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Anticancer activity of leaf extract of Andrographispaniculata on human breast cancer cell line

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ABSTRACT:

Aim: The investigation ofpresent study is to evaluate the Anticancer activity of leaf extract of *Andrographispaniculata* on human breast cancer MCF-7 cells.

Materials and methods: The cytotoxic effect of*A.panniculate*was evaluated by 3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Further, the morphological assessment in MCF-7 cells upon treatment with/without*A.paniculata*leaf derived crude extract were examine under phase contrast microscope. All data obtained from the experiments were analysed and interpreted statistically (SPSS Student Version Software Package; SPSS Inc., Chicago, IL, USA) using student t-test and one-way ANOVA.

Results: The aqueous extract of *A.paniculata* showed the cytotoxic potency against the MCF-7 cell line which further confirmed with greater morphological changes at 24 hrs treatment respectively. The MTT results confirmed that the *A.paniculata* has greatly inhibits the cell growth when the concentration was increased at 24hrs treatment. The P value is <0.001

Conclusion: The present study shows 50% cytotoxic effect (IC_{50}) at 10 µg/mL of aqueous extract of *A.paniculata* against human breast cancer MCF-7 cell line.

Keywords: Cytotoxicity, MCF-7, *Andrographispaniculata*, MTT assay

INTRODUCTION:

Breast cancer is among the most frequent illnesses among women globally, with Asian nations reporting the bulk of instances during the last two decades. Currently, numerous researches have been carried out globally to identify the active new chemicals from plants for cancer therapy. (1) Chemotherapy and radiation therapy are the most common cancer treatments today, but they are also the costliest and have the greatest potential for adverse effects for patients. As a result of the high costs and adverse effects of chemo and radiation treatments, natural items have become more popular as cancer medicines. (2)(3) Several disorders, including cancer, have been linked to natural products as a primary source of medications. There are many phytochemicals found in plants, and more than 75% of the anti-infective medications have been isolated from plant sources. Plant-derived antioxidants have the potential to affect the microenvironment and influence cancer cell activity. From therapeutic plants, a variety of secondary metabolites, including alkaloids,

polyphenols, flavonoids, and triterpenes, were extracted and purified. On the other hand, it is important to analyse and create a personalized chemopreventive medicines that can assess cytotoxicity and apoptosis induction in cancer cell lines.

Nature has equipped mankind with a broad range of beneficial sources, particularly plants, which have been used in the discovery and development of medicines to combat a number of debilitating ailments. The use of traditional herbs is an useful method for treating cancer as well as a number of other ailments. It has been discovered that medicines derived from medicinal plants have less side effects with lower toxicity levels. Andrographispaniculata is a member of the Acanthaceae family and is a medicinal plant that is popularly referred to as the "King of bitters". (4)This herbaceous plant's roots and leaves were traditionally used in the treatment of a variety of infectious and chronic conditions, including respiratory infections, sore throats, and others. It is indigenous to India and Sri Lanka, although most commercial production takes place in southern Asian countries(5)(6). This plant has many medicinal activities such as antimicrobial ,anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective activity, and nephroprotective activity.(7) The plant extract also exhibits anti-typhoid, antifungal, antimalarial anti-thrombogenic, anti-snake venom and antipyretic properties. Besides this it is also used as an immunostimulant agent(8). The aim of this study is to perform an anticancer activity of leaf extract of *Andrographispaniculata* on human breast cancer cell lines. Our team has extensive knowledge and research experience that has translate into high quality publications(9–13),(14),(15),(16),(17),(18),(19),(11,20,21),(22–26),(27),(28)

Materials and methods:

REAGENTS:
MCF-7 cell lines were obtained from the National Centre for Cell Science (NCCS) in Pune. Sigma Chemicals Co., St. Louis, USA, provided DMEM (Dulbecco's modified Eagle's media), sodium bicarbonate solution, bovine serum albumin (BSA), and MTT. The Fetal bovine serum (FBS), 0.25 percent Trypsin-EDTA solution, antibiotic/antimycotic solution, and DMSO were all acquired from Gibco BRL (CA, USA). Thermo Fisher brand, India, supplied sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid, and methanol. The cells were cultivated in T25 culture flasks with 10% FBS in DMEM medium.

PREPARATION OF *A. panniculate* Leaf EXTRACT:
The air-dried A. paniculate leaf powder was purchased directly from Aravindh Herbal Labs (P) Limited, Chennai. The extraction was performed three times using the maceration technique (3x24 h) at room temperature (25–28°C). The crude methanol extract from each of the samples was then concentrated using a rotary evaporator (made by Steroglass, Italy), and then freeze-dried (Zirbus, Germany). The freeze-dried leaf extract was accurately weighed and diluted in 1 mg/ml DMSO as a stock solution. This solution was then diluted to various concentrations ranging from 20 to 300 g/ml for MTT experiment.

MTT ASSAY:
The MTT (3-(4, 5-dimethylthiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) test was used to determine the cytotoxic impact of A. Panniculate on MCF-7 cells. (29) In addition, the vitality of MCF-7 cells after drug treatment was evaluated, and then a trypan blue exclusion test was performed. Cells were seeded in 96-well plates at a density of $5X10^3$/100l for 24 hours before being treated with various concentrations (0, 20, 40, 80, 100, 200, and 300 µg) of A. paniculata. 20 µl of MTT stock solution, 5 mg/ml, was applied to each well and incubated for 4 h at 37 °C. The formazan crystals were solubilized in DMSO and analysed at 570 nm (SpectraMax M5, Molecular Devices, USA). It has been shown that the percentage of live cells may be calculated as the ratio of the absorbance at 570 nanometers (A570) in treated cells to the absorbance in control cells with 0.1 % DMSO (A570). When compared to the absorbance of the DMSO-treated control, the sample concentration that was determined to be necessary for achieving an IC_{50} reduction of fifty percent was determined. The following equation was used to determine the percentage of live cells in a sample:

$$\%\ of cell viability = 100 - \frac{OD of test}{OD of control} \times 100$$

STATISTICAL ANALYSIS:
All of the data were calculated statistically using one-way ANOVA using SPSS/10 software for Windows, version 20; SPSS Inc., Chicago, Illinois, USA. All of the data were represented as the mean along with the standard error (SE) and were obtained from three separate experiments. The LSD was used to do post-hoc testing for inter-comparisons. In every experiment, the threshold of statistical significance was determined to be $p<0.05$, and this was maintained throughout the study.

Results:
Morphological investigation of cytotoxic effect of *Andrographispaniculata*on MCF-7 Breast cancer cells

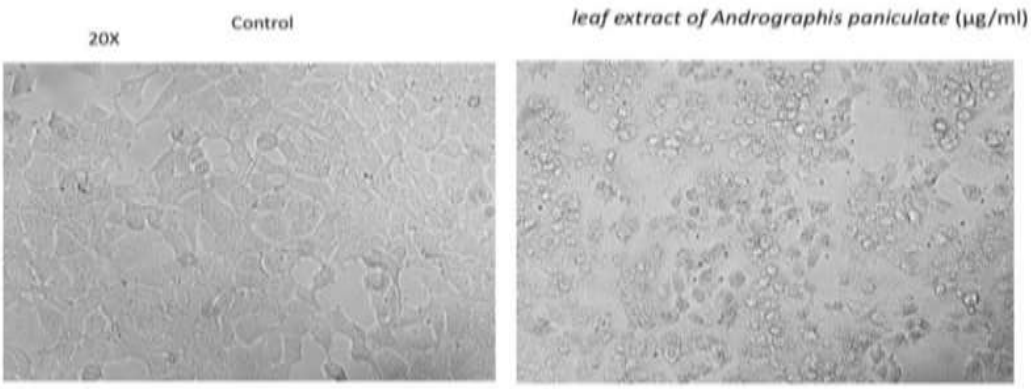

Figure 1: Represents the morphological changes that occurred in the control breast cancer cell line as well as the breast cancer cell line that was treated with A. paniculata. The photos were captured using a phase contrast microscope with a magnification setting of 20x.

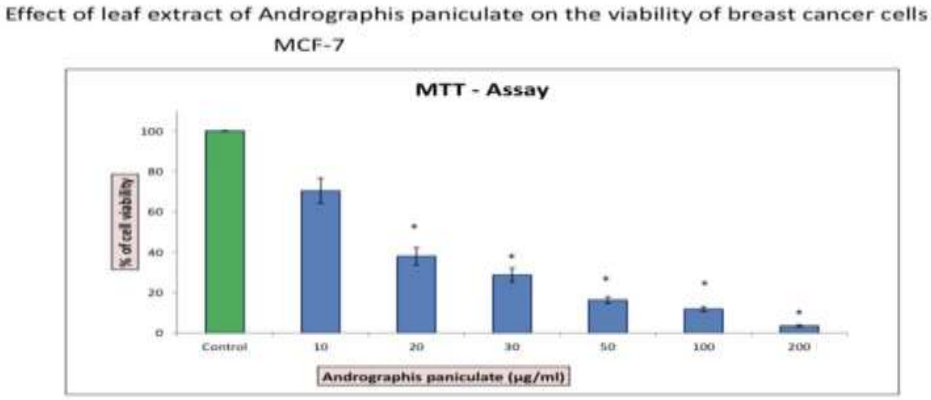

Figure 2: The cytotoxic effects of A.paniculata on MCF-7 cells were evaluated using the MTT test. During this period of time, the cells were exposed to a series of concentrations, comprising 0, 20, 40, 80, 100, 200, and 300 g. At a concentration of 15 micrograms per millilitre, which has been shown to be the IC50 value for the herbal extract, there was a fifty percent inhibition of cell growth. Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

DISCUSSION:

Plants used in medicine are non-toxic and may treat a wide range of conditions, including cancer, without negatively impacting a person's overall health and well-being. These medicinal herbs have beneficial immunomodulatory and antioxidant characteristics, both of which contribute to the plants' anticancer actions (30). Antioxidant phytochemicals shield the cells from the destructive effects of free radicals. Consuming a diet high in antioxidant plant material may therefore bring health benefits. These natural chemicals are proposed to minimize DNA damage by interacting with free radicals, hence preventing cancer. (31) Andrographispaniculata is a plant whose aerial parts, including its stems and leaves, have a long history of usage in traditional medicine for the treatment of a wide variety of conditions, including diabetes, hypertension, and cancer.

In addition, it has been shown that an extract of Andrographispaniculata may boost both the antigen-specific and the nonspecific immune systems in mice. (32) Immunomodulatory and anticancer activity of A.paniculata plant extract and its constituents in human cells is unknown. The anticancer action shown by the plant extract is mostly attributable to the presence of andrographolide (33). The other two components do not contribute substantially to the anticancer activity.

This bioactive component found in plants may prevent carcinogenesis by limiting metabolic activation, boosting detoxification, or giving alternate targets for electrophonic metabolites (34). They may work by limiting the interaction of chemical carcinogens or endogenous free radicals with DNA, lowering the amount of damage and consequent mutations that contribute not only to cancer development but also to increasing genomic instability and overall neoplastic transformation (35). The quantitative contents of chemicals indicated varying concentrations across three plants, with

*A.paniculata*leaves having the greatest concentration and Ferocactus sp. leaves having the lowest (36). This inhibitory activity might be related to the nature of the substances contained in each crude extract and how they interact with the metabolic nature of each kind of cancer cell, or it could be due to the efficacy of specific enzymes that serve as antioxidants, particularly in cancer cells (37). The study's drawback is that it does not include any in vivo testing, therefore its effectiveness cannot be determined. This opens the door to a variety of future investigations, such as seeing drug activity in in vivo tests and understanding about the extract's detrimental consequences.

CONCLUSION:

Based on the results, the extracts of A.paniculata were shown to be cytotoxic to the murine cells. Additionally, it induces apoptosis and cell death in breast cancer cells, which contributes to its significant anticancer impact in breast cancer cells. However, further research is required to understand the anticancer action of the plant in order to produce a new anticancer medicine that may be used to treat a variety of malignancies, including breast cancer.

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