



## The *in vitro* Potential Oncolytic Effect of Lentogenic and Velogenic Newcastle Disease Viruses on MCF-7 and Caco-2 Cell Lines Compared to Chemotherapies

Alaa O. Abd El-Khalek<sup>1</sup>, Mohamed H. Khodeir<sup>2</sup>, Azza Hassan<sup>3</sup>, Amani A. Saleh<sup>2</sup>, Sahar S. Abdelrahman<sup>3\*</sup>

<sup>1</sup>Poultry department, Al-Badrashein Veterinary Unit, Giza, Egypt.

<sup>2</sup>Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

<sup>3</sup>Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.

\*Corresponding Author: Sahar S. Abd El-Rahman, E-Mail: [Saharsamirmahl@cu.edu.eg](mailto:Saharsamirmahl@cu.edu.eg)

### ABSTRACT

Cancer is the leading cause of death worldwide, with breast and colorectal cancers being the two most common cancer forms. The present work was designed to investigate the probable oncolytic effect of lentogenic and velogenic Newcastle disease viruses on MCF-7 and Caco-2 cell lines compared to the commonly used chemotherapies as an *in vitro* preliminary study to further prelude an *in vivo* study. The cytotoxic effects of Newcastle disease virus strains NDV/chicken/Egypt/Giza/2015 (velogenic NDV genotype VIID) and Lasota strains, as well as the commonly used chemotherapies (Paclitaxel or Doxorubicin) were investigated on MCF-7 and Caco-2 cell lines at different concentrations. Both the human colorectal adenocarcinoma (Caco-2) and the Michigan Cancer Foundation-7 (MCF-7) human breast cancer cell lines were inoculated with NDV VIID and LaSota at concentrations of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ , Paclitaxel (for MCF-7) at concentrations of 0.5, 1, and 2  $\mu$ M, and Doxorubicin (for Caco-2) at 0.1, 1 and 10  $\mu$ M in four replicates each. The cytotoxic effect was performed using a neutral red assay for both virus strains and in combination with chemotherapeutic agents. The present study clarified that both VIID and LaSota strains of NDV, particularly at titers of  $10^{-3}$  and  $10^{-4}$  TCID<sub>50</sub>/ml, respectively, displayed a significant ( $P \leq 0.05$ ) cytotoxic effect on both MCF-7 and Caco-2 cell lines. Moreover, the combined treatment of the TCID<sub>50</sub> (Tissue Culture Infective Dose 50) doses of both NDV strains and the tested chemotherapies showed a more significant ( $P \leq 0.05$ ) cytotoxic effect than the sole use of each. Depending on the results, we can conclude that this study opens the way for further *in vivo* studies aiming to provide more safe treatment for human cancers, save human lives, and avoid dramatic ends.

**Keywords:** Caco-2, MCF-7, Oncolytic; lentogenic; Velogenic *J. Appl. Vet. Sci.*, 9(1) : 32-41. Newcastle disease viruses.

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### INTRODUCTION

Cancer is still the tenth most common cause of death around the globe. Breast cancer is the most common malignancy, according to the 2007 National Cancer Registry Report, followed by colorectal cancer (Omar *et al*, 2011).

Patients with breast cancer can receive a variety of treatments, such as surgery, radiation, and chemotherapy. Moreover, various anti-cancer chemotherapeutic medications have been available since their discoveries, including tamoxifen (Jordon, 1993), trastuzumab (Goldenberg, 1999), and

bevacizumab (Rugo, 2004). Although these medications have numerous adverse effects.

Conventional cytotoxic medicines frequently destroy cancer cells at the expense of harming healthy tissues, leading to toxicities that are intolerable. Therefore, the ultimate objective of all oncologists and cancer researchers is to destroy cancer cells without resulting in collateral damage (Zhou *et al*, 2018). As a result, various attempts have been made by researchers worldwide to investigate and create tailored medication delivery methods with the fewest possible side effects that will specifically target cancer cells without damaging healthy cells in the body.

The practice of modifying a virus to act as an anti-cancer agent has become more popular recently. The only virus type that selectively targets cancer cells while causing minimal harm to normal cells is the oncolytic virus (Parato *et al.*, 2005).

Since the mid-1800s, tumour regressions associated with natural infections have been seen, leading to the original inspiration for employing bacteria and viruses to treat human malignancies (Zhou *et al.*, 2018; Hemminki *et al.*, 2020).

Many oncolytic viruses from various families demonstrate various tumour-selectivity mechanisms. For instance, malignant glioma has been treated using the herpes simplex virus mutant G207 from the Herpesviridae family (Markert *et al.*, 2000). Mumps virus, a member of the Paramyxoviridae family, has been used to treat ovarian cancer in the past (Myers *et al.*, 2005), while Sindbis virus, a member of the Togaviridae family, has been suggested as a treatment for cervical and ovarian malignancies (Unno *et al.*, 2005). Apart from all these viruses, the oncolytic Newcastle disease virus (NDV) also has anti-cancer potential (Zamarin and Palese, 2012).

Newcastle disease virus (NDV) is a negative single-strand RNA avian paramyxoviruses. Six genes make up the viral genome: hemagglutinin-neuraminidase (HN), matrix protein (M), fusion protein (F), nucleoprotein (NP), large protein (L), and phosphoprotein (P). Based on their virulence in poultry, NDV strains are categorized into three classes; velogenic strains of NDV are the most virulent (Kumar *et al.*, 2012). Mesogenic NDV strains are mid-virulent and lentogenic, or attenuated strains that are not virulent (Pecora *et al.*, 2002).

NDV was demonstrated to be a strong oncolytic drug with an alluring safety profile in phase I and II clinical trials in humans (Freeman *et al.*, 2006). With both pathogenic (MTH-68/H, Ulster, and PV701) and non-pathogenic (Hitchner-B1, LaSota, 73-T, and HUU) virus strains, the oncolytic potential of NDV has been proven in cell cultures, in experimental animal models, and in clinical trials (Freeman *et al.*, 2006; Zamarin *et al.*, 2009).

When compared to other oncolytic medicines being developed, NDV shows a number of benefits. First of all, NDV is a specific pathogen for avian species and the virus's use as an avian vaccine for more than 50 years has shown that its genome is extremely stable (Schirmacher, 2016). Second, according to serological research, ~96% of the population is seronegative for NDV, which eliminates the issue of preexisting immunity in people, which is a potential issue with adenovirus, herpes simplex

virus, and vaccinal virus (Schirmacher *et al.*, 2019). Last but not least, because the NDV receptor is so widely distributed, a variety of malignancies can be treated with it. The present work was designed to investigate the oncolytic effect of lentogenic and velogenic Newcastle disease viruses on MCF-7 and Caco-2 cell lines compared to chemotherapies as an *in vitro* preliminary study to further prelude an *in vivo* study.

## MATERIALS AND METHODS

### 1. Chemicals

Paclitaxel (Taxol)<sup>®</sup>; Ebewe Pharma (Austria), Doxorubicin; Ebewe Pharma (Austria). A stock solution for neutral red 4mg/ml was prepared by dissolving 40 mg neutral red dye (SERVA) in 10 ml PBS, this preparation was kept in the dark at 4°C (Repetto *et al.*, 2008).

### 2. Cell culture solutions

Phosphate buffered saline (PBS, pH 7.2) (Gibco™ Catalog number: 20012027) was used for preparation of trypsin versine solution and as a diluent for the used treatments.

**2.1.** Trypsin versine solution: 0.25% solution of 1/250 trypsin (Gibco™ Catalog number: 15040066) was prepared in PBS with 0.1% ethylene diamine tetra acetic acid (EDTA) sterilized by filtration and used for cell culture passages as cell dispensing agent.

**2.2.** Fetal bovine serum (FBS) (Gibco™, Paisley, Scotland, UK, P.B.35, virus and mycoplasma screened) was used in the ratio of 10% as a supplement for cell culture growth media while 2% of such serum was added to maintenance media.

**2.3.** Minimum essential medium (MEM) with Hank's salts, L-glutamine and without sodium bicarbonate was supplied by Gibco™ G80 Catalog number: 12491015, Paisley, Scotland, U.K.) prepared according to the manufacture directions. MEM was used with 10% new FBS as growth medium while it was used with 3% serum as maintenance medium for Vero cell cultures.

**2.4.** RPMI-1640 medium Biological, USA was used for passaging and maintenance of MCF7 cell line.

### 3. Specific pathogen free chicken embryonated eggs (SPF)

SPF embryonated eggs were supplied by the SPF farm Qom Osheim El-Fayoum Governorate, Egypt and were used for propagation NDV.

### 4. Preparation of Chicken Red Blood Cells (CRBCs) for virus titration

Chicken RBCs were collected from brachial veins under the right wing of a healthy, unvaccinated chicken using a heparinized syringe to draw about 2 ml of blood. The chicken RBC suspension was prepared by adding 1 ml

of blood to 7 ml of cold PBS and mixing using a centrifuge for 10 min at 1000 rpm, 4° C, and 10 min. Then the sediment will be washed three times with PBS. The supernatant was discarded, the pellet (RBC) was collected, and then 0.1 ml of the washed RBCs was added to 10 ml of PBS to prepare a 1% RBC solution for the Hemagglutination test (Al-Ziaydi *et al.*, 2020).

## 5. Newcastle disease viruses (NDV)

Newcastle disease virus strains; NDV/chicken/Egypt/Giza/2015 (Velogenic NDV genotype VIID) (Fawzy *et al.*, 2020) and Lasota strains was donated by Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Egypt.

### 5.1. Viral propagation

The original stock of each viral strain was amplified by passage through 10-day-old chick embryos, and then purified from debris by centrifugation (at 3000 rpm and 4 °C for 30 min). The NDV titer was quantified through the hemagglutination test (HAT), aliquoted, and stored at -80 °C. Later the virus was passaged three successive times in the Vero cell line, and the viral titers were determined through a 50% tissue culture infective dose (TCID<sub>50</sub>) titration on Vero cells following the standard procedure according to Al-Shammari *et al.*, (2014).

## 6. Cell lines

**6.1. African green monkey kidney (Vero) cell line** was graciously provided by VSVRI; and was used for the initial propagation of both strains of NDV.

### 6.2. Cancer cell lines

Both the human colorectal adenocarcinoma (Caco-2) and the Michigan Cancer Foundation-7 (MCF-7) human breast cancer cell lines were originally acquired from the Cell Lines Bank of VACSERA (Cairo, Egypt). Both cell lines were principally grown in DMEM (Dulbecco's Modified Eagle Medium) (Lonza) with 10% foetal bovine serum (LSP) and 1% antibiotics, penicillin G potassium (SERVA) and streptomycin (Sigma), at 37°C in humidified air with 5% CO<sub>2</sub>. The Faculty of Agriculture's cell culture lab at Cairo University Research Park served as the site for all in-vitro research.

## 7. Determination of cell viability/cytotoxicity of NDV (VIID and LaSota strains), Paclitaxel, and Doxorubicin

Neutral red uptake assay was used to assess the anticancer activity of both NDV strains as well as the tested chemotherapies on both MCF-7 and Caco-2 cells in accordance with (Repetto *et al.*, 2008).

Cultures of MCF-7 and Caco-2 cells at 1 x 10<sup>5</sup> and 2 x 10<sup>4</sup> cells per well respectively, were

established in 96-well microtiter plates. MCF-7 cells were either treated with NDV VIID and LaSota at concentrations of 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, and 10<sup>-5</sup> (Xu-Feng *et al.*, 2015) or Paclitaxel at concentrations of 0.5, 1, and 2 μM (Luo *et al.*, 2010) in four replicates each. In contrast, Caco-2 cells were treated in four duplicates with or without NDV VIID, LaSota at the same previous concentrations, or Doxorubicin at 0.1, 1 and 10 μM (Eid *et al.*, 2012). A phase-contrast inverted microscope (LEICA DMI 3000 B) was used to evaluate cultures 48 hours after incubation, and any changes in the morphology of the cells were noted. By comparing the OD of neutral red extract at 540 nm in a microtiter plate reader spectrophotometer (BioTek, ELX808) to a blank containing no cells as a reference, cell viability was determined. Cell viability (%) = mean tested OD / control OD x 100%; the results were expressed as percentage of cell survival relative to the untreated control (100%).

## 8. Determination of the half maximal inhibitory concentration (IC50)

At the utilized concentrations of NDV VIID and LaSota, Paclitaxel, and Doxorubicin, which were necessary to induce a 50% suppression of MCF-7 and Caco-2 cells viability, dose-response curves were created, and IC50 values were graphically established. The relative cell viability was represented on the Y-axis while the drug concentration was plotted on the X-axis.

## 9. Assaying the cytotoxic effect of the combined treatment of NDV strains (VIID and LaSota) with chemotherapies in relation to their sole treatments on MCF-7 and Caco-2 cell lines

In two further experiments, cultures of 1 x 10<sup>5</sup> and 2 x 10<sup>4</sup> MCF-7 or Caco-2 cells, respectively, were established in 96-well microtiter plates. MCF-7 cells were inoculated with 10<sup>-3</sup> for the VIID strain and 10<sup>-4</sup> for the LaSota (a chosen concentration; lower than the IC50) of either VIID and LaSota and/or 1μM paclitaxel (Luo *et al.*, 2010). Caco-2 cells were inoculated with the same chosen concentration of either VIID and LaSota and/or 1μM doxorubicin (Eid *et al.*, 2012). The cell viability was examined after 48 hours, and changes in morphology were recorded. Next, neutral red uptake assays were performed as previously mentioned to determine the cell viability (%).

## 10. Statistical analysis

Data is shown as means SD. Using SPSS, one-way analysis of variance (ANOVA) and the LDS correction test were used to compare data from various groups. Statistics were judged significant at P<0.05.

## RESULTS

**The cytotoxic effect of VIID and LaSota strains of NDV, Paclitaxel, and Doxorubicin on MCF-7 and Caco-2 cell lines at different doses expressed as cell viability**

Under a phase-contrast inverted microscope, the sensitivity of MCF-7 to NDV VIID, LaSota, or Paclitaxel at various concentrations and that of Caco-2 to NDV VIID, LaSota, or Doxorubicin at various doses were assessed 48 hours after exposure. The cytotoxic effects of the tested compounds presented by alterations in cellular morphology were recorded, including severe inhibition of cell growth, vacuolations, rounding, cellular detachment, and clumping (Figs. 1A and B), as opposed to the control negative cells, which had the appearance of confluent sheets.

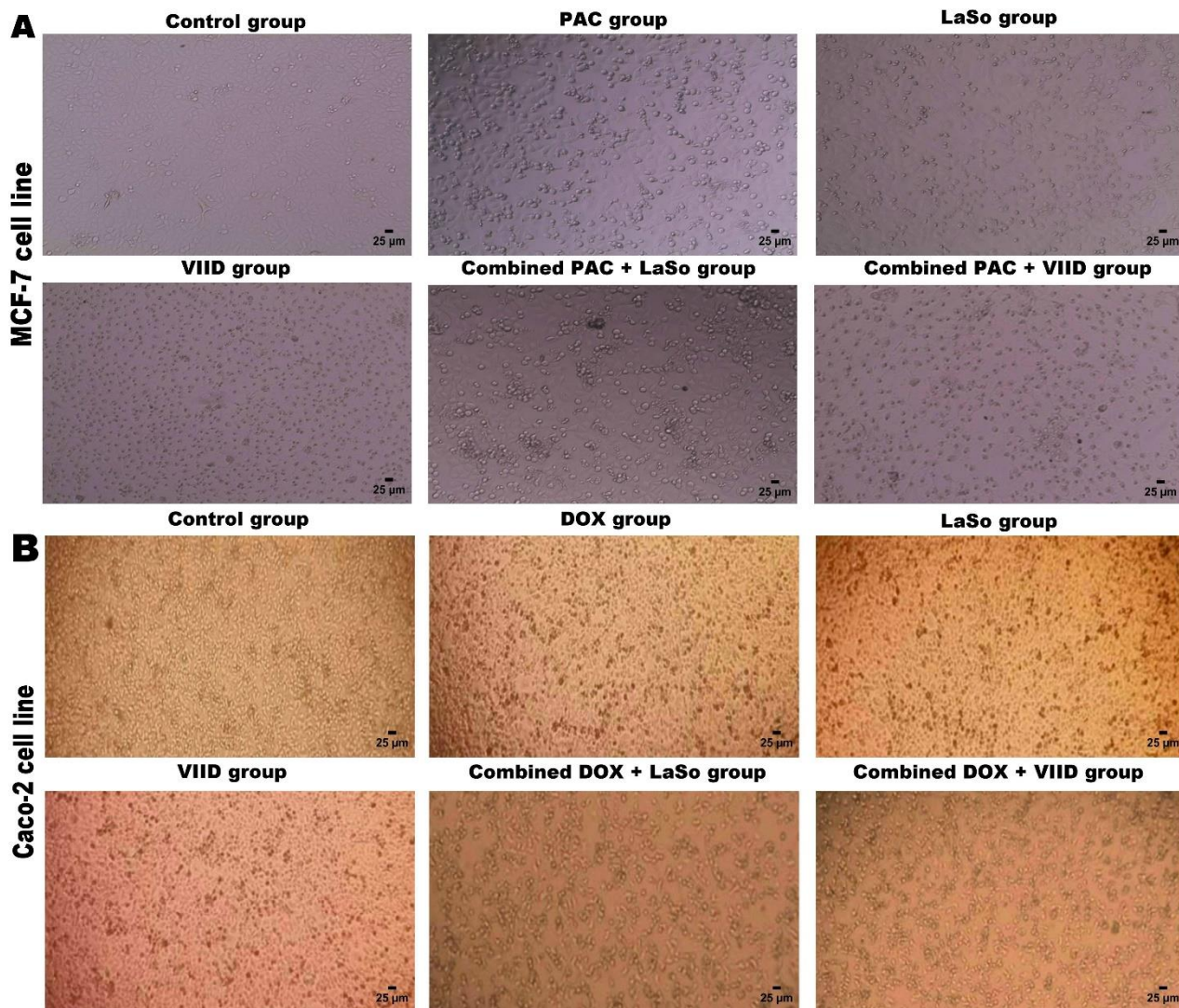


Fig.1: Photomicrographs of (A) MCF-7 and (B) Caco-2 cell lines 48 hours after incubation showing the morphological changes of the cytotoxic effect of NDV VIID, LaSota, Paclitaxel on MCF-7, and Doxorubicin on Caco-2 presenting vacuolation, rounding, cellular and detachment, and clumping.

In general, a dose-dependent, significant ( $P \leq 0.05$ ) decrease in cell viability was noticed in various treatments. With an increase in virus concentration or incubation time, cell viability decreased. NDV-VIID and LaSota treatments significantly ( $P \leq 0.05$ ) reduced the cell viability percentage of MCF-7 by 44%, 60%, and 40%, respectively, at doses of  $10^{-3}$ ,  $10^{-4}$ . Moreover, both strains decreased the cell viability of Caco-2 cells by 39%, 66% and 35%, 60%, respectively, compared to untreated control cells. On the other hand, at doses of 1 and 2 µM, Paclitaxel significantly ( $P \leq 0.05$ ) reduced cell viability in MCF-7 samples by 35% and 47%, respectively. At dosages of 1 and 10 µM, doxorubicin reduced Caco-2 cell viability by 32% and 72%, respectively (Figs. 2A and B).

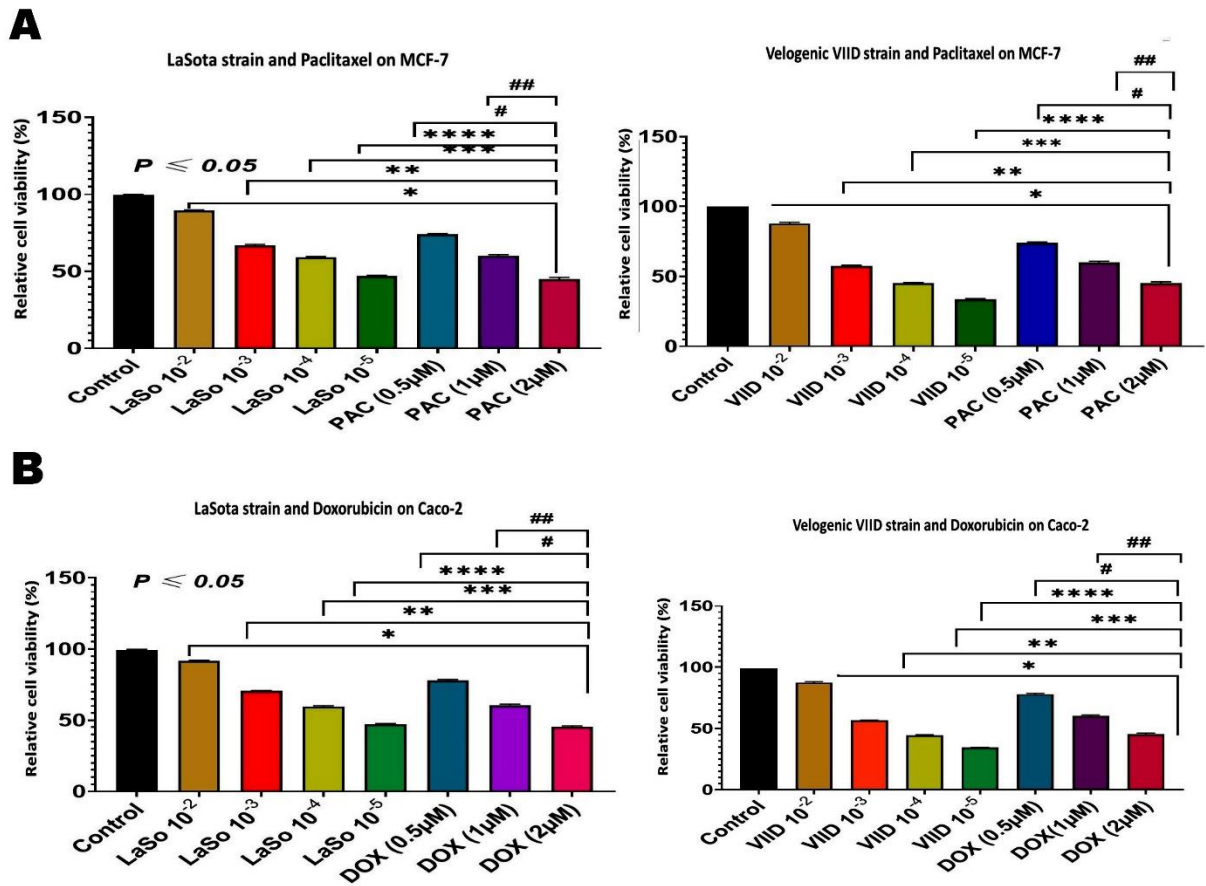


Fig. 2: The cytotoxic effect of NDV-VIID, LaSota, Paclitaxel, and Doxorubicin on MCF-7 and Caco-2 cell lines at different doses expressed as cell viability. Values are expressed as mean  $\pm$  SE. Data were analyzed by using one way ANOVA followed by Tukey post-hoc test. \*, \*\*, \*\*\*, \*\*\*\*, # and ## were significant differences at  $P \leq 0.05$ , where; \* significantly different when compared to control group, \*\* when compared to LaSota 10<sup>-2</sup> group, \*\*\* significantly different when compared to LaSota 10<sup>-3</sup>, \*\*\*\* significantly different when compared to LaSota 10<sup>-4</sup>, # when compared to LaSo 10<sup>-5</sup> and ## significantly different when compared to PAC (0.5μM).

### The IC<sub>50</sub> of NDV VIID and LaSota strains, Paclitaxel and Doxorubicin on MCF-7 and Caco-2 cell lines

It was found that the IC<sub>50</sub> value of NDV VIID and LaSota which caused 50% growth inhibition was 10<sup>3</sup> TCID<sub>50</sub> for the VIID strain and 10<sup>4</sup> TCID<sub>50</sub> for the LaSota strain on both MCF-7 and Caco-2 cells. On the other hand, paclitaxel's IC<sub>50</sub> on MCF-7 cells was 2.2 μM, whereas doxorubicin's IC<sub>50</sub> on Caco-2 cells was 5.3 μM (Fig.3A-C).

### The cytotoxic effect of the combination treatment of NDV VIID and LaSota with each type of the chemotherapeutic drugs on MCF-7 and Caco-2 cell lines compared to their separate treatments

The cytotoxic effects of each of the chemotherapeutic drugs administered in combination with the chosen concentrations of NDV VIID and LaSota (10<sup>-3</sup> for the VIID strain and 10<sup>-4</sup> for the LaSota) were compared to those of the sole use of Paclitaxel (1 μM), Doxorubicin (1 μM), NDV VIID (10<sup>-3</sup>), and LaSota (10<sup>-4</sup>). In general, 48 hours after exposure to the combined treatments, dramatic changes in cell morphology, including apoptosis, cellular rounding, and detachment as well as decreased cell viability, were found compared to the use of NDV VIID (10<sup>-3</sup> TCID<sub>50</sub>), LaSota (10<sup>-4</sup> TCID<sub>50</sub>), Paclitaxel (1μM), and Doxorubicin (1μM), alone. The combination treatments of NDV VIID (10<sup>-3</sup> TCID<sub>50</sub>) and LaSota (10<sup>-4</sup> TCID<sub>50</sub>) with Paclitaxel and Doxorubicin on MCF-7 and Caco-2 cell lines, respectively, exhibited a cell viability percent of 21, 33% and 35, 42%, as opposed to 65% for Paclitaxel on MCF-7 cells and 68% for Doxorubicin on Caco-2 as presented in Fig.4A-C. Contrarily, only NDV VIID (10<sup>-3</sup> TCID<sub>50</sub>) and LaSota (10<sup>-4</sup> TCID<sub>50</sub>) demonstrated 53% and 55%, respectively, and 60% and 61%, respectively, cell viability in MCF-7 and Caco-2 cell lines.

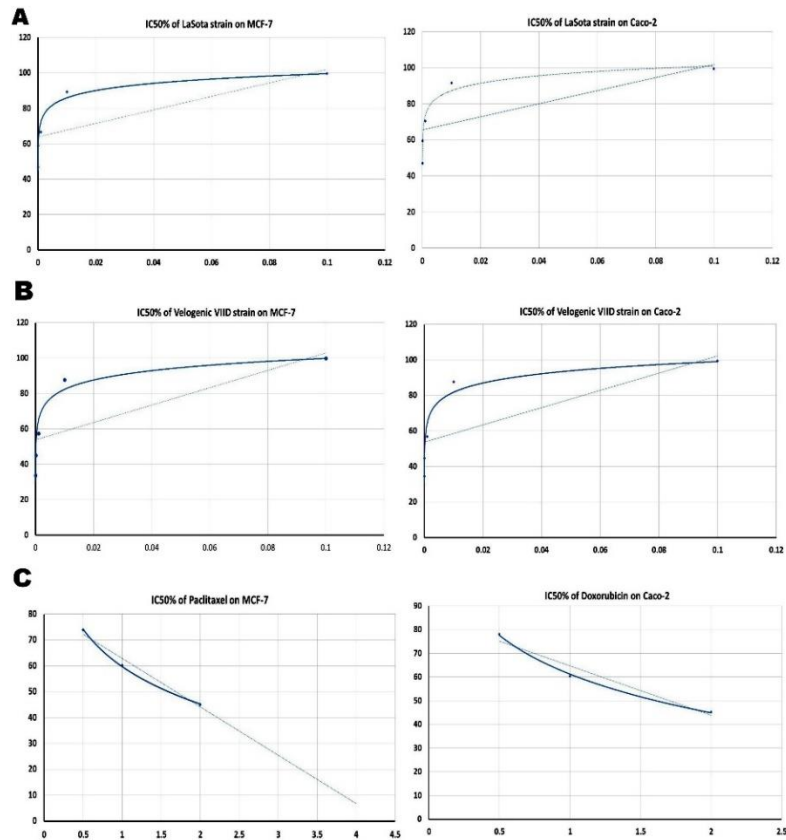


Fig.3: The IC50 of NDV VIID, LaSota, Paclitaxel and Doxorubicin on MCF-7 and Caco-2 cell lines.

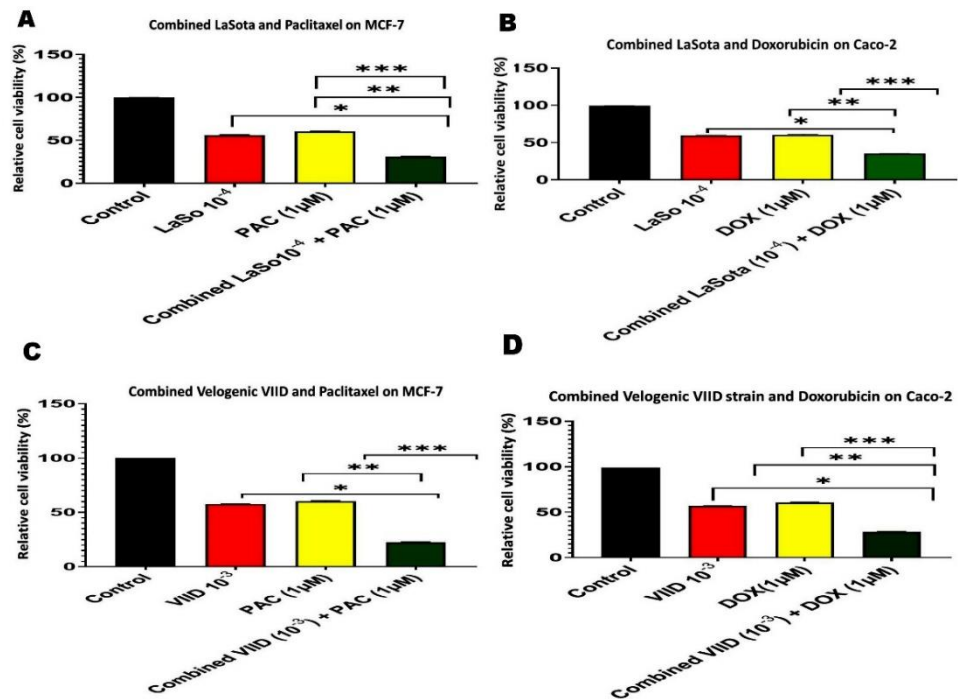


Fig.4: The cytotoxic effect of the combined treatment of each viral strain (VIID and LaSota) of NDV with each type of the chemotherapeutic drugs (paclitaxel and doxorubicin) on MCF-7 and Caco-2 cell lines compared to their separate treatments. (Values are expressed as mean ± SE. Data were analyzed by using one way ANOVA followed by Tukey post-hoc test. \*, \*\*, and \*\*\* were significant differences at  $P \leq 0.05$ , where; \* significantly different when compared to control group, \*\* when compared to the sole viral strain group, \*\*\* significantly different when compared to the sole chemotherapeutic drug group).

## DISCUSSION

Cancer is the leading cause of death worldwide, with breast and colorectal cancers being the two most common cancer forms (Arnold *et al.*, 2017; Bray *et al.*, 2018). Despite the fact that chemotherapeutic drugs are frequently used today to treat most cancer types, drug resistance remains a major issue for chemotherapy treatments. As a result, efflux pump activity, drug absorption, detoxification enzyme activity, drug targets, and cell apoptosis all increased (Shain and Dalton, 2001).

In the current study, we investigated the potential impact of combining two strains of NDV (lentogenic LaSota and velogenic VIID) with two widely used chemotherapeutic drugs, paclitaxel or doxorubicin, on both MCF-7 and Caco-2 tumour cell lines, as all recent studies have focused on finding novel agents to solve this problem.

In *in vitro* tissue culture, the colorimetric cell viability and cytotoxicity technique, the neutral red absorption assay, relies on the ability of healthy living cells to concentrate this weakly basic vital stain within their lysosomes after nonionic diffusion. Neutral red absorption is reduced by cytotoxic treatments because they damage the exposed cells' structural integrity. The dye is subsequently removed from the living cells using an acidified ethanol solution (Repetto *et al.*, 2008). Neutral red absorption assay usage has been documented in a number of earlier cytotoxicity and cell viability studies (Parker *et al.*, 2010 and Thay *et al.*, 2014).

In the current work, we found that both VIID and LaSota strains of NDV particularly at titers of  $10^3$  and  $10^4$  TCID<sub>50</sub>/ml respectively, displayed a significant cytotoxic effect on both MCF-7 and Caco-2 cell lines. The later effect was detected by marked alteration in cellular morphology as well as diminished cell viability of MCF-7 by 44%, 60% and 40%, 56% respectively, and 39%, 66% and 35%, 60% of Caco-2 cells. It was reported that NDV, a member of the oncolytic virus family, offers a variety of ways for inhibiting cancer cells, and all NDV strains are linked to immunological action against tumours (Zamarin and Palese, 2012).

It was demonstrated that *in vitro* NDV could kill a wide range of human cancer cells, including tumour cell lines of ecto-, endo-, and meso-dermal origin (Li *et al.*, 2016). NDV strains were categorized as lentogenic (avirulent, no clinical signs), mesogenic (intermediate, mild illness), and velogenic (virulent strain producing high mortality) based on their pathogenesis in birds.

None of these strains have been connected to any serious human illnesses. Lentogenic strains are commonly employed as anticancer medicines due to

their low environmental risk; however, the oncolytic effect of NDV will be slightly limited (Lam *et al.*, 2017). In recent years, NDV's oncolytic activities have been demonstrated to cause tumour cell death. Additionally, NDV can cause the immune system to destroy tumour cells, and it can infect tumour cells more potently than normal cells (Song *et al.*, 2019).

Several mechanisms are incorporated into the oncolytic activity of NDV. NDV has been shown to mediate its oncolytic effects through apoptosis induction in infected cells (Elankumaran *et al.*, 2006); additionally, it showed a potent antitumor effect via induction of autophagy (Xu-Feng *et al.*, 2015). Formation of syncytium is a direct cancer cell killing of NDV-mediated oncolysis (Galanis, 2010), as well as stimulation of systemic antiviral innate immunity such as the production of IFNs, other cytokines and enhancing the immunogenicity of cancer cells (Matveeva *et al.*, 2015).

The oncolytic effect of NDV is attributed to two main factors: the per se oncolytic capability of the virus and the host cancer cells' vulnerability to viral infection. Numerous studies showed that the NDV virion's hemagglutinin-neuraminidase (HN) and fusion (F) proteins play crucial roles in the NDV-induced oncolytic action by mediating the fusion of infected cells with their nearby uninfected cells and the development of syncytia (Welch *et al.*, 2014; Ji *et al.*, 2017). Moreover, the F gene is crucial for NDV's *in vivo* oncolytic capabilities.

Liu *et al.*, (2021) mentioned that neoplastic cells differ in their susceptibility to NDV treatment. The production of alpha-sialic acid acyltransferase and interferon was linked to the NDV treatment's greater efficacy in cells that were sensitive.

In the current study, the IC<sub>50</sub> value of NDV-VIID and LaSota, which resulted in a 50% growth inhibition on MCF-7 and Caco-2 cells, was  $10^{-3}$  for the VIID strain and  $10^{-4}$  for the LaSota strain. While that for Doxorubicin in Caco-2 cells was 5.3 M, whereas the IC<sub>50</sub> for Paclitaxel in MCF-7 was 2.2 M.

A microtubule toxin, paclitaxel, is thought to stop cells in the middle of their mitosis, improve microtubule stability, and cause apoptosis. Chromosome mis-segregation on aberrant mitotic spindles without mitotic arrest is another possible definition of paclitaxel-mediated cell death (Zasadil *et al.*, 2014).

The findings of Luo *et al.*, (2010) and Sivakumaran *et al.*, (2018) provided support for our findings, as they mentioned that the IC<sub>50</sub> of paclitaxel was in the range of 2.2 - 2.3 M on the MCF-7 cell line. However, one of the most commonly utilized treatments for treating various solid and hematopoietic malignancies is doxorubicin,

which is known for intercalating between DNA strands and hydrolyzing those (Lentacker *et al.*, 2010). Also, our findings were consistent with those of Li *et al.*, (2018) and Zhou and Wink (2018) who reported that the respective IC50 values for doxorubicin on Caco-2 cell lines were 4.97 and 5.40 1.27 M.

In addition, we found that the used VIID and LaSota strain NDV had a greater cytotoxic effect on MCF-7 and Caco-2 cell lines than either Paclitaxel or Doxorubicin alone. On the other hand, the combined use of chemotherapeutic medicines and NDV strains on both the MCF-7 and Caco-2 tumour cell lines has shown a wonderful effect on the viability of the tumour cells, surpassing that of the solitary use of NDV strains, Paclitaxel, or Doxorubicin alone. The later result demonstrates that the combination of chemotherapies with the employed VIID and LaSota strain NDV OMVs may be a promising new therapeutic approach for treating tumours.

Moreover, the potential anticancer activity of NDV could also be attributed to its inability to induce immune escape mechanisms in mammalian cells (Zamarin and Palese, 2012). So, it might work well as an immunologic adjuvant in a vaccination for human cancer (Washburn and Schirmacher, 2002).

Further, the lethal oncolytic activity of NDV may be related to its ability to induce apoptosis, autophagy, ERs, and mitochondrial dysfunction in cancer cell lines (Keshavarz *et al.*, 2020) which may contribute to other pathways. The later attribution could be the cause of the morphologic changes we observed in MCF-7 and Caco-2 when inoculated by both strains of NDV.

Through our work, it was noticed that cellular morphological alterations were higher in the VIID strain than in LaSota, which was in parallel with the results of the cellular viability percent coming in agreement with those of Ahlert *et al.*, (1990) who stated that the oncolytic characteristics of NDV strains in birds are correlated with their pathogenic categorization. While lentogenic strains often exhibit greater attenuation due to the lack of activation of the F0 protein, velogenic strains can easily carry out multicycle replication in various tested human cancer cells with effective cell lysis. Further, syncytia formation has been demonstrated to greatly increase NDV-mediated lysis of cancer cells for fusogenic NDV strains, both in vitro and in vivo (Zamarin *et al.*, 2009 and Song *et al.*, 2010).

## CONCLUSION

According to the current research, NDV strains (VIID and LaSota) demonstrated a promising *in vitro* anticancer impact. This suggests that they

could be a unique and promising choice for cancer treatment and that they could also be a useful adjuvant to reduce the dosage of chemotherapy medications. Moreover, combining both virus strains with chemotherapy drugs may be a promising way to treat cancer. This combination may improve the efficiency of chemotherapy drugs, prevent the problem of drug resistance that happens during the course of chemotherapy, and lessen the negative effects on healthy cells by reducing the dosage of the chemotherapeutic drugs. The *in vivo* anticancer efficacy of NDV strains VIID and LaSota and their safety in surviving lab animals require more investigation.

## Conflicts of interest

The authors declare that there is no conflict of interest regarding the research data and tools used in this study.

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