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Research Article

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A Comparative study to evaluate the anti-cancer activity of siddha drugs "Veera Rasa Padhangam" and "Panchamuga Chendhuram" with the standard drug taxol

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Abstract

Background: Cancer remains to be the highest cause of mortality in the world and claims millions of human lives every year. Many systems are paying attention on cancer research to invent new interventions. It is evident that there are many anti cancerous drugs available in Siddha System of medicine. This study claims Veera Rasa Padhangam **(*VRP*), P**anchamuga Chendhuram **(*PMC*)** are two drugs commonly prescribed by Siddha physicians for *puttru*, it can be equated to cancer. However there is no scientific validation for this claim and this made the investigator to fix the hypothesis for its efficacy. **Materials and methods:** The Siddha drugs *VRP* and *PMC* tested for anti-cancer activity on human breast cancer cells MCF-7 was morphologically examined by phase contrast microscopy and the cell viability was determined by MTT assay. **Results:** *In vitro* studies showed that after 24 hrs and 48 hrs incubation, the IC 50 values of *VRP* were 63.62±0.03 µg/ml, 90.16±0.02 µg/ml and *PMC* were 65.03±0.05 µg/ml, 70.51±0.01 µg/ml compared with standard drug taxol 72±2.4, 75±3.2 µg/ml. **Conclusion:** Thus this study takes up scientific evidence the efficacy of the Siddha drugs *VRP* and *PMC* against the human breast cancer cell line MCF-7 and confirming its traditional use in cancer treatment.

Keywords: Siddha drug, Breast cancer, Cytotoxicity, Padhangam, Chendhuram, MCF-7

Introduction

Cancer is the third leading cause of death worldwide, preceded by cardiovascular and infectious diseases. It is a generic term for a group of more than 100 diseases that can affect any part of the body. Over the past few decades, breast cancer has become an increasingly important public health problem in developing countries, which currently contributes to half of the disease burden worldwide[1]. "One in eight Indians is likely to develop cancer in their lifetime" A Nandakumar, Head of National Cancer Registry said. For the year 2016; 1.5 lakh (10% of all cancers) women were newly diagnosed with breast cancer[2]. Although there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases[3]. Since an increasing proportion of cancer patients are acquiring resistance to chemotherapeutic agents, it is necessary to search for new compounds that provide suitable specific cytotoxic effects that can be developed as anticancer agents.

In siddha system of medicines there are so many preparations in classical text indicated for cancer. Cancer is known as '*Puttru*' in Siddha Medicine which literally means 'Termite mound' because of its proliferative nature[4].

Siddha system of medicine is divided into 3 major divisions, Plant kingdom, Inorganic compounds (IOC) and Animal kingdom. In Inorganic compounds there are 4 subdivisions they are Metals (Ulogam)-12, Minerals (Karasaram)-64, Hydrochemicals (Uparasam)-24 and Toxins (Paasanam)-120[5]. IOCs are usually made into preparations such as parpam, chendhuram, chunnam, padhangam, kattu, kalangu etc.

Herbo-mineral formulations are very vital in Siddha Medicine. This unique alchemical process was practiced by our Siddhars, even before this scientific era.

Importance of herbo-mineral formulations[6]:

1. Effective even in minimal dose.
2. Challenges incurable diseases.
3. Increased bioavailability.
4. Shelf life is higher compared to plant products.
5. Therapeutic efficacy is high.
6. Quick remedy.
7. The great specialty of Herbo-mineral formulation is adoptogenicity. (ie) the same drug can be successfully used for various diseases.

The metals and minerals present in these Herbo-mineral formulations are not present in elemental form. The finished form after reaction with several organic and inorganic materials of herbal origin is finally responsible for action, changing the properties of the toxic metal, making it therapeutically effective and provide safety[7]. *VRP and PMC* were used since ages for several purposes, almost negligible attention was provided by the scientific community for the scientific validation of these formulations.

Materials and Methods

Raw drugs were purchased from R. N. Rajan and co., Parry's, Chennai. Authentication were made by Pharmacognosist, Siddha Central Research Institute (SCRI), Chennai and Purification were made as per the Siddha classical literature.[8]

Panchamuga chendhuram preparation[9] ingredients:

- Purified *Rasam* (Hydragyrum) -100g,
- Purified *Gandhagam* (Sulphur) -100g,
- Purified *Thalagam* (Arsenic trisulphidium) -100g,
- Purified *Lingam* (Mercury II sulfide) -100g,
- Purified *Veeram* (Mercuric chloride) -100g,
- *Piper betel* leaf juice Q.S.

Procedure:

All the ingredients were grinded well for 1 day and made into (*villais*) pellets. The pellets were allowed to dry, then it is ignited for small flame (*Deepaagni*)- 6 hrs, moderate flame *(Kamalaagni)*-6hrs, high flame (*Kaadaagni)*-9hrs. After self cooling, the product was again subjected to grinding for 1 day. The final product (PMC) was weighed and stored in an air tight container.

Veerarasa padhangam preparation[10]:

Veeram (Mercuric chloride) - 100 g and *Urukku* (Steel)-100 g were grinded in a stone mortar, mercury was expelled out from the mixture. The mixture was subjected to the process of sublimation for 12 hrs. After self cooling the sublimated product was again subjected to sublimation for 12 hrs. The final product was weighed and stored in a glass vessel.

Cell Culture

Human breast cancer cells MCF-7 were obtained from National Center for Cell Science (NCCS), Pune, India. The cells were maintained in medium Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich, St. Louis, Mo, USA), with 100 U/mL penicillin and 100 µg/mL streptomycin as antibiotics (Himedia, Mumbai, India) in a humidified atmosphere of 5% CO_2 and 95% air in a CO_2 incubator (Heraeus, Germany).

Cell Viability Assay

To evaluate the cytotoxic property of VRP and PMC, the MTT colorimetric assay was performed. *VRP and PMC* drugs, in the concentration range of 0–50 µg/mL, dissolved in DMSO (Sigma-Aldrich), were added to the wells, 24 h after seeding of 5×10^3 cells per well of 96-well plate. DMSO was used as the solvent control. After 24 and 48 h of incubation, 20 µL of MTT solution (5 mg/mL) in phosphate-buffered saline (PBS) was added to each well, and the plates were wrapped with aluminum foil and incubated for 4 h at 37°C. The purple formazan product was dissolved by addition of 100 µL of 100% DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, USA). Data were collected for three replicates each and used to calculate the means and the standard deviations. The percentage inhibition was calculated from this data using the following formula:

$$\frac{\text{Mean OD of untreated cells (control) } - \text{Mean OD of treated cells}}{\text{Mean OD of untreated cells (control)}} \times 100$$

The IC_{50} concentration was determined as the dose that would be required to kill 50% of the cells with the respective preparation and duration.

Statistical analysis

The IC_{50} is the concentration of toxic compound that reduces the biological activity by 50 %. Numerical data are expressed as mean ± standard deviation (SD). Statistical differences were evaluated by a one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) software (Version 18.0 (SPSS) Inc., Chicago, Ill, USA). Posthoc test was performed for comparisons using the least significant difference (LSD) test. Differences were considered statistically significant when $P < 0.05$.

Results

After treatment with *VRP and PMC* drugs for 24 and 48 h, the cells morphology was transformed in both *VRP (Figure 1) and PMC (Figure 2)* drugs at a concentration of 50µg/mL. The results of cytotoxicity (MTT) assay showed that both *VRP and PMC* drugs, inhibited proliferation of MCF-7 breast cancer cells in time and dose dependent manner. Concentration *VRP and PMC* drugs increased from 10 – 50 µg/mL, and the percentage of inhibition increased from 63.62% to 90.16% for VPR at 24 to 48 h and 65.03 % to 70.51 % for PMC at 24 to 48 h (**Table 1**). Of these two drugs subjected to the test, VPR was more efficacious since it affected viability of the cells its statistically significant at the level of $P < 0.05$ *(Figure 3 & 4)*.

Figure 1: Phase contrast image of the MCF-7 treated with VRP

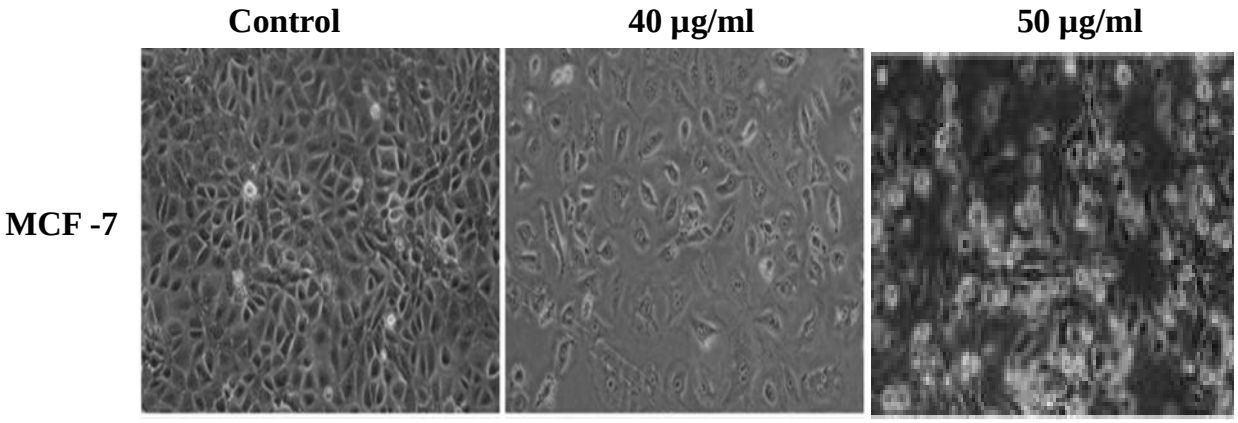

Figure 2: Phase contrast image of the MCF-7 treated with PMC

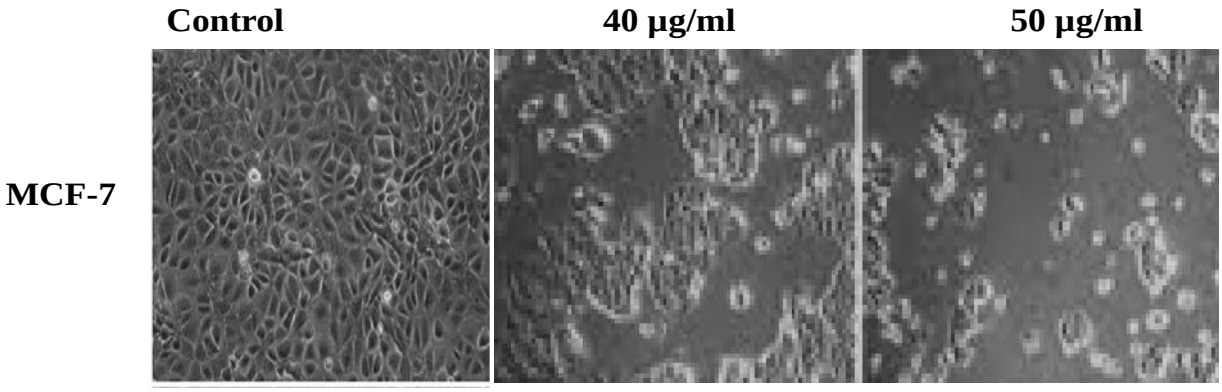

Table 1: Inhibitory concentration (IC_{50}) of VRP and PMC in human breast cancer cell line MCF-7.

Cell line	IC_{50} (50 µg/mL)			
	VRP		PMC	
	24 h	48 h	24 h	48 h
MCF-7	63.62± 0.03	90.16±0.02*	65.03 ± 0.05	70.51 ± 0.01

Figure 3:

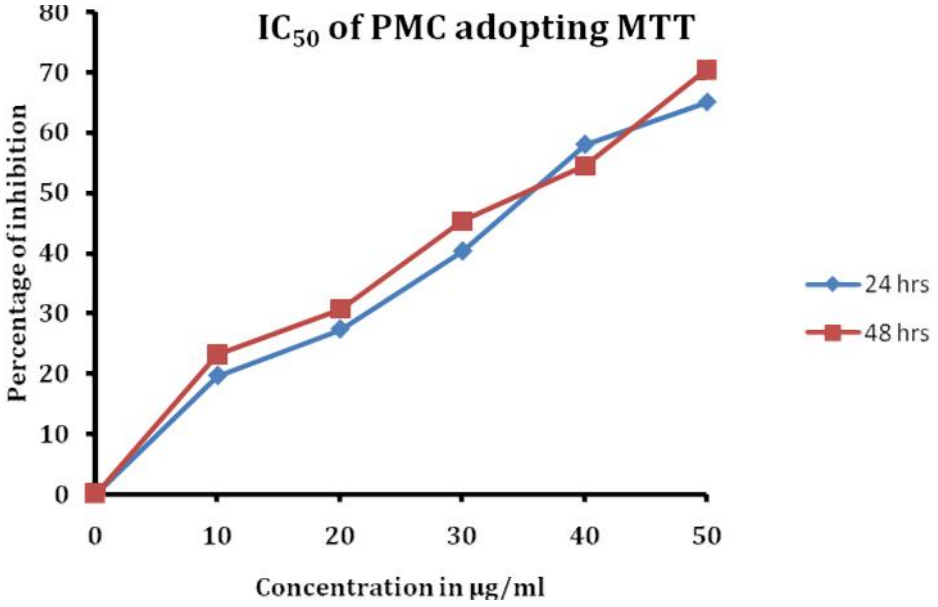

Figure 4:

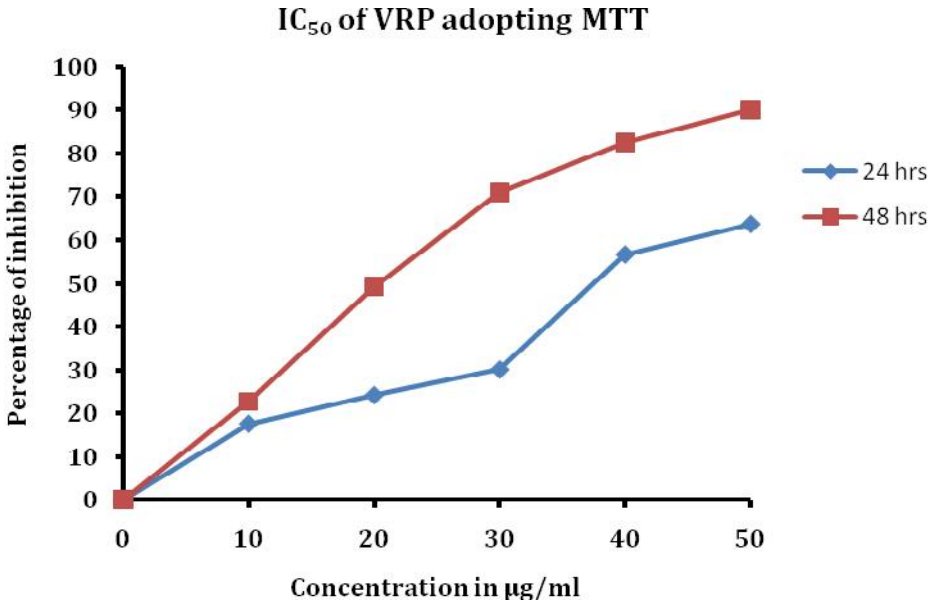

Table 2: IC_{50} values of taxol on MCF-7 (60 µg/ml) cancer cell lines

Cell line	IC_{50} (60 µg/mL)	
	24 h	48 h
MCF-7	72 ±2.4	75 ± 3.2

Discussion

Since ancient times, plant-based formulations have been practiced as remedies against diverse ailments **(Ahirrao and Patil, 2010)**. Over the past two decades, interest in traditional medicines has increased considerably in many parts of the world (Muthu et al., 2009). The principles are based on the interaction of several crude drugs or several ingredients even in a single crude drug. Therefore, the apparent combined effects are equivalent to the sum of effects of those components which underwent addition, potentiation, subtraction, and modulation (**Kimura and Tsunek et al, 2005**).

The focus of the present study was to find if *VRP and PMC* drugs would inhibit the proliferation of human breast cancer cells MCF-7. The result of cytotoxicity assay showed that there is a significant difference in the two different time points at higher concentration (50 µg/mL) of both *VRP and PMC* drugs. The morphology of the MCF-7 also indicated that these siddha drugs *VRP* and *PMC* showed good anti-proliferant activity when compared with taxol as positive control.

The data obtained in this study suggest that, when used in combination, formulation can potentially produce synergistic effect *in vitro*.

Conclusion

The Siddha drugs PMC and *VRP* are having potent anti-cancer activity and it would be a potential alternative medicine for human breast cancers. In the future, we hope that many research groups will attempt to study and develop Siddha Herbo-mineral formulations for therapeutic applications with improved efficacy, safety and at reduced cost.

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