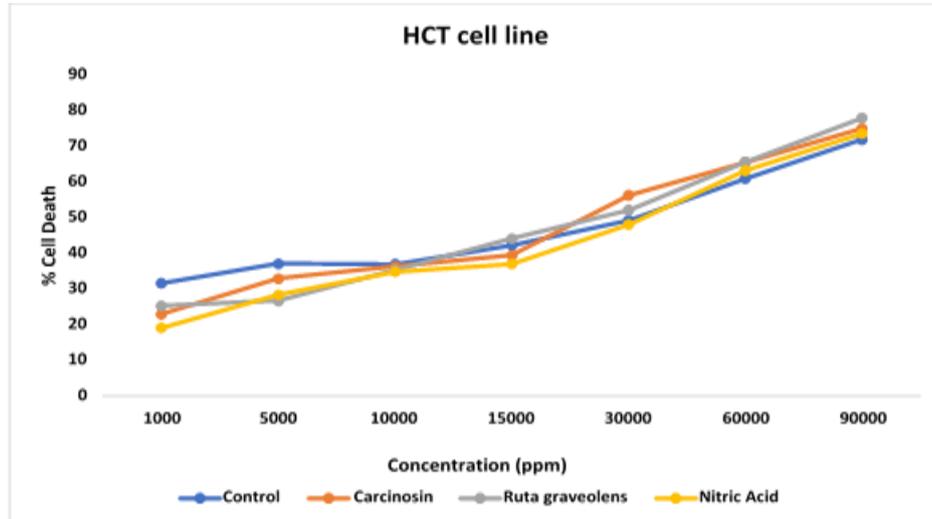
RESULTS



Picture – 3: HCT 116 Cell Line under Microscope

LC50 Values

S.NO	HOMEOPATHIC MEDICINES	LC50 VALUE (IN PPM)
1	Carcinosinum 0/1	19466.23
2	Ruta graveolens 0/1	20308.15
3	Nitric acid 0/1	25440.05
4	Control – Globules medicated with 90% alcohol, dried and then used	21506.55



Graph - 3: Cell viability results showing percentage cell death at varying concentrations of tested drugs on HCT 116 cells

CONCLUSION

We were unable to make any conclusions about the anti-cancer qualities of homeopathic medications based only on these findings. In-vitro studies have focused on a single mechanistic action and the mechanistic effect of homeopathy has yet to be investigated. Because the selected drugs

have proved effective in the treatment of cancer clinically. Animal research will be done in the future to better understand the mechanism of action and anti-cancer effects.

Declaration by Authors

Ethical Approval: Approved

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