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Research Article

IN VITRO ANTI-CANCEROUS ACTIVITY OF NAVAMANI CHENDURAM (NMC) IN OVARIAN CANCER CELL LINE (PA-1) USING MTT ASSAY

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Abstract

Ovarian cancer is one of the leading gynaecological issues that contribute to morbidity and mortality, worldwide. Siddha system of medicine has various medicines which can be considered as an alternative form of therapy in cancer management with proising therapeutic efficacy and minimal contraindications.. The present study is to indicate the anticancer property of *Navamani Chenduram*, Siddha Metallo mineral formulation through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium assay in a dose dependent manner. MTT assay (cytotoxic study) is performed by Serial dilutions of *Navamani Chenduram* (10, 50, 100, and 200 µg/ml) and methotrexate 10 µg/ml, as a standard comparison drug on human metastatic ovarian cancer PA-1 cell line. The study was evaluated by assessing the inhibition of the percentage of cell viability and they had minimum viability of cell (40.38 ± 3.122 %,) and high anti-ovarian cancer activities dose-dependently against PA-1. Our results prove that *Navamani Chenduram* induces Apoptosis of carcinogenic cells, thus it inhibits the growth of metastatic ovarian cancer cells.

Keywords: Anti Cytotoxic Action, Navamani Chenduram, PA1Human ovarian cancer cell line, Methotrexate..

INTRODUCTION

Ovarian cancer is the fourth-deadliest malignancy in women and it was the cause of 4.4% of all cancer-related deaths in women [1,2]. According to 2019 cancer data, there were an anticipated 22,240 new cases and 14,170 fatalities [3]. The ovarian surface epithelium is the origin of 90% of ovarian cancer. The main obstacle to the clinical management of ovarian cancer is metastasis [4]. The condition is linked to three different histology types. Epithelial Ovarian Cancer is the most typical type. Only 45.6% of patients with this deadly illness survive five years [5]. If accurate early-stage identification is achievable, the overall survival rate rises to 70%. The rate of early-stage illness detection is as low as 20%. The Draghici *et al.,* 2020 majority of patients, have a miserable survival rate of 35% due to late-stage identification and advanced cancer stage. There is currently no effective treatment for recurrent Epithelial Ovarian Cancer. Almost 10%–15% of familial OCs are caused by BRCA1 and BRCA2 breast cancer gene mutations [6]. Chemotherapy, radiation therapy, and surgery are all common cancer treatments. The usual cancer treatment is linked to a wide range of systemic, psychological, and neurological side effects, so the use of plant-based pharmaceuticals called phytomedicines in cancer chemotherapy is a growing area of research. A significant development in the field of cancer will be the introduction of such medications as an alternative therapy with therapeutic efficacy on par with or better. Many number of anti cancer medicines were mentioned in siddha system. All these medicines were in the form of herbs and metals/minerals [7]. Lack of scientific documentation masks their prevalence to the human community. Only a very few herbs has been selected and validated scientifically till date.

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For instance,vincristine and vinblastine derived from Catharanthus roseus and campothecins topotecan) from Campotheca accuminata has made a revolution in the treatment of lymphoma, leukemia, ovarian cancer, and lung cancer. [8]. All the herbal and metallic/mineral preparations mentioned in siddha literature are to be validated scientifically. As a part of this revolution we have chosen *Navamani Chenduram* (NMC),a Metallomineral formulation for validation [9]. Usage of Metals or minerals are restricted because, those are considered to be toxic. But, Fotunately, the toxicity present in heavy metals like Mercury and other minerals will be reduced or will be made zero,once these substances are convertedas herbomineral formulation. In these herbomineral formulations the ingredients are converted into nano sized particles.[10,11]. For instance, Hua-Feng-Dan (HFD), that has cinnabar (HgS) and realgar (As4S4), as a main ingredientpromoted in the treatment of neurodegenerative diseases [12] and Tamra Bhasma, prepared from copperintended for some human disorders, proved safe and nongenotoxic [13]. Such safety would be assured only if those formulations of metal origin strictly adhere to their native method of preparation mentioned in the mother literatures. [14]. Green synthesized metal nanoparticles exhibit antitumor and antiviral activities[15]. This is the first study on the evaluation of the cytotoxic effect of *Navamani Chenduram* (NMC)on the PA1 human ovarian cancer cell line.

METHODOLOGY

Reagents used

Trypsin, Dulbecco's Modified Eagle Medium, Dulbecco's Phosphate Buffered Saline(PBS), Fetal Bovine Serum (FBS), Pen strip. The test drug NMC was procured from Earth India Pharmaceuticals, Chennai, Tamilnadu.

Preparation of test solutions

After inoculation 1ml test drug NMC was taken and diluted with Dimethyl sulfoxide (DMSO) and chosen in serial dilutions such as (10, 50, 100 and 200 µg/ml). Methotrexate was fixed as atandard drug and 1mg was taken and diluted using the agent Dimethyl sulfoxide (DMSO) to attain the concentration 10 µg/ml. With all the above-mentioned concentrations of the sample and the standard, the cytotoxic property (anticancer) was evaluated on Human ovarian (PA-1) cancer cell culture and media using MTT {3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide} assay. Human ovarian (PA-1) cancer cell culture and media were acquired from NCCS, PA-1 cell lines were acquired and stock cells were cultivated in medium augmented DMEM, streptomycin (100 ìg/ml), penicillin (100 IU/ml), The plates were incubated for 24 hr at 37˚C in CO2. Then the cells were detached with Sterile filtered Trypsin Phosphate Versene Glucose (TPVG) Solution (0.1% Trypsin, 0.02% EDTA, 0.05% Glucose, and Phenol red in Dulbecco's Phosphate Buffered Saline). The viability of the cells was examined, and they were centrifuged. A 96-well plate with an additional 50,000 cells per well was seeded. Incubation was done for 24 hours at 37 °C with 5% CO2.

Anti- proliferation assay

Using appropriate medium containing 10% FBS, the monolayer cell culture was trypsinized and the cell count was increased to 1.0 x 105 cells/ml. A 100 l portion of the diluted cell suspension (50,000 cells/well) has been added to each well of the 96-well microtiter plate. A partial monolayer formed after 24 hours; the supernatant was wiped off, the monolayer was rinsed once with media, and 100 l of each of the test drug concentrations were added to the partial monolayer in microtiter plates. The plates were then incubated for 48 hours at 37°C in a 5% CO2 environment. Following incubation, the test solutions in the wells were removed, and 100 l of MTT (5 mg/10 ml of MTT in PBS) has been added. The plates were incubated for 4 hours at 37°C with 5% CO2. To dissolve the formazan that had formed, 100 l of DMSO was added after the supernatant had been eliminated from the plates. At a wavelength of 570 nm, the absorbance was calculated using a microplate reader. The following method was used to calculate the percentage of survival rate, and the dose-dependent curves for each cell line were used to determine the concentration of test drug required to inhibit cell growth by 50% (IC50).

$$\text{Survival rate}(\%) = \frac{\text{Asample} - \text{Ab}}{\text{AC} - \text{Ab}} \times 100$$

IC50 Value

Half maximal inhibitory concentration (IC50) was used to gauge a mixture's effectiveness and capacity for impairing biological and physiochemical function. It signifies the amount of the drug or ingredient (inhibitor) required to obstruct a specific enzyme, cell, cell receptor, or microorganism by half. The IC50 of a medicine can be calculated using a dose-dependent curve. Examining the agonistic action of the standard's reversal allows one to ascertain the antagonistic action of various test drug concentrations. By determining the concentration required to block a portion of the agonist's strongest biological response, one can evaluate the IC50 estimates for a certain antagonist.

MTT Assay

The in vitro determinations of anti-proliferative effects of the test formulation have been performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate, and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water-soluble tetrazolium salt upon incubation MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. The coloured solution formed finally was examined spectrophotometrically. The percentage of cell viability was calculated. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the NMC (Roshni *et al.,*2022).

RESULTS

Table 1. Effect of various concentrations of Test drug NMC on Cell viability of Human ovarian (PA-1) cell line with Methotrexate as the standard comparison drug

S.No	Concentration µlg/ml	% cell Viability
1	10 µg/ml	89.42 ± 1.827
2	50 µg/ml	72.5 ± 2.388
3	100 µg/ml	59.78 ± 4.613
4	200 µg/ml	40.38 ± 3.122
5	STD (METHOTREXATE 10 µg/ml)	9.896 ± 4.579

Table 2. Shows the ability of various dilutions of Test drug NMC to produce cell death on Human ovarian (PA-1) cell line with Methotrexate as the standard comparison drug

S.No	Concentration in µg/ml	% cell Death
1	10 µg/ml	10.58 ± 1.827
2	50 µg/ml	27.5 ± 2.388
3	100 µg/ml	40.22 ± 4.613
4	200 µg/ml	59.62 ± 3.122
5	STD (METHOTREXATE 10 µg/ml)	90.1 ± 4.579

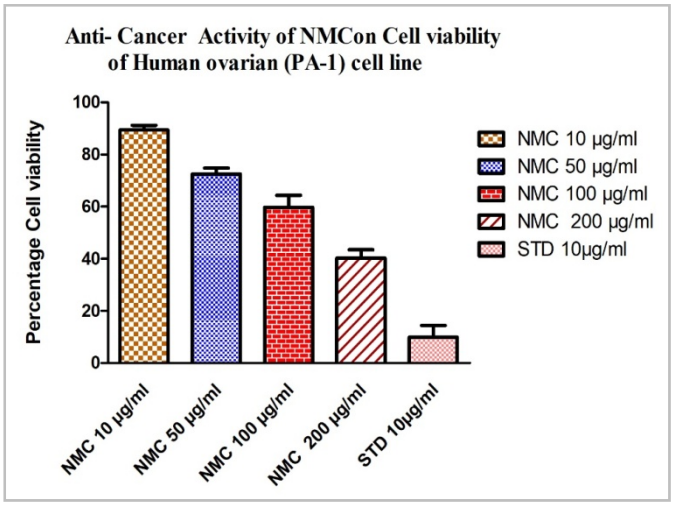
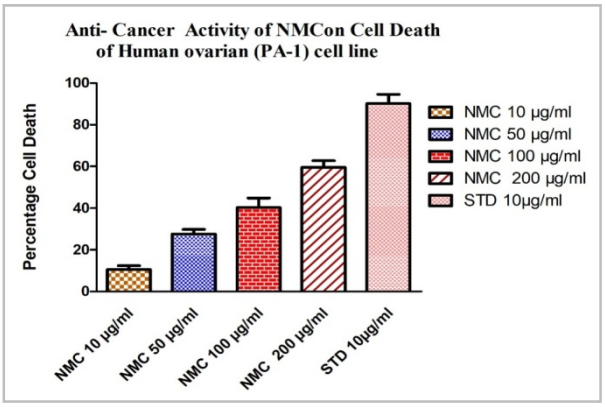


Fig. 1. Pictorial Representation of Anticancer Activity of NMC on Cell Viability of Pa-1 Cell Line



*NMC – Navamani Chenduram

Fig. 2. Pictorial Representation of Anticancer Activity of NMC on Cell Death of Pa-1 Cell Line

In-vitro anti-cancer evaluation of test drug NMC on the cell viability against Human ovarian (PA-1) cell line was performed at varying concentration ranges from 10 to 200 µg/ml. The reuslt obtained from the study reveals that the percentage of cell viability of PA-1 celline line viability decrease with increase in concentration of the test drug NMC.

Least viability of cell was observed at the concentration of 200µg/ml was 40.38 ± 3.122 %, followed by this at 100 µg and 50 µg showing59.78 ± 4.613%, 72.5 ± 2.388, similarly 10 µg/mlshowing89.42 ± 1.827 % cell viability in MTT assay. The corresponding IC50 value was found to be 153.1 ± 9.583 µg/ml. It was concluded from the result of the present study that the formulation NMC possesses promising anti-cancer activity.

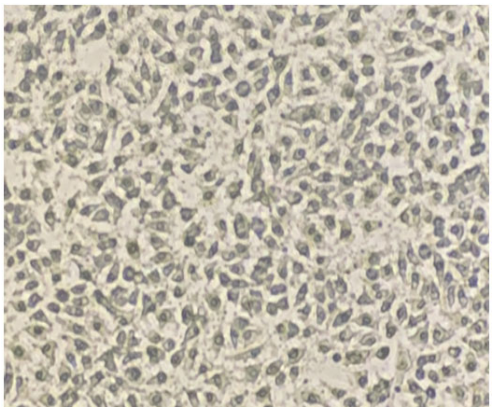


Fig.3. PA-1 Control

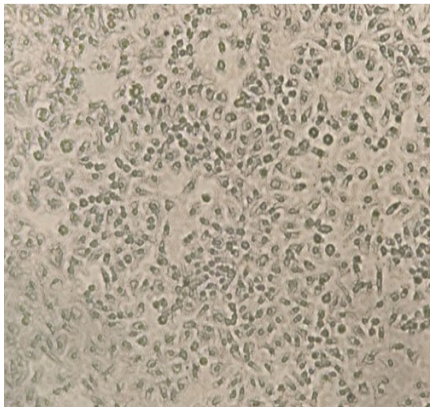


Fig. 4. Test Drug NMC – 10 µl

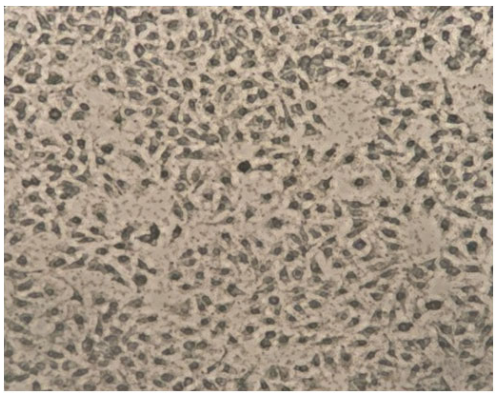


Fig. 5. Test Drug NMC – 50 µl

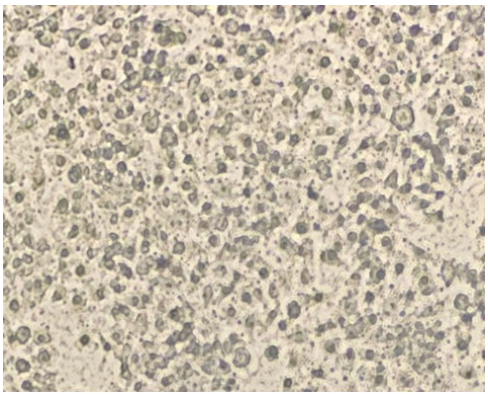


Fig. 6. Test Drug AH – 100 µl

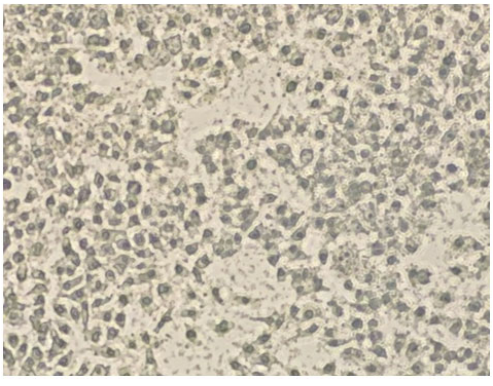


Fig. 7. Test Drug NMC-200 µl

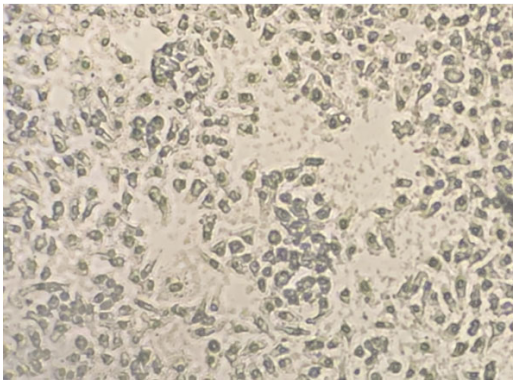


Fig. 8. Methotrexate(STD Drug) – 10 µg/ml

DISCUSSION

In the current investigation, we aimed to clarify the impact of NMC on apoptosis in the metastatic PA-1 cell line of human ovarian cancer. Using methotrexate as the reference drug, we determined that 200 g/ml of NMC is an efficacious concentration. NMC decreased the cell viability of PA-1 cells in a dose- and time-dependent manner. Metals in nanoform, such as cinnabar, sulfur, potassium nitrate, mercuric perchloride, and mercuric sub-chloride, were the main ingredients of NMC. According to an antiquated methodology, harmful mercury can be changed into a safe medicinal medication by employing a plant extract [16]. Based on conventional wisdom, Mukhi *et al.,* 2017. created the mercury-based medication Rasasindur and discovered its efficacy.[17] The effectiveness of the long-used process of turning the hazardous element mercury into a medication with antioxidant properties was also confirmed by Gokarn *et al.,* 2015.[18] It is well established that traditional medical formulations are effective at eliminating the toxicity from metallic compounds to create safe herbomineral medications [19]. The usage of nanoform of heavy metals like mercury, sulphur etc., have gained a great interest in the biomedical field and cancer activity in particular [20]. Cancer treatments that only trigger apoptotic cell death without also causing intrinsic cytotoxicity are likely to kill more normal cells than tumor cells [21]. Drugs that are both cytotoxic and apoptotic are necessary for an effective cancer treatment [22]. The NMC chosen in this study may used as a promising chemo-preventive medication due to its inherent cytotoxicity and apoptotic activities..

Conclusion

As a result, the MTT assay, which was conducted using Methotrexate as the reference medication and *Navamani Chenduram* as the sample, demonstrated the NMC's potent anticancer properties. Higher test sample concentrations of NMC revealed its efficient cytotoxic impact. To confirm the therapeutic applicability of the aforementioned test sample, additional animal research and clinical trials are needed.

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Author contribution : EN: methodology, validation, data curation, writing – original draft; PC: data, curation, writing – review and editing; EN: formal analysis, resources; KS,PE: writing – review and editing; KS: conceptualization; EN: conceptualization, writing – review and editing; PC: conceptualization, supervision.

Conflict of Interest: Nil

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