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Anticancer and Cytotoxic Evaluation of Padikara Parpam Combined with L-Ascorbic Acid (PPLAA)

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ABSTRACT

Cancer is still one of the leading causes of death globally a lot of research is being done to develop safer, more effective anticancer drugs that come from natural sources. Because of their mineral and botanical components, traditional Siddha medicines have demonstrated exceptional medicinal potential. The present study analyzes the in vitro anticancer and cytotoxic characteristics of Padikara Parpam coupled with L-Ascorbic Acid (PPLAA) against MCF-7 (human breast cancer) and VERO (normal kidney epithelial) cell lines utilizing the MTT assay.

The findings showed that PPLAA significantly inhibited the growth of MCF-7 cancer cells in a dose-dependent manner, with the lowest cell viability (3.4%) occurring at the highest concentration (500 µg/mL). On the other hand, VERO cells showed negligible cytotoxicity (7.0% drop in cell viability at 500 µg/mL), suggesting a good therapeutic selectivity. The findings suggest that PPLAA could serve as a potential natural anticancer agent with limited toxicity toward normal cells.

INTRODUCTION

Uncontrolled cellular proliferation, invasion, and metastasis are hallmarks of cancer, which is one of the most serious health issues of the contemporary era. Despite its effectiveness, conventional chemotherapy frequently has harmful side effects and damages healthy tissues. As a result, there is increasing interest in investigating traditional and natural medicine formulations that have anticancer properties and few side effects.(1) Originating in Tamil Nadu, Siddha is a unique medicinal method that maintains the ratio of Vasam, Pitam, and Kapam in an attempt to alleviate the underlying cause of disease. An ancient saint by the name of Siddhar is credited with creating the Siddha medical system.(2)

When an alkaline metal or group sulphate, such as ammonium, potassium, or sodium sulfate, is combined with magnesium, chromium, aluminum, or ferrum sulphate, a double sulphate known as padikaram (alum) is produced.(3) In siddha medicine, Padikara Parpam is frequently used to treat menorrhagia, hematuria, urinary tract infections, and frequent urination.(4)

Researchers can uncover new phytomedicines or pharmacological combinations by studying synergistic interactions, but also in the avoidance of unnecessary harmful synergies. Medicinal combinations can help eliminate drug resistance.(5)

A range of Parpams or calcined mineral compounds, are used in Siddha medicine, one of the traditional Indian medical systems, to treat chronic conditions like tumors, inflammation, and ulcers. Traditionally made from alum (Padikaram), Padikara Parpam is renowned for its antibacterial, wound-healing, and detoxifying qualities.

Padikara Parpam was mixed with L-Ascorbic Acid (Vitamin C), a strong antioxidant and immune-modulating substance, to increase its biological effectiveness and create PPLAA.

By inducing apoptosis in cancer cells and neutralizing free radicals through redox-mediated pathways, vitamin C is essential. It's thought that the mineral and vitamin-based components work in concert to enhance antioxidant defense and cytotoxic capabilities against cancer cells.

Therefore, the purpose of the current study is to assess the cytotoxic and anticancer effects of PPLAA on MCF-7 breast cancer cells and VERO normal kidney cells using quantitative analysis using the MTT assay.(6)

MATERIALS AND METHODS

Preparation of Padikara Parpam and Combination with L-Ascorbic Acid

The Padikara Parpam that was purchased was made in accordance with the conventional Siddha purification and calcination methods. PPLAA was created by combining the resultant powder with L-ascorbic acid in a certain ratio. The

purpose of adding L-ascorbic acid was to increase the formulation's overall bioavailability and antioxidant capacity.

Cell Lines and Culture Conditions

Two types of cell lines were used in this study:

- MCF-7 (Human Breast Cancer Cell Line) – used to evaluate anticancer activity.(6)
- VERO (Normal Kidney Epithelial Cell Line) – used to evaluate cytotoxicity on normal cells.

MTT Assay for Cell Viability

The MTT assay was performed to determine the cytotoxic potential of PPLAA. Cells were seeded in 96-well plates with appropriate growth medium and allowed to attach overnight. Different concentrations of PPLAA were added to the wells and incubated for 24 hours.

After incubation, MTT reagent was added to each well, and the plates were further incubated for 4 hours. The purple-colored formazan crystals formed by viable cells were dissolved using DMSO, and absorbance was measured at 540 nm using a microplate reader. The IC_{50} value concentration causing 50% inhibition) was determined graphically.(7)

RESULTS

Anticancer Activity on MCF-7 Cell Lines

PPLAA exhibited a potent, dose-dependent cytotoxic effect on MCF-7 cells. At 500 µg/mL concentration, the lowest cell viability (3.4%) was noted, suggesting a considerable reduction of the growth of cancer cells. For the common medication Cisplatin, the IC_{50} value was found to be 24.96 µg/mL. These findings show that PPLAA efficiently kills breast cancer cells at higher concentrations. The data unambiguously show a concentration-dependent decline in cell viability, indicating that PPLAA effectively inhibits MCF-7 cell proliferation. (Table 1, Fig 1)

Table 1: Anticancer Activity of PPLAA against MCF-7 Cell Line

Concentration (µg/mL)	Absorbance (540 nm)	% Cell Viability
500	0.03	3.4%
250	0.09	10.4%
125	0.18	20.9%
62.5	0.27	31.3%
31.2	0.39	45.3%
15.6	0.50	58.1%
DMSO	0.86	100%
Control	0.86	100%

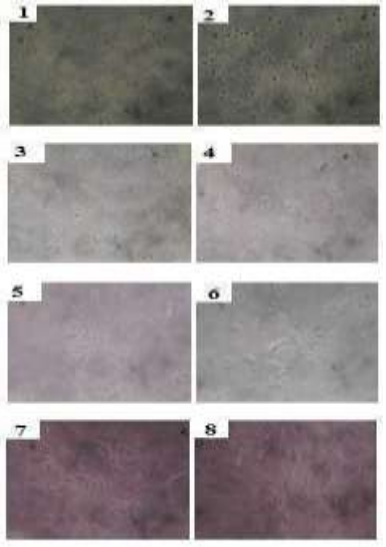

Fig 1: Anticancer activity of PPLAA against MCF-7 cell line
(1. 500µg, 2. 250µg, 3. 125µg, 4. 62.5µg, 5. 31.2µg, 6. 15.6µg, 7. DMSO, 8. Control)

Cytotoxicity Evaluation on VERO Cell Lines

Cytotoxicity was assessed using VERO cells to ascertain whether PPLAA is harmful to healthy cells. At the highest dose (500 µg/mL), the extract showed just a 7.0% decrease in cell viability, indicating low toxicity. When compared to VERO cells, Cisplatin's IC_{50} value was 87.45 µg/mL, which was notably higher confirming that PPLAA has a safer profile compared to standard chemotherapy agents. The results confirm that PPLAA selectively targets cancer cells without affecting normal healthy cells.(Table 2, Fig 2)

Table 2: Cytotoxicity of PPLAA against VERO Cell Line

Concentration (µg/mL)	Absorbance (540 nm)	% Cell Viability
500	0.09	7.0%
250	0.23	18.1%
125	0.42	33.0%
62.5	0.76	59.8%
31.2	1.05	82.6%
15.6	1.26	99.2%
DMSO	1.25	98.4%

Concentration (µg/mL)	Absorbance (540 nm)	% Cell Viability
Control	1.27	100%

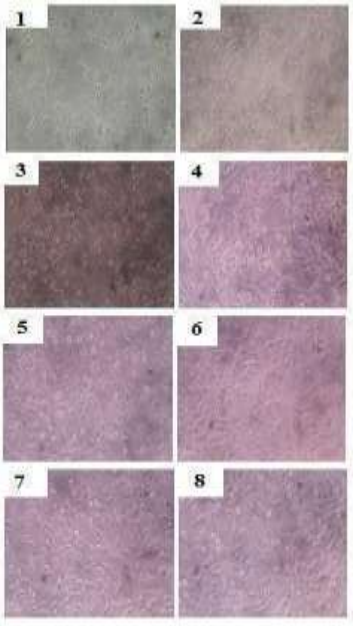

Fig 2: Cytotoxicity activity of PPLAA against VERO cell line
(1. 500µg, 2. 250µg, 3. 125µg, 4. 62.5µg, 5. 31.2µg, 6. 15.6µg, 7. DMSO, 8.Control)

DISCUSSION

Natural and synthesized alkaloids have medical use, including analgesic, antisplasmodic, and bactericidal properties, antioxidant activity, and usage in renal disorders.(8,9) A synergistic mechanism of anticancer action is validated by the combination of Padikara Parpam and L-Ascorbic Acid (PPLAA).(6,7)

The majority of cancer treatments use the primary mechanism known as apoptosis to influence carcinogenesis. However, the majority of currently available medications exhibit variable degrees of adverse effects.(10) Strong natural medications require care, and conventional Siddha medical references to date have insisted on the use of certain mineral compounds as target-specific anticancer treatments. Alum, also known as alumen (potassium aluminum sulphate), or padikaram in Tamil, is one of the most often utilized inorganic salt minerals in Siddha medicine. This mineral's strong potential as a styptic agent in circumstances like bleeding, discharge, and diarrheal illnesses makes it therapeutically indicated in both raw and processed formulations (internal and external).(11)

Due to its mineral base, Padikara Parpam may affect the oxidative metabolism and ionic balance of cancer cells, causing apoptosis. At therapeutic quantities, L-ascorbic acid increases redox activity and helps produce reactive oxygen species (ROS), which specifically cause oxidative stress in cancer cells.(12)

Additionally, vitamin C is known to alter immune response and collagen formation, which may prevent tumor angiogenesis and metastasis.(13) PPLAA's minimal cytotoxicity toward healthy VERO cells indicates that it retains cellular selectivity, which is a crucial quality for a possible anticancer medication.(7)

Earlier studies highlighted the efficacy of Padikara Parpam with L-Ascorbic Acid against Bacterial Pathogens and In Vitro Evaluation of Antioxidant Activity of Padikara Parpam (PP), L-Ascorbic Acid (LAA), and combination of Padikara Parpam and L-Ascorbic Acid(PPLAA).(14,15)

The findings are consistent with earlier studies that have shown antioxidant-enriched metal oxides can trigger apoptosis by causing DNA breakage and mitochondrial damage. Hence, the anticancer effect of PPLAA may result from oxidative stress induction, cell cycle arrest, and inhibition of cancer cell proliferation.(6)

CONCLUSION

The present study successfully demonstrates that Padikara Parpam combined with L-Ascorbic Acid (PPLAA) possesses significant anticancer activity against MCF-7 breast cancer cell lines while exhibiting negligible cytotoxicity toward normal VERO cells. The dose-dependent inhibition of cancer cell growth suggests that PPLAA is a promising candidate for further development as a natural, safe, and effective anticancer formulation.

Future work should focus on identifying the bioactive components, elucidating molecular mechanisms of action, and conducting in vivo studies to validate its therapeutic potential.

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