Research Article

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COMPARATIVE *IN-VITRO* ANTICANCER ACTIVITY OF *THANGA PARPAM* (SIDDHA GOLD DRUG) IN BREAST, LIVER, PROSTATE AND LUNG CANCER CELL LINES

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ABSTRACT

Thanga parpam is a gold nanoparticle used in Siddha for many chronic and challenging diseases including cancers. Clinically it shows benefit in some cancer types, but not to all types. This study was aimed to compare the *in-vitro* anticancer activity of Thanga parpam in various cancer cell lines. The particle size and elements were evaluated using Scanning Electron Microscope coupled with Energy Dispersive X-Ray Spectroscopy. Thanga parpam was added to breast adenocarcinoma cells, Human hepatocellular carcinoma cells, Human prostate cancer cells and Human lung adenocarcinoma epithelial cells for 24 hours. MTT assay was used to evaluate the cell viability. Graph was drawn from the % cell viability and dose required to kill 50 % cells was calculated. The gold particles are irregular disk shaped, but agglomerated to each other and not in uniform size. The particle size varies from 17.8 nm – 448 nm. About 9.3% of gold element was present in oxide and sulfide form. It showed dose dependent killing effect on all cancer cell lines. IC50% value was 0.63, 3.51, 6.65 and 11.01 µg/ml for breast, liver, prostate and lung cancer cells respectively. Thanga parpam is a potent anticancer drug to all the four cancer cells, however higher efficacy was observed in breast, liver and prostate cancer cells.

Keywords: Siddha, Ayurveda, metal, gold, cancer, breast cancer, gold nanoparticle

INTRODUCTION

"*Ver paaru thazhai paaru – minjinakkaal, mella mella parpam chendooram pare*" – It means always treat with shoots and roots, if not responding Gradually start metal (*parpam/chendooram*) based drugs, which is an important guideline to choose drug therapy in Traditional Siddha medical system.[1] Gold based drugs are being used in Siddha for the treatment of cancers, tuberculosis, wasting diseases, impotency, autoimmune diseases, severe infections, AIDS, etc.[2-4]

The modern pharmacology industry developed gold nanoparticles and gold salts (sodium aurothiomalate, aurothioglucose, aurothiosulfate, auranofin, etc.) for the use in rheumatoid arthritis to prevent disease progression.[5] Ayurveda gold preparations (swarna bhasma), which are different from Siddha preparation, have shown free radical scavenging activity, anti-depressant, analgesic, immunomodulatory activities and also significantly reversed the global ischaemia in animal models of cerebrovascular diseases.[6]

So far, no literature is on chemistry, particle size and comparative anticancer activity the Siddha gold among different caners. So, this study was aimed to compare the cytotoxic activity of Siddha gold among various cancer cell lines.

MATERIAL AND METHODS

Thanga parpam – particle size and chemical analysis

The test drug was procured from SRM Siddha Medicine Manufacturing Company, Chennai, India. The particle size and elements were evaluated using Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-Ray Spectroscopy (EDX) using standard protocol.[7]

Cell culture

In this study, we used four cancer cell lines; Human breast adenocarcinoma cells (MCF7), Human hepatocellular carcinoma (Hep G2), Human prostate cancer cell line (PC3), and Human lung adenocarcinoma epithelial cells (A549). Cell lines were obtained from National Centre for Cell Sciences (NCCS, India). Cell culture supplies were obtained from Invitrogen Pvt LTD, India. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Cell lines were sub cultured and seeded at 104 cells/cm^2 in six-well plates using Ham's F-12 medium (A549, PC3) or Dulbecco's modified Eagle's medium (DMEM) (HepG2, MCF7) supplemented with 10% foetal bovine serum. Antibiotic gentamycin was added and maintained in a humidified atmosphere of 95% air / 5% CO_2 at 37° C.

Treating cells with Thanga parpam and MTT assay

All the cells were harvested from the medium and resuspended to a final concentration of 10,000 cells/100µL of fresh medium containing 10% foetal bovine serum (FBS). Cell suspensions (100 µL) were dispensed into individual wells of a 96-well tissue culture plate with a lid (Falcon, Oxnard, CA). These were kept for attachment to the 96-well tissue culture plate for a period of 24 hours. Four plates were prepared for the test. In the respective plates, blank column was kept containing medium alone and control column containing cells but no drugs. Thanga parpam dissolved in dimethyl sulfoxide (DMSO) solution at different concentrations (10, 20, 50, 100, 200, 500, µg/ml) were then added (100 µL) to cell suspensions. Cell lines were incubated with the

drug for 24 hours. Viable cell growth was determined by MTT reduction assay for all well. MTT values were observed at absorbance of 540 nm. The % viability was calculated using the formula: (AT – AB) / (AC – AB) × 100, in which AT was the Absorbance of drug (test) treated cells, AB was the Absorbance of blank (only media) and AC was the Absorbance of control (untreated). The % cytotoxicity was calculated using the formula: (100 – % cell survival). From this data, the dose-response (% cell viability) graph was plotted in excel sheet. The linear trendline with formula (Y = mX + c) was drawn. The IC_{50} value was calculate using the formula; x = (50-c)/m.

RESULTS AND DISCUSSION

Particle size and elemental analysis

The gold particles are irregular disk shaped, but agglomerated to each other and not in uniform size. The particle size varies from 17.8 nm – 448 nm (Figure 1). Earlier study has shown that the Ayurvedic gold drug with 28-35 nm size got absorbed orally and released Au (I) ions in a sustained manner to exert its pharmacological action. They neither induced blood cell aggregation nor protein adsorption, but they opened the tight junctions in Caco-2 cell experiments.[8] In our study, we observed the particle size of 17.8 nm and above.

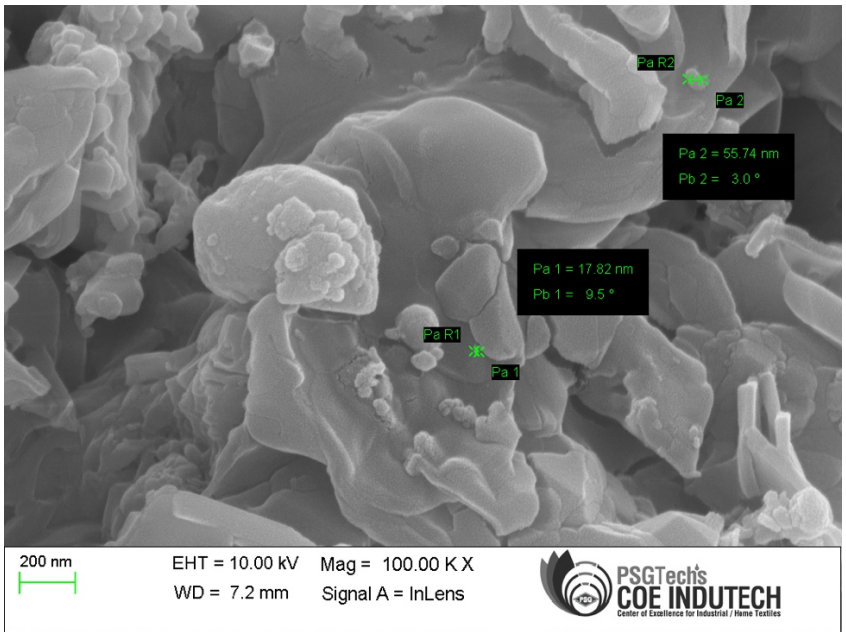

Figure 1: Scanning Electron Microscopy of Thanga parpam

About 9.3% of gold element is present in Thanga parpam, mostly as oxide and sulfide form, along with other elements as minor quantity (Figure 2, Table 1).

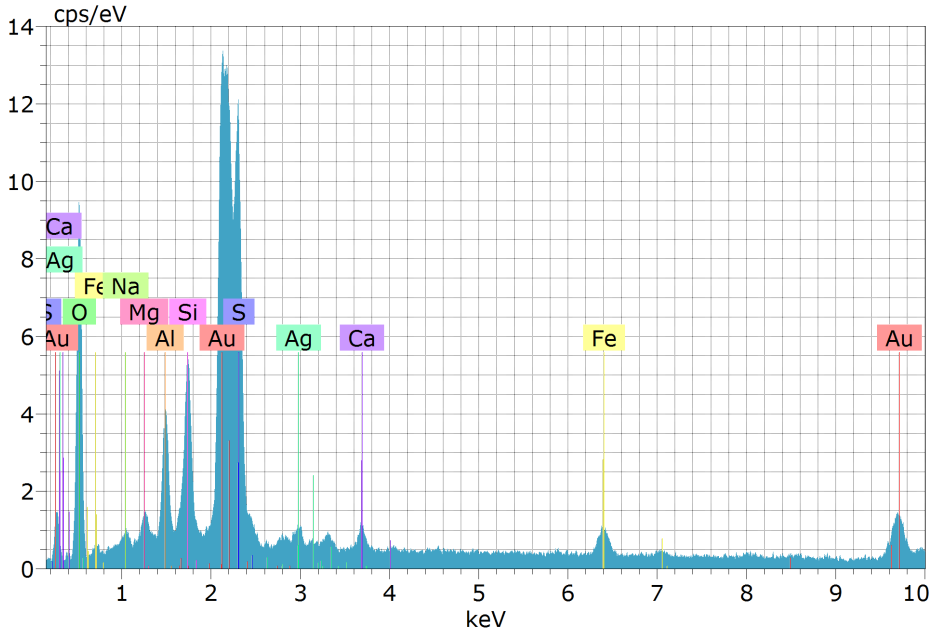

Figure 2: Elemental analysis of Thanga parpam using Energy Dispersive X-Ray Analysis

Table 1: Elemental analysis of Thanga parpam using Energy Dispersive X-Ray Analysis

Elements	Weight (%)	Atomic (%)
Gold	50.51	9.30
Oxygen	31.28	70.92
Sulphur	10.27	11.62
Silicon	2.14	2.76
Iron	2.53	1.64
Aluminium	1.91	2.57
Silver	0.51	0.17
Calcium	0.45	0.41
Magnesium	0.27	0.40
Sodium	0.13	0.21

Anticancer activity

Thanga parpam showed dose dependent killing effect on all cancer cell lines. Breast cancer cells and liver cancer cells were killed even at lowest doses. The highest cytotoxic activity was seen in breast cells, followed by, liver, prostate and lung cells. The dose of Thanga parpam required to kill 50% of cancer cells (IC50 values) are 0.63, 3.51, 6.65 and 11.01 µg/ml for breast, liver, prostate and lung cancer cells respectively (Figure 3, 4).

Many studies have shown that the gold nanoparticles enter cells by receptor-mediated endocytosis and Macropinocytosis, where they get accumulated in membrane-bound vesicles or vacuoles, respectively. Beaudet, *et al* concluded that the Ayurvedic gold particles are pharmacologically inert large particles, acts as a carrier, so the other ingredients might be exerting the actions, but the exact mechanism of action is poorly understood.[9] In our study, the drug showed cytotoxic effect. Further research must be done what decides the cell killing effect or inert role.

In a clinical trial, Ayurvedic gold preparation was administered to solid cancer patients (lung, liver, pancreas, gall bladder, rectal caners) for one year and followed for the clinical improvement. Results showed best beneficial effect in rectal cancer compared to other types.[10] So, it reveals that the gold drug could exhibit varying anticancer activity among different cancer types. *In-vitro* and preclinical studies could be helpful to draw the conclusions. Our study showed the highest cytotoxic activity in breast cells, followed by, liver, prostate and lung cells. So, this result would be helpful to move on further into research or clinical practice.

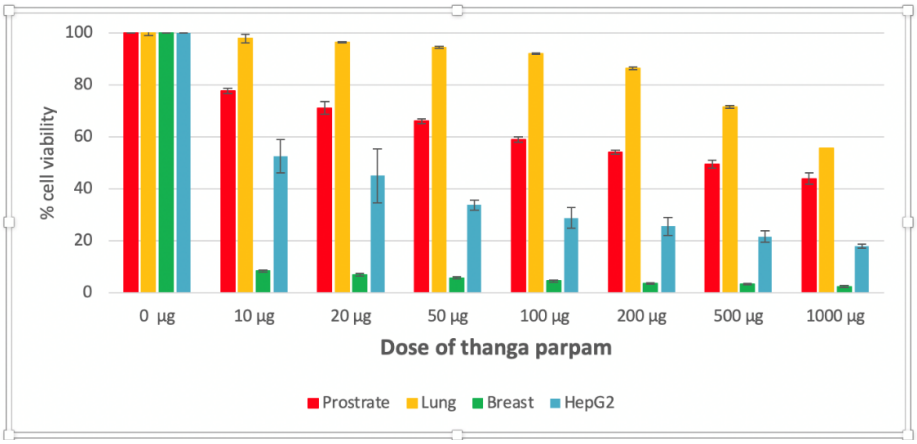

Figure 3: Effect of Thanga parpam in % cell viability among various cell lines

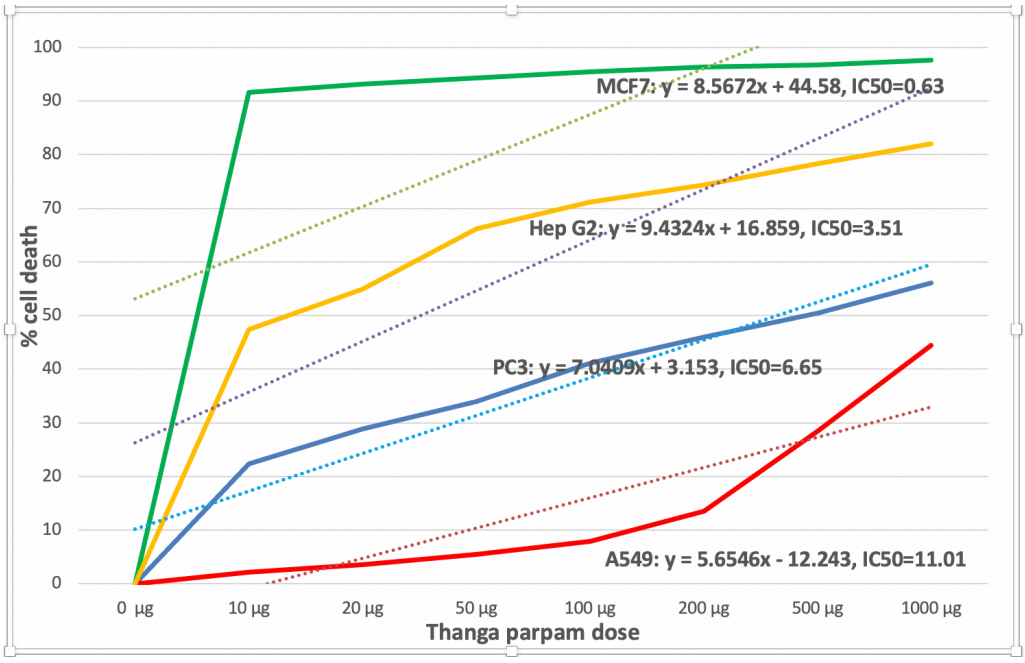

Figure 4: Effect of Thanga parpam in % cell death and IC50 among various cell lines

CONCLUSION

The anticancer drugs usually show IC_{50} values < 25 µg/ml, which means lesser the IC_{50} values, higher the anticancer property. Hence, Thanga parpam is the potent anticancer drug to all the four cells. It could be a potent anticancer drug for cancers in breast, liver, prostate and lung.

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