Evaluation of Invitro Anticarcinogenic Activity of Pirandai Uppu Using MTT Assay

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ABSTRACT:

Introduction:

The pirandai uppu (PU) is a simple formulation derived from ash of aerial parts of Cissus quadrangularis. Since documentation regarding therapeutic effects of PU is lacking we aimed in evaluating the anticancer activity of PU through MTT assay.

Aim and Objective:

The present study is aimed to evaluate the anticancer potential using *in vitro* assay system.

Materials :

MCF-7 cells (1×10^5 cells/ml) were seeded in the 96-well plate and incubated at 37˚C in CO2 incubator. After 24 hr of incubation, the plates were washed thrice with 0% media (no serum added) and Pirandai Uppu (PU) treated with following concentration - 5, 25, 50, 75, 100µg with 10% DMEM medium. . After incubation, media was carefully removed and DMSO were added in plate for the purple color formation, which is directly proportional to the number of viable cells. The 96 plate was subjected to OD value at 570 nm using a microplate reader (The percentage of cell viability was calculated.

Results: From the results, it is evident that the prepared PirandaiUppu (PU) enormously regulate the cell viability . 5, 25 and 50µg of PU was regulate the cell proliferation upto 25-30% than control. Meanwhile 100µg of PU negatively influence the cell proliferation on the whole. Notably, the 5µg and 25µgPU will be the effective target for cancer treatment .

Conclusion:

Thus Pirandai uppu can be used as an effective anticancer drug in addition to anti inflammatory.

Key words: Cissus quadrangularis, MTT, MCF-7 cells, Apoptosis

Introduction

Globally, cancer is the most serious health complication suffering by a human being, with an estimated new cases total of 19.3 million and nearly 10.0 million cancer-related deaths in 2020 [1]. Phytochemicals and their derivatives are capable to enhance therapeutic efficacy in cancer patients and reduce side effects. Phytochemical compounds regularly act by controlling molecular signaling pathways that are implicated in the development of cancer. The particular mechanisms such as augmenting antioxidant status, carcinogen inactivation, inhibiting proliferation, initiation of cell cycle arrest and apoptosis, and regulation of the immune system[2].

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Pirandai uppu (salt derived from *Cissus quadrangularis*) can be used to cure many ailments like stomach ache, hemorrhoids, dysentery, dysmenorrhea, skin diseases etc[book]. All forms of this plant is used to treat inflammatory conditions. It is rich in flavonoids and triterpenoids[3] Most common cancer among women is breast cancer.

According to global cancer statistics 2020 most commonly diagnosed cancer is breast cancer exceeding the cases of lung cancer[4].The MCF-7 breast cancer cell line originated from a 69-year-old Caucasian woman who previously underwent two mastectomies in a five-year span. The tissue removed at the first mastectomy was benign, but the second operation found a malignant adenocarcinoma at Michigan Cancer Foundation (MCF). The MCF-7 cell line was derived from this pleural effusion in 1970. ero cells are lineages of cells used in cell cultures. The Vero lineage was isolated from kidney epithelial cells extracted from African green monkey (Cercopithecus aethiops). The lineage was developed on 27 Mar 1962, by Yasumura and Kawakita at the Chiba University in Chiba, Japan[5]. Thereby indicating that this formulation with its combined activity can bind with the estrogenic receptors present in the MCF-7 cell line promising potent anti-carcinogenic activity. This is the basis of our study and our objective is to establish the therapeutic potential of this potent simple herbal salt based formulation.

Methodology

Pirandai uppu ws purchased from SKM Pharmaceuticals, Chennai.

MTT assay

To determine the cell viability, MCF-7 cells (1 × 10 5 cells/ml) were seeded in the 96-well plate and incubated at 37˚C in CO2 incubator. After 24 hr of incubation, the plates were washed thrice with 0% media (no serum added) and Pirandai Uppu (PU) treated with following concentration - 5, 25, 50, 75, 100µg with 10% DMEM medium. The plates were incubated for 24 hr at 37˚C in CO2 incubator and following that MTT (stock 5mg/10ml 1X PBS) (Sigma; Chennai, Tamil Nadu, India. Catalogue: 11465007001)) were added in 96 well plate and incubated for 2 hours in dark condition. After incubation, media was carefully removed and DMSO (Himedia; Mumbai, India. Catalogue: MB058-100ml) were added in plate for the purple color formation, which is directly proportional to the number of viable cells. The 96 plate was subjected to OD value at 570 nm using a microplate reader (BioTek Epoch Microplate Spectrophotometer; United States). The percentage of cell viability was calculated using the formulae namely (Treatment average OD/Control Average OD*100).

Results

According to the results, the Pirandai Uppu (PU) enormously regulate the cancer cell viability(Fig . 1) . Briefly, 5, 25 and 50µg of PU regulate the cell proliferation up to 25-30% than control (non-treated) , meanwhile in 100µg of PU completely negatively influences cell proliferation.

Discussion

Notably, the 5µg and 25µg PU will be the effective target for cancer treatment because we predict these concentrations may don’t affect non-cancerous cell proliferation.

In approach to identify novel anti-cancer drugs it is important to ensure that it doesn't affect non-cancerous proliferation normally taking place in the human body. Comparative reports explore that MCF-7 proliferation is directly proportional to inflammation (Coussens LM,Werb Z., 2002).Hence the capability of suppressing cancerous proliferation indicates the ability to regulate inflammation as well.Anti-cancer property of Pirandai uppu has not been explored nor documented.This study concludes that Pirandai uppu has potent anti-carcinogenic activity and can be used in the treatment of cancer.

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Figure

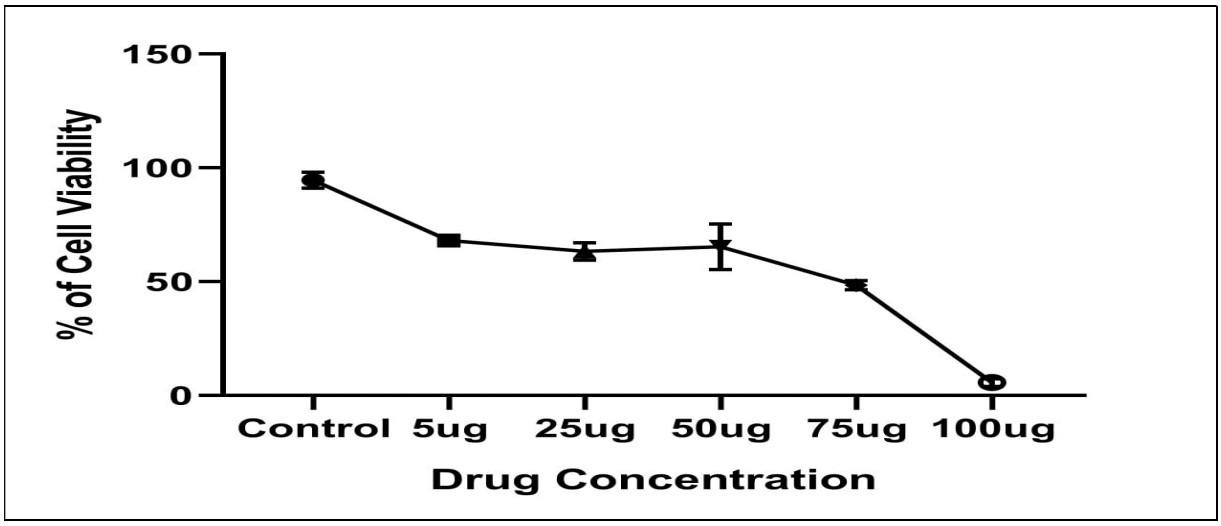

Fig:1 Graph to show % of cell viability with respect to various concentration of pirandai uppu

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