Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.05.040

Evaluation of The Anti-Cancer Efficiency of Lavender Oil and Newcastle Disease Virus (NDV) Through Induction of Apoptosis

Fatema Fawzi Younis Al-Habeeb[1], Ali Abd Alateef Abd Alhassan Al Ali[2]
[1,2]Department of Biology, College of Education for pure sciences, University of Basrah, Basrah, Iraq
***Corresponding author:** Fatema Fawzi Younis Al-Habeeb, Department of Biology, College of Education for pure sciences, University of Basrah, Basrah, Iraq, Email: ff.alhabeeb87@gmail.com

Submitted: 16 January 2023; Accepted: 08 February 2023; Published: 04 March 2023

ABSTRACT

Due to their high effectiveness and lack of adverse effects, numerous researches have recently concentrated on the use of biological agents and natural products as an alternative method of treating many incurable diseases, such as cancer. In this study, NDV and lavender oil were tested on cancer cell lines (AMJ-13 and HCAM) as well as normal cell line (HBL-100). The study's findings demonstrated that the study materials (lavender oil, NDV) were cytotoxic to malignant cell lines when tested using the MTT assay, where the IC50 values for NDV were MOI0.0065 for the cancerous cell line AMJ-13 and MOI0.0068 for the cancerous cell line HCAM, and the IC50 values for lavender oil were 5.77 µg / ml for the cancerous cell line AMJ-13 and 5.55 µg / ml for the HCAM cell line, Pathological abnormalities appeared in the treated cells when they were examined with hematoxylin and eosin (H & E), indicating that each substance's harmful effects, both separately and in combination, had an impact on the cells' appearance . Additionally, to examine the occurrence of programmed cell death, cells were stained with acridine orange and ethidium bromide (AO/EB).

Keywords: *Cancer cell lines, lavender oil, Newcastle disease virus (NDV), Apoptosis*

INTRODUCTION

Regardless of age, gender, ethnicity, or diet, cancer is a multifaceted illness that can attack any component of the body (Ravindra et al., 2009). A genetic abnormality that promotes unregulated growth and causes the body to produce an abundance of aberrant cells is what causes cancer. Human cancer is primarily activated by a number of key variables, including aging, alterations in lifestyle, hormonal changes, and exposure to toxic chemicals. (Sharma et al., 2022). Statistics show that cancer is the second-leading cause of mortality in the globe, with approximately 9.6 million deaths occurring in 2018 and 10 million in 2020. (Ferlay et al., 2020). There are around 17 million cancer diagnoses each year, and this figure is anticipated to rise in the near future (Velazquez-Kronen & Nelson, 2020). Additionally, it is anticipated that by 2030, there will be 11.4 million cancer deaths annually. (W.H.O., 2017). As a first line of treatment, radiotherapy, chemotherapy, and surgery are all considered to be traditional cancer treatments. Even though these therapies have shown some effectiveness in reducing tumor growth, they have not been able to eliminate the problem of malignancies and have left the patient

with a host of serious side effects. include medication sensitivity, nausea, vomiting, hair loss, and leukopenia. Radiotherapy is typically used to treat the systemic spread of cancers such as esophageal cancer, lung cancer, nasopharyngeal cancer, and skin cancer; however, once the cancer has spread to the tissues, radiation therapy is no longer effective. Chemotherapy is characterized by low selectivity and severe toxicity (Khan et al., 2019). Most chemotherapeutic drugs have a negative effect on healthy cells and tissues because to their lack of selectivity (Fahmy et al., 2020). Regardless of the significance of some synthetic drugs in the treatment of various illnesses, there are many naturally occurring molecules and compounds with distinctive properties that have been used for medical purposes. Natural chemicals are increasingly used and in demand over the world these days. A more contemporary method that "depends on boosting the body's immune system to attack cancer cells" is the use of biological treatments to treat cancer, such as gene therapy, immunotherapy, and viral treatment (Rasoul et al., 2019). A cutting-edge biotechnology method known as "virotherapy" modulates viruses to treat diseases and manages to combine them with medications that can treat a variety of illnesses (Hemminki et al., 2020). Several Oncolytic Viruses (OVS), including the Newcastle disease virus (NDV), have been investigated for usage as a promising therapeutic agent for tumors since it has the capacity to increase immunity against cancer and has been demonstrated to be able to spontaneously proliferate in cancer cells, killing them while maintaining normal cells. In numerous clinical trials, it has been utilized for more than thirty years (Russell et al., 2018). Utilizing medicinal plants is one of the alternative treatment approaches since they are a gift from nature that have been successful in treating a number of human-killing diseases (Sabo & Knezevic, 2019). Due to their crucial significance in the development of contemporary treatments, natural plant products have been used extensively in medicine. Several plants and their potent constituents have undergone testing as tumor-suppressing medicines (Rejhová et al., 2018). Some essential oils among these natural compounds have pharmacological effects against cancer, viruses, bacteria, and free radicals (Pejin et al., 2017). In the fields of medicine and the food business, a variety of essential oils have been the focus of in-depth investigation (Zheljazkov et al., 2018). The Lamiaceae family, which has its roots in the western Mediterranean, includes the Lavandula lavender plant, which yields lavender oil. Additionally, herbal remedies can be made from the lavender plant's flowers and leaves (Gezici, 2018). There is a substantial body of research that supports the antibacterial, antioxidant, and anticancer properties of lavender oil (Sokovic et al., 2010) and it is additionally employed in the treatment of rheumatism, gastrointestinal disorders, agitation, and other conditions. (Zu et al., 2010).

MATERIALS AND METHODS

Cell Lines and Culture

HBL-100 (Human Breast Normal), AMJ-13 (Human Breast Cancer), and HCAM (Human Hepatocyte Cancer) cell lines were used in this study. These cell lines have been selected from the Cell Culture Laboratory of the Department of Biology in the College of Education for Pure Sciences at the University of Basrah. Were grown in flasks on Roswell Park Memorial Institute Medium RPMI 1640 (US Biological, USA) with 10% fetal bovine serum (FBS, Biowest, USA) and 1% antibiotics (100 U/ml gentamycin and 100 g/ml penicillin) at 37°C in a humidified CO2 (5%) incubator. They were transferred using trypsin-EDTA (US biological, USA) (Al-Ali et al.,2022).

Chemical Analysis of Lavender Oil

The bioactive components of lavender oil were discovered using the Gas Chromatography - Mass Spectrometry (GC-mass) assay.

Cytotoxicity assay

The pure lavender essential oil's cytotoxicity should be evaluated (Lavandula augustifolia) (Purelyblack, Australia), NDV LaSota strain (Volvoc®, Germany), In a 96-well plate (Korea), the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay

experiment was performed. After trypsinization the HBL100, AMJ-13 and HCAM cells were seeds at 1×104 cell/well in 100 µl medium serum-free. Following the formation of a monolayer, each cell was individually treated with various doses of lavender oil (0, 1, 3, 4, 6, 9, 15 g/ml) as well as various infectious multiplicities (MOI) of NDV (0.001, 0.0015, 0.01, 0.03MOI). Control cells were those that had not been treated, and the experiment was incubated in an incubator at 37 °C and 5% Co2 for a period of 72 hours. Following the conclusion of the treatment period, the cells' viability was evaluated using an MTT test. Then 10 µl of 5 mg/ml MTT solution prepared in RPMI medium was added to each well after discarding the medium, and the cells were then incubated for 2 h at 37 °C. After the MTT solution was removed, tetrazolium crystals were dissolved in each well by adding 100 µl of dimethyl sulfoxide (DMSO, Scharlau, Spain). The 96-well plate was incubated in dark conditions for 20 minutes at room temperature. Using a microplate reader (BioTek), the absorbance was measured at 490 nm. The experiment was performed in three replicates. By this equation (t-co)/co × 100 the rate of cell growth inhibition was calculated, where co is the average uptake of untreated wells and t is the mean uptake of treated wells (Nadri et al.,2014). Three replicates were used for each concentration and each experiment was repeated three times. Using GraphPad Prism 7 software, the inhibitory concentration (IC50) killing 50 percent of cells was calculated (Falih et al.,2022).

The Combined Effect of Lavender Oil and NDV

Firstly, Trypsin was used to separate the cells, which were subsequently sown in 6-well plates (5*105) and cultured for 24 hours at 37°C with 5% CO2. Cells were treated after they formed a monolayer and were exposed to lavender oil and NDV at the following concentrations: 1 g/ml + 0.002 MOI, 2 g/ml + 0.004 MOI, 4 g/ml + 0.006 MOI, 8 g/ml +0.01 MOI, 10 g/ml +0.02 MOI, and 12 g/ml +0.03 MOI as for the control cells, they were not treated. In order to allow virus particles to penetrate the cells, NDV was first delivered to cell lines and then maintained at room temperature for two hours. The cells were treated with various dosages of lavender oil. They were incubation for 72 hours in a CO2 incubator at 37 °C and 5% humidity, and their absorbance was assessed using the MTT assay. In order to assess the degree of synergy or antagonism of cytostatic drugs based on the Combination index values as given in Table 1, the data were then analyzed by the statistical application Compusyan Isobologram. For each treatment, the experiments were run three to five times. (Falih et al.,2022).

TABLE 1: Using CI Analysis, describe the values for the synergistic and antagonistic effects (Al-Jassim et al.,2014)

CI	Description
<0.1	Very Strong Synergism
0.1 - 0.3	Strong Synergism
0.3 – 0.7	Synergism
0.7 – 0.85	Moderate Synergism
0.85 – 0.90	Slight Synergism
0.90 – 1.10	Nearly Additive
1.10 – 1.20	Slight Antagonism
1.20 -1.45	Moderate Antagonism
1.45 – 3.3	Antagonism
3.3 – 10	Strong Antagonism
>10	Very Strong Antagonism

Morphological Study

Cells were sown into a 6-well plate with 5 * 105 covers, and then treated with lavender oil and NDV at respective IC50 concentrations. Although the cover slide, which acted as the control, was not exposed to the study's elements. After that, the plate was sealed with adhesive paper and incubated for 48 hours at 37°C in a humid environment with 5% CO2. Prior to being examined under a microscope, each cover slide was stained with hematoxylin and eosin (Al-Shammari et al., 2019).

Apoptosis Study

Staining with Acridine Orange and Ethidium Bromide (AO/EB)

The IC50 doses of lavender oil and NDV were used to cover slides containing 5 * 105 cells in a 6-well plate. Contrarily, the control cover slide was not exposed to the study materials. Incubation took place for 48 hours at 37°C with 5% CO2 after the plate had been covered with adhesive paper. Each cover slide was immediately examined under the fluorescence microscope after being stained with AO (5 mg/ml)/EB (3 mg/ml) (Liu et al., 2015).

RESULTS

Chemical Analysis of Lavender Oil

GC-MS chemical analysis revealed the existence of 22 chemical components in lavender oil, with the top three compounds, linalool, linayl acetate, and alpha-terpinyl acetate, accounting for 23.004%, 17.62%, and 11.909%, respectively. The percentages of the remaining chemicals ranged from (0.29 to 9.31) Table (2).

TABLE 2: Bioactive Compounds of Lavender Oil via GC-mass

No.	ID	Rt	Concentation%
1	. alpha. -Pinene	7.367	1.8334
2	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	8.378	0.9559
3	3-Octanone	8.751	0.5138
4	.beta.-Myrcene	8.854	0.6423
5	(+)-3-Carene	9.176	0.5684
6	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	9.329	0.4215
7	D-Limonene	9.71	8.3112
8	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate	10.457	0.8103
9	Linalool	11.497	23.0042
10	(+)-2-Bornanone	11.907	3.8353
11	Isoborneol	12.229	2.7345
12	3,5,5-Trimethylhexyl acetate	12.493	9.3176
13	Terpineol	12.668	1.1657
14	Hexanal, 3-(hydroxymethyl)-4-methyl-	13.078	0.2943
15	Linalyl acetate	13.693	17.6273
16	2-(5-Methyl-furan-2-yl)-propionaldehyde	13.928	0.4175
17	Isobornyl acetate	14.125	2.9866
18	.alpha.-Terpinyl acetate	15.019	11.9097
19	Caryophyllene	15.927	7.0377
20	Coumarin	16.095	1.3142
21	Humulene	16.256	1.1095
22	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	28.198	2.629

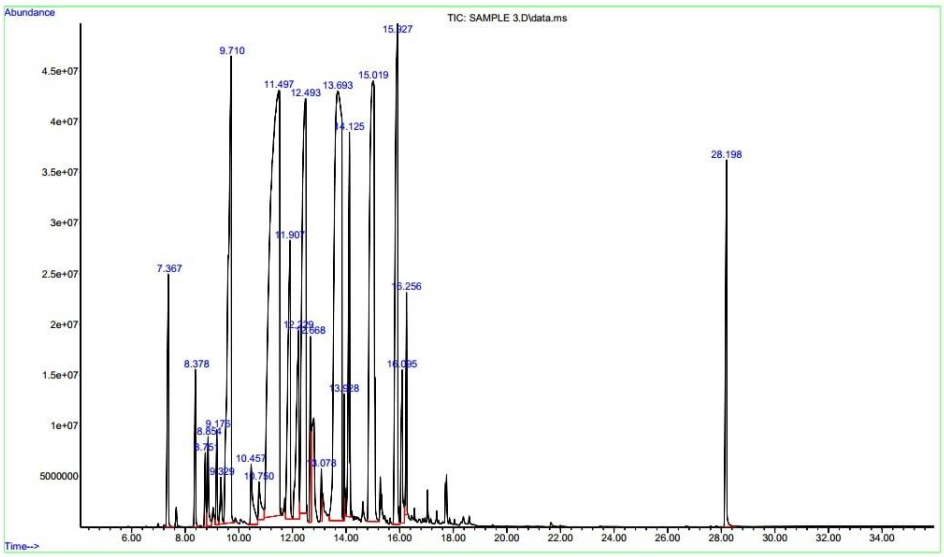


FIGURE 1: GC-Mass findings and composition of lavender essential oil.

Cytotoxicity Assay

Using the MTT test, the current study's findings revealed that NDV and lavender oil had an inhibitory effect on the cancer cell lines AMJ-13, HCAM, and normal HBL-100 after being applied for 72 hours. In NDV, an inhibitory effect on the vitality of cells was observed, as an increase in the percentage of inhibition was observed with an increase in the MOI. The highest inhibition percentage was recorded in the cancer cell lines AMJ-13 and HCAM at virus titers of 0.03 MOI, reaching 65.66% and 62.66%, respectively, while in the normal cell line HBL100 it reached 39.33%, the lowest inhibition percentage in cell lines treated with 0.001 MOI in cancer cell lines AMJ-13 and HCAM was 8.66% and 7.33%, respectively, and in the normal cell line HBL-100 it was 7%. The IC50 values for the cancer cell lines AMJ-13 and HCAM were 0.0065 and 0.0068 MOI, respectively, in the normal cell line HBL-100, the inhibition ratios did not reach the amount that could obtain the IC50 value (Figure 1). At a probability level of P≤0.05, the statistical analysis employing one-way anova analysis of variance revealed that the values of the IC50 of NDV in cancer cell lines did not differ significantly from one another (Figure 2).

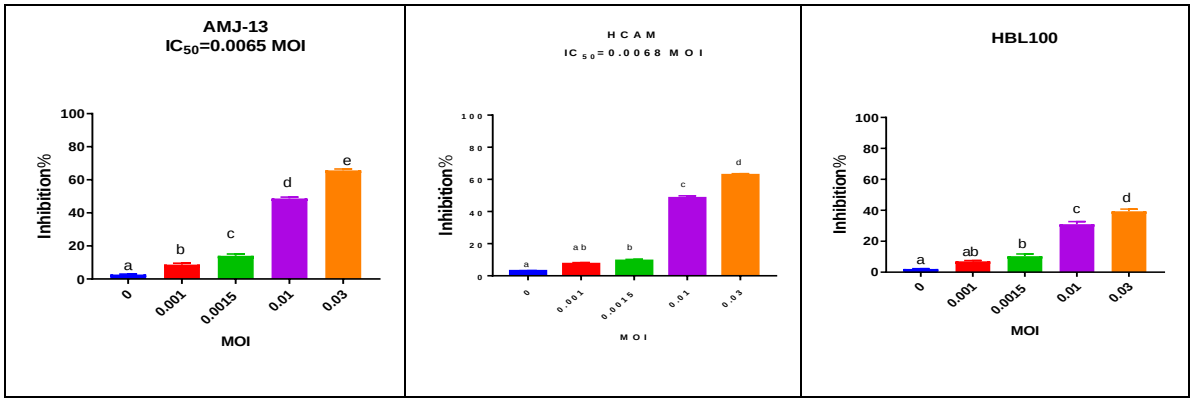


FIG 1: Effect of NDV on the Viability of Cell Lines After 72h of Treatment (mean±SD, n=4, P≤0.05).

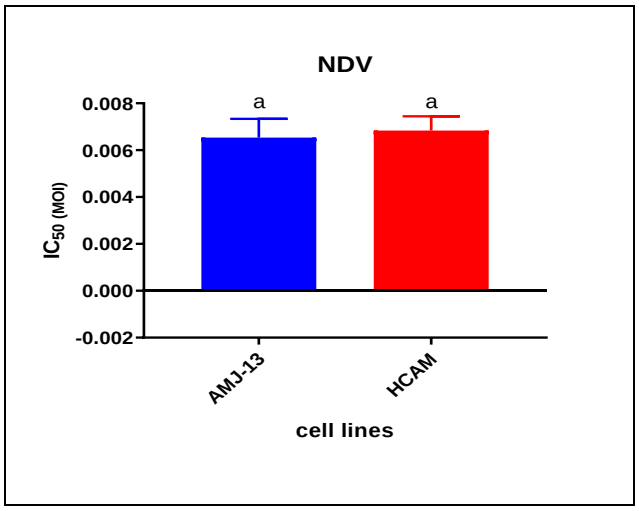


FIG 2: The IC50 rate of NDV on Cancer Cell Lines (mean±SD, n=4, P≤0.05).

While the normal cell line HBL100 and the cancer cell lines AMJ-13, HCAM, were all inhibited by lavender oil at various doses, a rise in the inhibition ratios was shown with increasing the concentration. The highest inhibition rates were recorded in the cancer cell lines AMJ-13 and HCAM treated at a concentration of 15 µg / ml reached 72.60% and 67.66%, respectively, while the percentage of inhibition in the normal cell line HBL-100 amounted to 43.66%, while the lowest percentage of inhibition was in the cancer cell lines AMJ-13 and HCAM treated at a concentration of 1 µg / ml It reached 11.76% and 12.93%, respectively, and in the normal cell line HBL100, it reached 5.60%. The IC50 values for the cancer cell lines AMJ-13 and HCAM were 5.77 and 5.55 µg / ml respectively, in the normal cell line HBL-100, the inhibition percentages did not reach the amount by which the value of the IC50 can be obtained (Figure 3). There were no significant variations between the values IC50 in various cancer cell lines at a probability level of P≤0.05, according to the findings of the statistical analysis of the IC50 values using one-way anova analysis of variance (Figure 4).

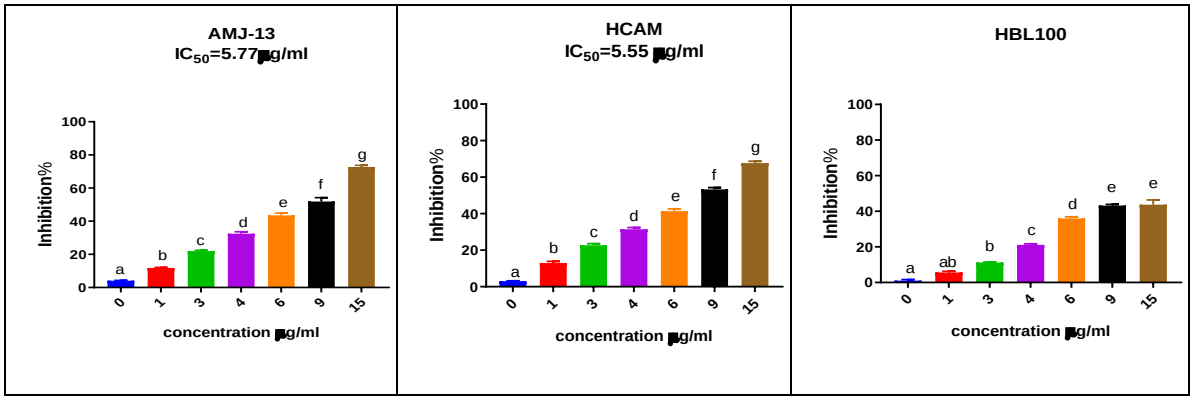


FIG 3: Effect of Lavender Essential Oil on the Viability of Cell Lines After 72h of Treatment (mean±SD, n=4, P≤0.05).

J Popul Ther Clin Pharmacol Vol 30(5):e391–e406; 04 March 2023.

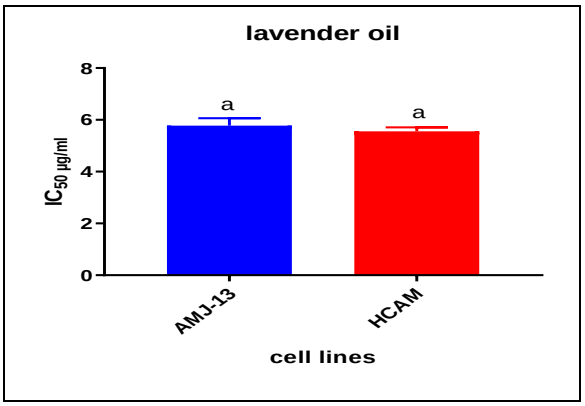
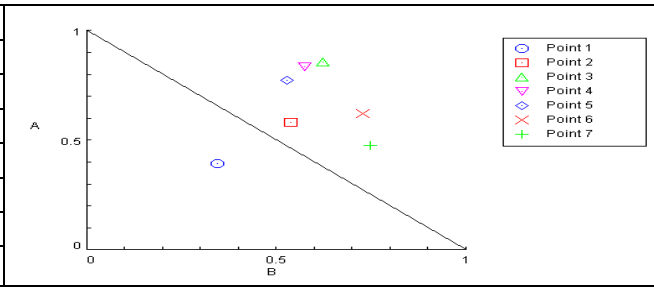
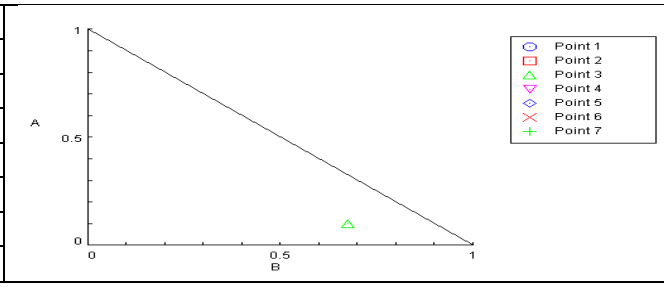
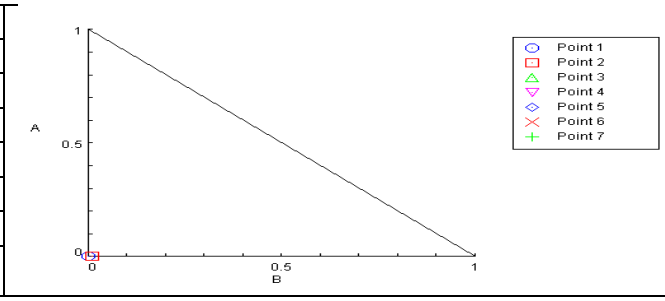


FIG 4: The IC50 rate of Lavender essential oil on cancer cell lines (mean±SD, n=4, P≤0.05).

The Combined Effect of Lavender Oil and NDV The findings of the compusyn Isobologram software after 72 hours of exposure reveal combination index (CI) data for 7 combined doses of both NDV and lavender oil. NDV and lavender oil have a synergistic effect on the AMJ-13 cell line in point (1), HCAM in point (3) and the HBL-100 cell line in point (2) (Table 3).

TABLE 3: The Combined Effect (CI) of NDV and Lavender Oil on Cell Line AMJ-13, HCAM and HBL-100

AMJ-13				
Dose Oil	Dose NDV	Effect	CI	
1µg/ml	0.002 MOI	0.256	0.73893	
2µg/ml	0.004 MOI	0.306	1.12239	
4µg/ml	0.006 MOI	0.363	1.48174	
6µg/ml	0.008 MOI	0.45	1.41132	
8µg/ml	0.01 MOI	0.526	1.30212	
10µg/ml	0.02 MOI	0.616	1.35027	
12µg/ml	0.03 MOI	0.7	1.22414	
HCAM				
Dose Oil	Dose NDV	Effect	CI	
1µg/ml	0.002 MOI	0.22	8.17969	
2µg/ml	0.004 MOI	0.32	2.13453	
4µg/ml	0.006 MOI	0.41	0.77808	
6µg/ml	0.008 MOI	0.49	4.61098	
8µg/ml	0.01 MOI	0.56	108.876	
10µg/ml	0.02 MOI	0.58	318.293	
12µg/ml	0.03 MOI	0.67	21150.5	
HBL-100				
Dose Oil	Dose NDV	Effect	CI	
1µg/ml	0.002 MOI	0.18	7.28E-5	
2µg/ml	0.004 MOI	0.253	0.00984	
4µg/ml	0.006 MOI	0.323	33.3123	
6µg/ml	0.008 MOI	0.353	25775.4	
8µg/ml	0.01 MOI	0.38	7818492	
10µg/ml	0.02 MOI	0.426	7.37E10	
12µg/ml	0.03 MOI	0.45	8.36E12	

J Popul Ther Clin Pharmacol Vol 30(5):e391–e406; 04 March 2023.

Cell Morphology Study

Figure shows how untreated cell lines behaved naturally in typical cultures, forming a monolayer and retaining their original morphological and structural characteristics (5,6and7A). Several changes in phenotypic and pathological characteristics were observed after 48 hours of treatment with study materials' half inhibitory concentration IC50, including changes to shape (changing to a sphere or irregular shape), and changes in size (cell atrophy and shrinkage), as the cells appear smaller in size than normal cells, changes in the nucleus such as Karyopyknosis and Hyperchromy, and the occurrence of degeneration in the cytoplasm of the cells, as the color of the cytoplasm is dull and vacuole, necrosis of the cells is observed, so they appear to have a lysis of cell membranes and the features of their external borders are blurred, and the appearance of empty spaces in cell cultures as shown in the figure (5, 6 and 7 B,C,D). When treated with NDV, the effect on the normal cell line was less than on the cancer cell lines, and the effect increased when combined treatment of all cell lines was used.

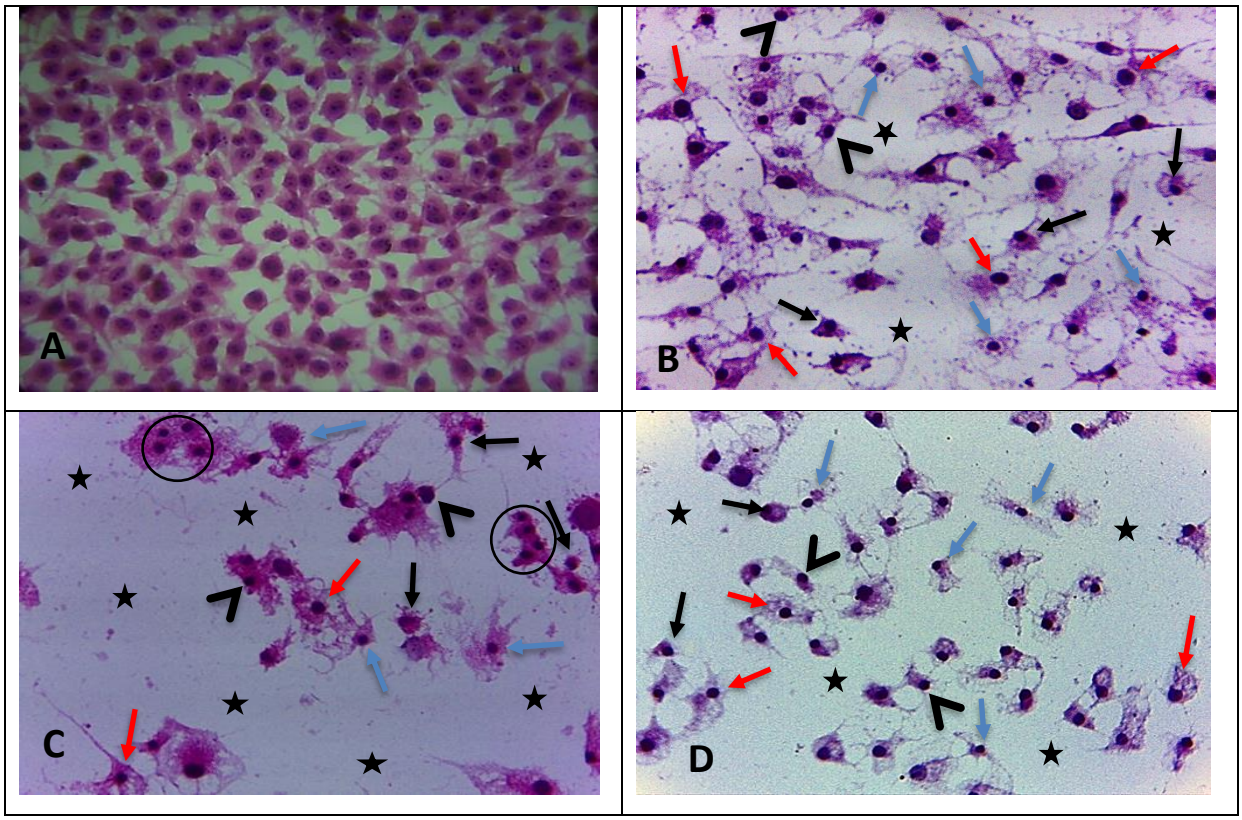


FIG 5: AMJ-13 Cell line stained with hematoxylin and eosin. A. Untreated cells appearing as monolayer and flat, with cytoplasmic appendages and spherical nucleus central location, 40x. B. Cells treated with IC50 of NDV, atrophy and puckering of cells (black arrow), degeneration (red arrow), necrosis (blue arrow), nucleus thickening and hyperpigmentation (arrowhead), empty spaces in cell culture (stars), 40x. C. Cells treated with IC50 of lavender oil, cell atrophy and roundness (black arrow), degeneration (red arrow), necrosis (blue arrow), nuclei thickening and hyperpigmentation (arrow head), cell aggregation (circular shape) and empty spaces in the Cell culture (stars), 40x. D. Cells treated with combination IC50 of NDV + IC50 lavender oil. Cell atrophy and roundness (black arrow), degeneration (red arrow), necrosis (blue arrow), nucleus thickening and hyperpigmentation (arrowhead), and empty spaces in cell culture (stars), 40x.

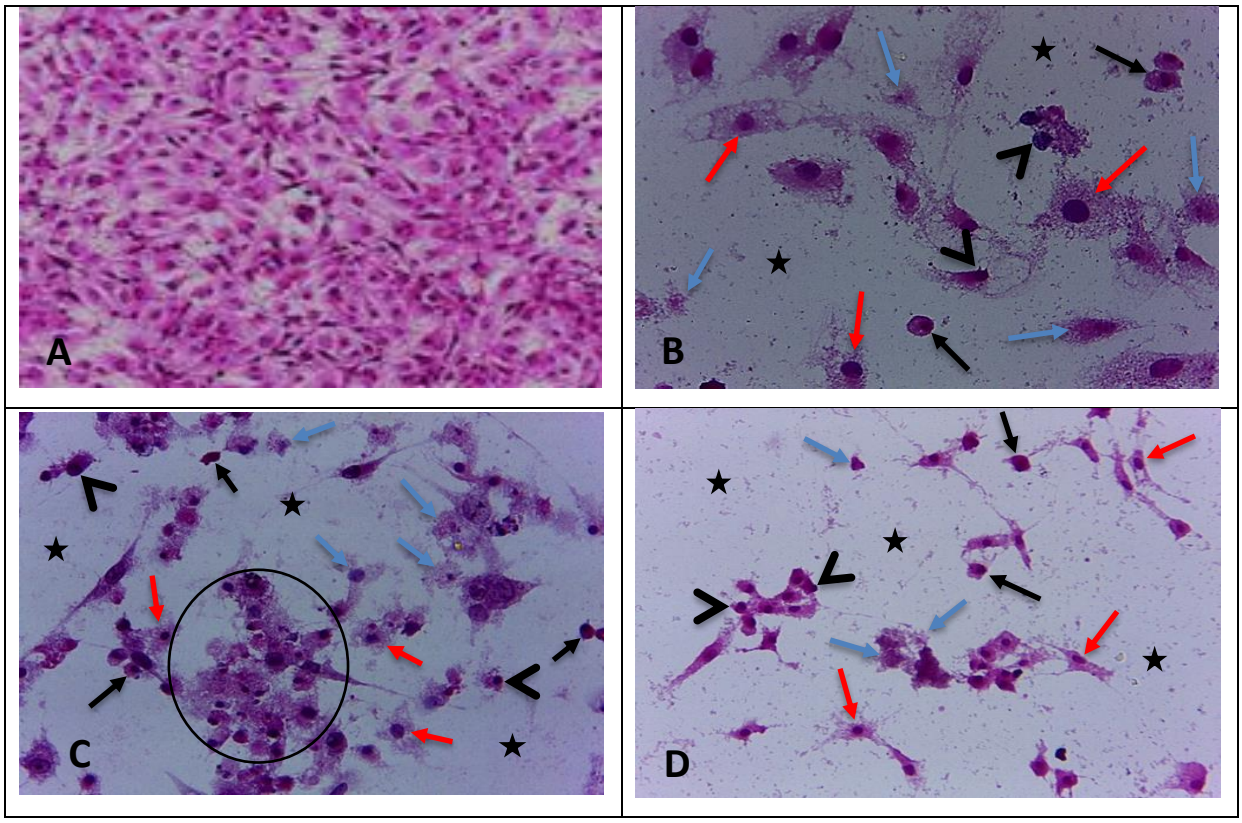
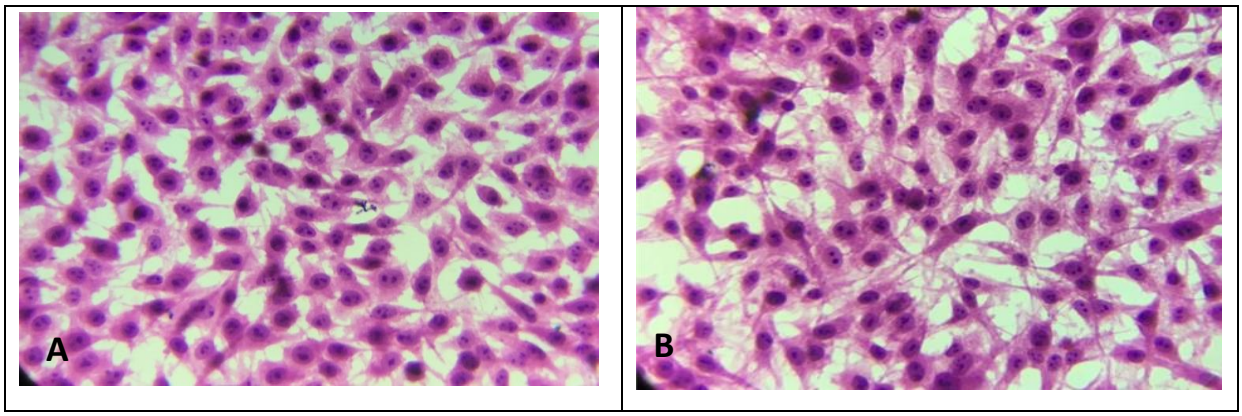


FIG 6: HCAM Cell line stained with hematoxylin and eosin. A. Untreated cells appearing as monolayer and flat, with cytoplasmic appendages and spherical nucleus central location, 40x. B. Cells treated with IC50 of NDV, atrophy and puckering of cells (black arrow), degeneration (red arrow), necrosis (blue arrow), nucleus thickening and hyperpigmentation (arrowhead), empty spaces in cell culture (stars), 40x. C. Cells treated with IC50 of lavender oil, cell atrophy and roundness (black arrow), degeneration (red arrow), necrosis (blue arrow), nuclei thickening and hyperpigmentation (arrow head), cell aggregation (circular shape) and empty spaces in the Cell culture (stars), 40x. D. Cells treated with combination IC50 of NDV + IC50 lavender oil. Cell atrophy and roundness (black arrow), degeneration (red arrow), necrosis (blue arrow), nucleus thickening and hyperpigmentation (arrowhead), and empty spaces in cell culture (stars), 40x.



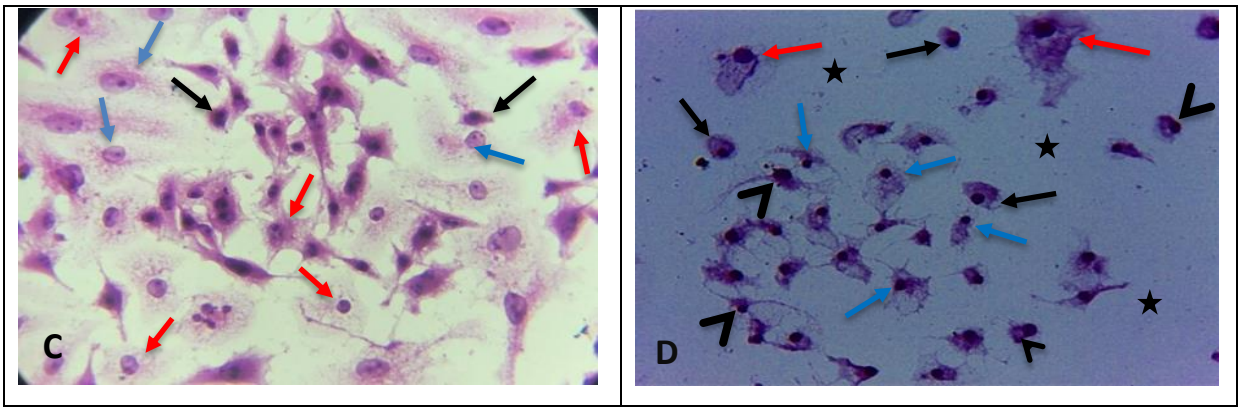


FIG 7: HBL-100 Cell line stained with hematoxylin and eosin stains: A. Untreated cells appearing as monolayer and flat, with cytoplasmic appendages and spherical nucleus central location, 40x. B. Cells treated with NDV MOI = 0.01 concentration, no obvious pathological effect was observed, 40x C. Cells treated with IC50 from lavender oil, cell swelling and atrophy (black arrow), cell degeneration (red arrow), necrosis (blue arrow), 40X. D. Cells treated with combination 0.01 MOI of NDV IC50 of lavender oil + IC50 of lavender oil. Cell swelling and atrophy (black arrow), degeneration (red arrow), necrosis (blue arrow), empty spaces in the cell culture (stars), nucleation thickening and hyperpigmentation were observed (arrowhead) 40X.

Apoptosis study

Acridine Orange/Ethidium Bromide (AO/EB)

After 48 hours of treatment, staining with acridine orange and ethidium bromide revealed green cytoplasm, indicating healthy cells Figure (8, 9 and 10 A). On the other hand, cells exposed to IC50 concentrations of NDV and lavender oil displayed morphological apoptotic alterations, including the formation of yellow-orange nuclei in early apoptotic cells and red nuclei in late apoptotic cells (Table 4; Figures 8, 9, 10, B, C, and D).

TABLE 4: The percentage of live and dead cells

Viable cells%	Dead cells%	Type of treated	Cell lines
100	0	Control	HBL-100
67.89	32.11	Oil	
73.55	26.45	NDV	
69.72	30.28	Combination	
97.538	2.461	Control	AMJ-13
51.023	48.976	Oil	
51.256	48.743	NDV	
57.778	42.222	Combination	
98.813	1.187	Control	HCAM
47.611	52.388	Oil	
47.504	52.495	NDV	
53.260	46.739	Combination	

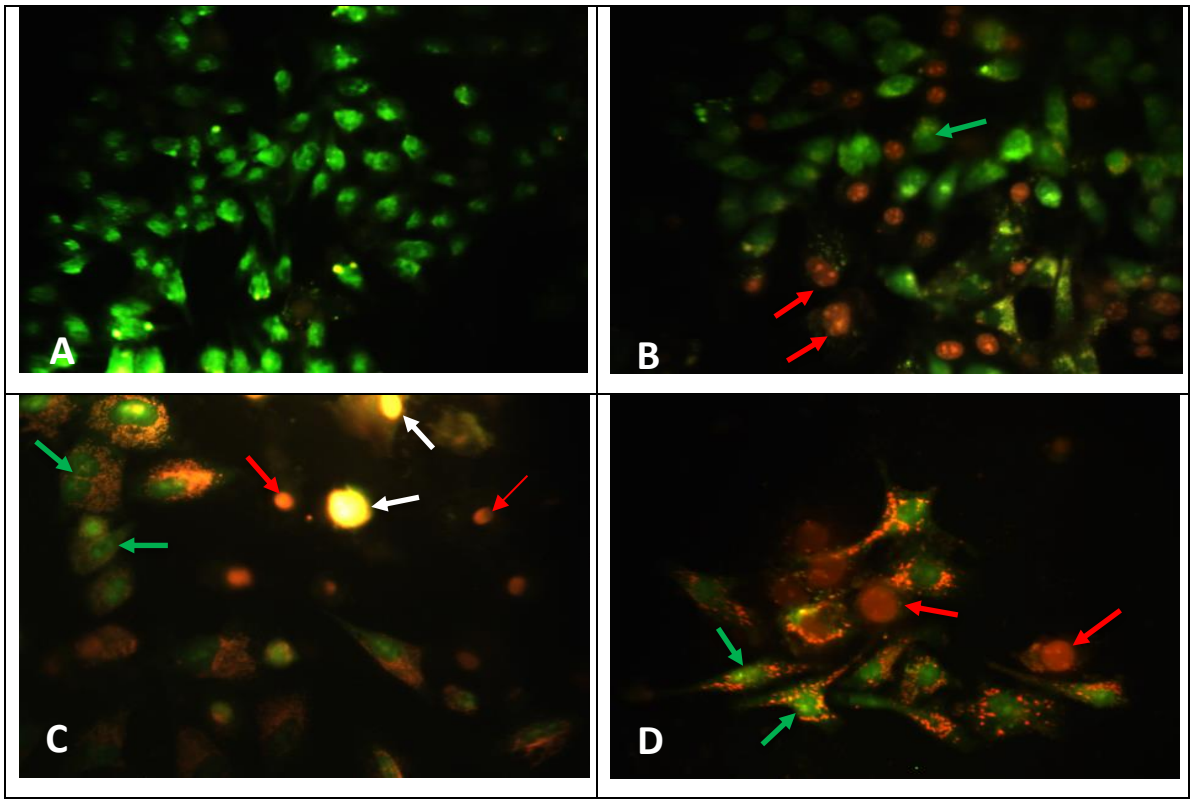


FIG 8: AMJ-13 Cell line the green arrow indicates healthy cells, the white arrow indicates cells suffering from pro-apoptosis, and the red arrow indicates cells suffering from late apoptosis. A. Untreated cells 400x. B. Cells treated with IC50 of NDV 400x. C. Cells treated with IC50 of lavender oil 400x. D. Cells treated with IC50 combination of NDV and lavender oil 400x.

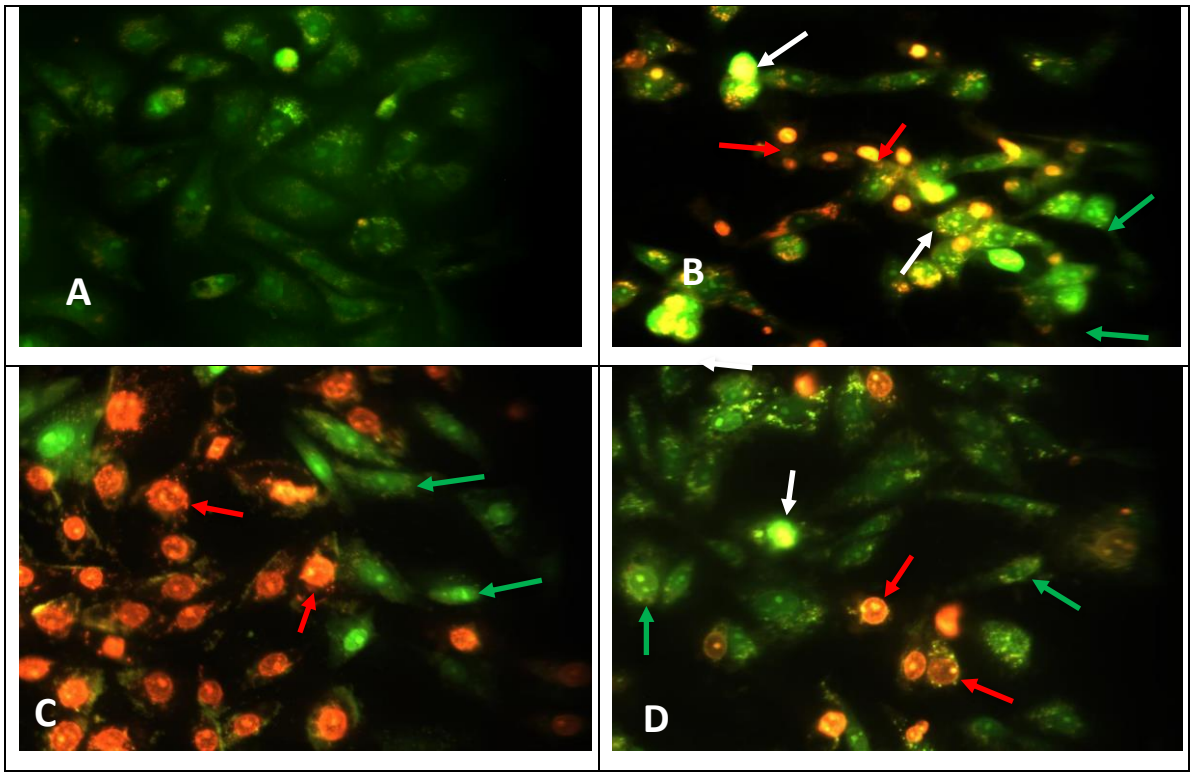


FIG 9: HCAM Cell line the green arrow indicates healthy cells, the white arrow indicates cells suffering from pro-apoptosis, and the red arrow indicates cells suffering from late apoptosis. A.

J Popul Ther Clin Pharmacol Vol 30(5):e391–e406; 04 March 2023.

Untreated cells 400x. B. Cells treated with IC50 of NDV 400x. C. Cells treated with IC50 of lavender oil 400x. D. Cells treated with IC50 combination of NDV and lavender oil 400x.

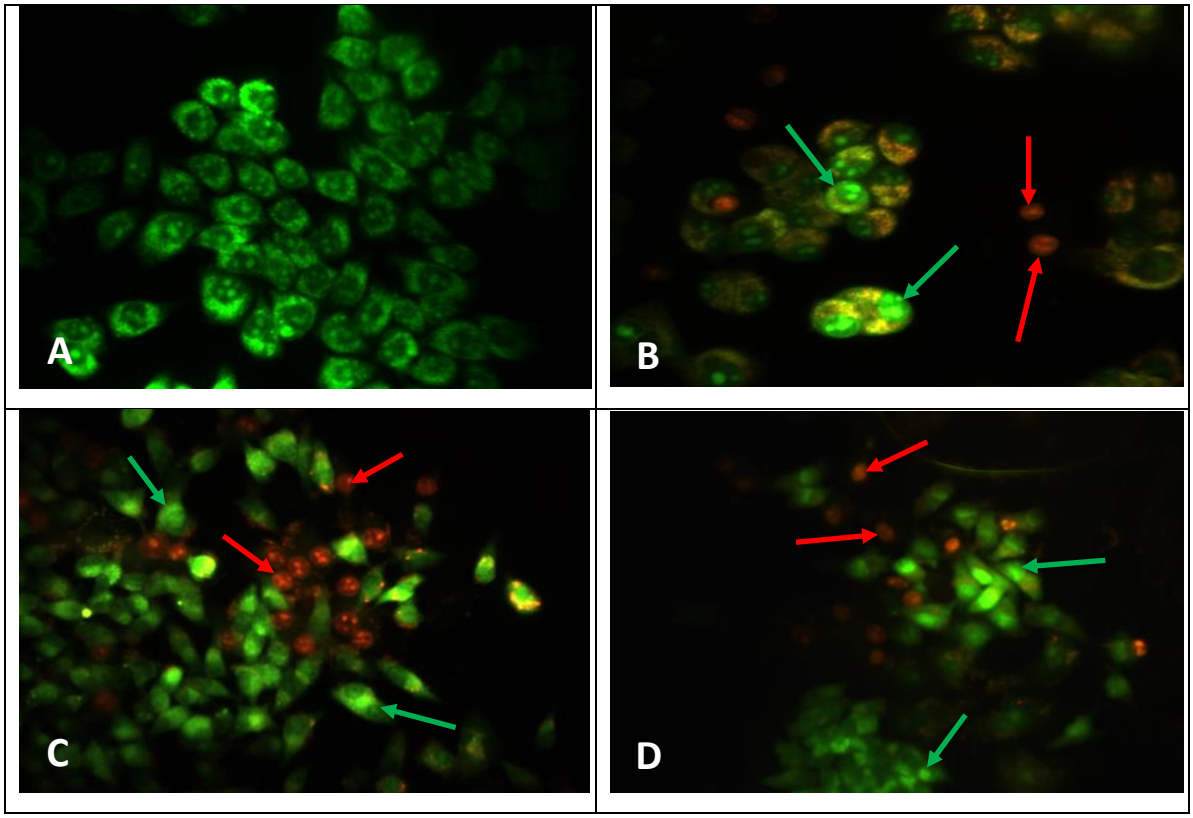


FIG 10: HBL-100 Cell line the green arrow indicates healthy cells, the white arrow indicates cells suffering from pro-apoptosis, and the red arrow indicates cells suffering from late apoptosis. A. Untreated cells 400x. B. Cells treated with IC50 of NDV 400x. C. Cells treated with IC50 of lavender oil 400x. D. Cells treated with IC50 combination of NDV and lavender oil 400x.

DISCUSSION

Due to its rising incidence and mortality rates, cancer has turned into an international health concern. Scientists anticipate that it will eventually pose a significant threat to human health. (Siegel et al., 2018). Scientists have primarily concentrated on examining the use of plant-derived natural chemicals with few or no side effects for cancer prevention and treatment because there has been an increase in drug resistance and undesirable effects of medications used in cancer treatment (Gezici and Sekeroglu, 2017). Natural ingredients have several benefits when used in medicinal procedures. They possess anti-oxidant (chemoprotective) and even pro-oxidant (pro-apoptotic) properties that have an impact on proliferation, development, angiogenesis, tumor spread, or cause tumor cell apoptosis. (Ruiz & Hernández, 2016).

The antibacterial and anti-cancer characteristics of plant essential oil have made it the subject of research in phytomedicine (Zhao et al., 2017). Our research demonstrates that low doses of lavender oil are highly cytotoxicity to the AMJ-13, HCAM, and HBL-100 cell lines. Lavender oil has a significant cytotoxic effect on the cancer cell lines A549, H1299, and C6 as demonstrated by numerous studies employing the MTT assay. Compared to other cells, it had a stronger impact on C6 glioma cells (Gezici, 2018), the cancer cell lines HepG2, PC3, A549, A431, HCT116, and MCF7 (Fahmy et al., 2022), the HCAM cell line (Allaftah & Hassan, 2022), and the cancer cell lines MCF7, H460, NCI, and MOLT-4 (Nikšić et

al.,2017). Linalool, linalyl acetate, alpha-terpinyl acetate, and other active compounds in lavender oil are some of those that support the essential properties of the oil. Chemistry GC MS was used for the analysis to determine these components, and the results were consistent with earlier research (Soulaimani et al., 2019; Fahmy et al., 2022; and Barkat & Laib, 2012).

The development of biological cancer treatments has been aided by the development of crucial instruments for assessing the viability of viruses thanks to advancements in recombinant DNA technology. The majority of oncolytic viruses used to treat cancer are attenuated strains, or strains that may infect and spread among people without seriously ill effects while also stimulate the host's immune system to identify and eliminate cancer cells (Mondal et al., 2020). NDV is a potent anti-cancer drug that enters the cytoplasm by endocytosis by receptors and has good cell binding properties (Ravindera et al., 2008). Increased expression of viral entry receptors is one trait that distinguishes some cancer cells (Hemminki et al., 2020). Because most tumor cells lack functional viral sensing mechanisms, NDV can detect the progression of tumors (De Queiroz et al., 2019).

The current study evaluated the effectiveness of tumor lysis using NDV LaSota strain on AMJ-13, HCAM and HBL-100 cell lines, and based on the MTT assay, the results of the study showed that the cancerous cell line AMJ-13, HCAM was affected. This is consistent with earlier research because NDV LaSota strain had a similar impact on the cancer cell line Hep-2(Al-Shammari et al., 2012), the cancer cell line TC-1 (Keshavarz et al., 2019), the cancer cell line MCF-7 (Kalantare et al.,2020). The normal cell line HBL-100 is less affected compared to the cancer cell lines because the percentage of inhibition did not reach the actual amount necessary to obtain the lethal concentration of IC50 cells. This is consistent with earlier studies on the normal cell line REF in comparison to the cancer cell line MCF-7. (Al-Ziaydi et al., 2020). This demonstrates the virus's capacity to kill cancer cells by causing programmed cell death and that an increase in the dose of the virus leads to an increase in the percentage of cells that suffer from programmed death (Hassan et al., 2020).

Additionally, several natural compounds have been shown to increase the sensitivity to conventional therapy through cytotoxicity, mutually strengthen the combined impact (Zhou et al.,2016), or solely influence malignant cells. Combination therapy uses a range of strategies to reduce the emergence of anticancer drug resistance since it targets several signaling pathwaysIn some instances, the combination of natural substances and conventional therapy may be able to defeat altered regulatory cell pathways, which may be the responsible of drug resistance mechanisms. Using natural compounds in combination with conventional chemotherapeutics brings up new possibilities for the study and treatment of cancer. It might be a promising approach to possibly reducing the negative effects connected with conventional chemotherapy (Rejhová et al.,2018). Oncolytic viruses help the therapy of cancer greatly when combined with other recognized medications (Jhawar et al., 2017). The present investigation demonstrated that when NDV and lavender oil were combined, the effects on the cancer cell lines AMJ-13 and HCAM were synergistic, despite the fact that each substance obviously causes cytotoxicity on cell lines via causing apoptosis. Because combined therapy allows for lower therapeutic doses, overall reduced toxicity, and decreased drug resistance when compared to monotherapy, this highlights how crucial it is (Ko et al., 2014).

The current study materials' cytotoxicity was reflected in the appearance of the cells exposed to them. The cells showed a wide range of phenotypic alterations with varying degrees of severity. The effect was stronger when the cells were exposed to both NDV and lavender oil at once. Cell morphological changes included cell atrophy, degeneration, necrosis, karyopyknosis, hyperpigmentation, and cell aggregation. Changes in form are brought on by biochemical and molecular processes (Al-Ali et al.,2022). When a potentially toxic chemical is present, the cell must defend itself by undergoing either hydrolytic degeneration (the collection of harmful substances within vacuoles and isolating

them from the rest of the cell) or hydrolytic degeneration (the buildup of fluid within the cell and subsequent enlargement) (Stevens et al., 2008). Chromatin condensation is an indication that a cell is dying. The loss of laminin proteins, which mediate the links between chromatin and the nuclear membrane and preserve the structure and integrity of the nucleus, results in chromatin condensation. This demonstrates that DNA fragmentation caused the death of cells that went through the apoptotic process.

The current study showed that programmed cell death might be brought about by staining cell lines with AO/EB dye (Ould-Moussa et al.,2014). The bulk of the study-treated cells were stained with EB dye, which appears red and indicates cell damage, and then had their membranes completely permeable, enabling observation of the study's results using a fluorescence microscope. As soon as the EB dye enters the cells and attaches to the genetic material, it is understood that the cells are in the final phases of programmed cell death (Ariizumi et al., 2009). Additionally, it was noted that certain cells had a yellow or orange stain, which denotes a partial breakdown of the cell membranes and a consequent reduction in the quantity of EB dye that can pass through, indicating that the cells are in an early stage of programmed cell death (Liu et al.,2015). This The integrity of the untreated cells' cell membrane was demonstrated by the staining of the cells with the green AO dye, which also inhibited the entry of the red dye into the cells (Ariizumi et al., 2009).

CONCLUSION

Generally speaking, Through the stimulation of both apoptosis and necrosis, the study demonstrated that lavender oil and NDV, when used either individually or in combination, have strong anticancer and antiproliferative actions against cancer cell lines. The search for novel cancer therapeutics that have fewer side effects than current therapies has recently gained international attention.

REFERENCES

1. AbbasAllaftah, A. N., & AlHassan, A. A. Y. (2022, January). Lavender oil anti-oxidative and anti-tumor activity. In AIP Conference Proceedings (Vol. 2386, No. 1, p. 020045). AIP Publishing LLC.
2. Al-Ali, A. A., Alsalami, K. A., & Athbi, A. M. (2022). Cytotoxic effects of CeO2 NPs and β-Carotene and their ability to induce apoptosis in human breast normal and cancer cell lines. Iraqi Journal of Science, 923-937.
3. Al-Jassim, Z. G., Al-Abbassi, M. G., & Yaseen, N. Y. (2014). The Cytotoxic Effect of 2-Deoxy-D-Glucose Combination with 5-Fluorourasil and NO-Aspirin on Mammary Adenocacinoma Cell Line. J Nat Sci Res www, 4(19), 2225-921.
4. Al-Shammari, A. M., Al-Esmaeel, W. N., Al Ali, A. A. A., Hassan, A. A., & Ahmed, A. A. (2019, April). Enhancement of oncolytic activity of newcastle disease virus through combination with retinoic acid against digestive system malignancies. In molecular Therapy (Vol. 27, No. 4, pp. 126-127). 50 HAMPSHIRE ST, FLOOR 5, CAMBRIDGE, MA 02139 USA: CELL PRESS.
5. Al-Ziaydi, A. G., Al-Shammari, A. M., Hamzah, M. I., Kadhim, H. S., & Jabir, M. S. (2020). Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. Virusdisease, 31(3), 341-348.
6. Ariizumi, T., Ogose, A., Kawashima, H., Hotta, T., Umezu, H., & Endo, N. (2009). Multinucleation followed by an acytokinetic cell division in myxofibrosarcoma with giant cell proliferation. Journal of Experimental & Clinical Cancer Research, 28(1), 1-6.
7. BARKAT, M., & LAIB, I. N. (2012). Antioxidant activity of the essential oil from the flowers of Lavandula stoechas. Journal of Pharmacognosy and Phytotherapy, 4(7), 96-101.
8. Cancer. World Health Organization. Available from: http://www.who.int/mediacentre/factsheets/fs297/en. [Accessed on 2017 Feb 17].
9. De Queiroz, N. M. G. P., Xia, T., Konno, H., & Barber, G. N. (2019). Ovarian Cancer Cells Commonly Exhibit Defective STING Signaling Which Affects Sensitivity to Viral OncolysisSTING Signaling in Ovarian Cancer. Molecular Cancer Research, 17(4), 974-986.
10. Fahmy, M. A., Abd-Alla, H. I., Hassan, E. E., Hassan, Z. M., & Heba-tollah, M. S. (2020). Genotoxicity and sperm defects induced by 5-FU in male mice and the possible protective role of Pentas lanceolata-iridoids. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 850, 503145.

11. Fahmy, M. A., Farghaly, A. A., Hassan, E. E., Hassan, E. M., Hassan, Z. M., Mahmoud, K., & Omara, E. A. (2022). Evaluation of the Anti-Cancer/Anti-Mutagenic Efficiency of Lavandula officinalis Essential Oil. Asian Pacific Journal of Cancer Prevention, 23(4), 1215-1222.
12. Falih, S. M., Al-Saray, S. T., Alfaris, A. A., & Al-Ali, A. A. (2022). The synergistic effect of eucalyptus oil and retinoic acid on human esophagus cancer cell line SK-GT-4. Egyptian Journal of Medical Human Genetics, 23(1), 1-12.
13. Falih, S. M., Al-Saray, S. T., Alfaris, A. A., & Al-Ali, A. A. (2022). The synergistic effect of eucalyptus oil and retinoic acid on human esophagus cancer cell line SK-GT-4. Egyptian Journal of Medical Human Genetics, 23(1), 1-12.
14. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2020 (https://gco.iarc.fr/today, accessed February 2021).
15. Gezici, S. (2018). Promising anticancer activity of lavender (Lavandula angustifolia Mill.) essential oil through induction of both apoptosis and necrosis. Annals of Phytomedicine, 7(2), 38-45.
16. Gezici, S. and Sekeroglu, N. (2017). Regulation of micro RNAs by natural products and bioactive compounds obtained from common medicinal plants: Novel strategy in cancer therapy. Ind. J. Pharm. Educ. Res., 51(3):483-488.
17. Hassan, S. A. H., Al-Shammari, A. M., & Allawe, A. B. Oncolytic Effect Of Non-Virulent Newcastle Disease Virus Lasota Strain In Human Breast Cancer Cell Lines In Vitro.
18. Hemminki, O., Dos Santos, J. M., & Hemminki, A. (2020). Oncolytic viruses for cancer immunotherapy. Journal of hematology & oncology, 13(1), 1-15.
19. Hemminki, O., Dos Santos, J. M., & Hemminki, A. (2020). Oncolytic viruses for cancer immunotherapy. Journal of hematology & oncology, 13(1), 1-15.
20. Jhawar, S. R., Thandoni, A., Bommareddy, P. K., Hassan, S., Kohlhapp, F. J., Goyal, S., ... & Zloza, A. (2017). Oncolytic viruses—natural and genetically engineered cancer immunotherapies. Frontiers in Oncology, 7, 202.
21. Kalantari, A., Keyvanfar, H., & Mortazavi, P. (2020). Evaluation of apoptosis induction by newcastle disease virus LaSota strain in human breast carcinoma cells. Archives of Razi Institute, 75(3), 367.
22. Keshavarz, M., Nejad, A. S. M., Esghaei, M., Bokharaei-Salim, F., DianatMoghadam, H., Keyvani, H., & Ghaemi, A. (2019). Oncolytic Newcastle disease virus reduces growth of cervical cancer cell by inducing apoptosis. Saudi Journal of Biological Sciences, 27(1), 47-52.
23. Khan, T., Ali, M., Khan, A., Nisar, P., Jan, S. A., Afridi, S., & Shinwari, Z. K. (2019). Anticancer plants: A review of the active phytochemicals, applications in animal models, and regulatory aspects. Biomolecules, 10(1), 47.
24. Ko, B. K., Lee, S. Y., Lee, Y. H., Hwang, I. S., Persson, H., Rockberg, J., ... & Lee, J. S. (2014). Combination of novel HER2-targeting antibody 1E11 with trastuzumab shows synergistic antitumor activity in HER2-positive gastric cancer. Molecular oncology, 9(2), 398-408.
25. Liu, K., Liu, P. C., Liu, R., & Wu, X. (2015). Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Medical science monitor basic research, 21, 15.
26. Liu, K., Liu, P. C., Liu, R., & Wu, X. (2015). Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Medical science monitor basic research, 21, 15.
27. Mondal, M., Guo, J., He, P., & Zhou, D. (2020). Recent advances of oncolytic virus in cancer therapy. Human Vaccines & Immunotherapeutics, 16(10), 2389-2402.
28. Nadri, M. H., Salim, Y., Basar, N., Yahya, A., & Zulkifli, R. M. (2014). Antioxidant activities and tyrosinase inhibition effects of Phaleria macrocarpa extracts. African Journal of Traditional, Complementary and Alternative Medicines, 11(3), 107-111.
29. Nikšić, H., Kovač-Bešović, E., Makarević, E., Durić, K., Kusturica, J., & Muratovic, S. (2017). Antiproliferative, antimicrobial, and antioxidant activity of Lavandula angustifolia Mill. essential oil. Journal of Health Sciences, 7(1).
30. Ould-Moussa, N., Safi, M., Guedeau-Boudeville, M. A., Montero, D., Conjeaud, H., & Berret, J. F. (2014). In vitro toxicity of nanoceria: effect of coating and stability in biofluids. Nanotoxicology, 8(7), 799-811.
31. Pejin, B., Iodice, C., Bogdanović, G., Kojić, V., & Tešević, V. (2017). Stictic acid inhibits cell growth of human colon adenocarcinoma HT-29 cells. Arabian Journal of Chemistry, 10, S1240-S1242.
32. Rasoul, L. M., Ali, L. F., & Mohammed, N. S. (2019). The Promising Anti-Tumor Impact of Newcastle Disease Virus Expressing IL-2 and P53 Genes in Many Cancer Cell Lines In vitro. Iraqi journal of biotechnology, 18(2).
33. Ravindra, P. V., Tiwari, A. K., Ratta, B., Chaturvedi, U., Palia, S. K., Subudhi, P. K., ... & Chauhan, R. S. (2008). Induction of apoptosis in Vero cells by Newcastle disease virus requires viral replication, de-novo protein synthesis and caspase activation. Virus research, 133(2), 285-290.

34. Ravindra, P. V., Tiwari, A. K., Sharma, B., & Chauhan, R. S. (2009). Newcastle disease virus as an oncolytic agent. Indian Journal of Medical Research, 130(5).
35. Rejhová, A., Opattová, A., Čumová, A., Slíva, D., & Vodička, P. (2018). Natural compounds and combination therapy in colorectal cancer treatment. European journal of medicinal chemistry, 144, 582-594.
36. Rejhová, A., Opattová, A., Čumová, A., Slíva, D., & Vodička, P. (2018). Natural compounds and combination therapy in colorectal cancer treatment. European journal of medicinal chemistry, 144, 582-594.
37. Ruiz, R. B., & Hernández, P. S. (2016). Cancer chemoprevention by dietary phytochemicals: Epidemiological evidence. Maturitas, 94, 13-19.
38. Russell, L., Swanner, J., Jaime-Ramirez, A. C., Wang, Y., Sprague, A., Banasavadi-Siddegowda, Y., ... & Kaur, B. (2018). PTEN expression by an oncolytic herpesvirus directs T-cell mediated tumor clearance. Nature communications, 9(1), 1-16.
39. Sabo, V. A., & Knezevic, P. (2019). Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: A review. Industrial crops and products, 132, 413-429.
40. Sharma, M., Grewal, K., Jandrotia, R., Batish, D. R., Singh, H. P., & Kohli, R. K. (2022). Essential oils as anticancer agents: Potential role in malignancies, drug delivery mechanisms, and immune system enhancement. Biomedicine & Pharmacotherapy, 146, 112514.
41. Siegel, R.L.; Miller, K.D and Jemal, A. (2018). Cancer statistics, 2018. CA. A Cancer Journal for Clinicians, 68(1):7-30.
42. Soković, M., Glamočlija, J., Marin, P. D., Brkić, D., & Van Griensven, L. J. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules, 15(11), 7532-7546.
43. Soulaimani, B., Nafis, A., Kasrati, A., Rochdi, A., Mezrioui, N. E., Abbad, A., & Hassani, L. (2019). Chemical composition, antimicrobial activity and synergistic potential of essential oil from endemic Lavandula maroccana (Mill.). South African Journal of Botany, 125, 202-206.
44. Stevens, A., Lowe, J. S., & Scott, I. (2008). Core pathology. Elsevier Health Sciences.
45. Velazquez-Kronen, R., & Nelson, J. (2020). World Cancer Day 2020–reflecting on a decade of NIOSH cancer research.
46. Zhao, Y., Chen, R., Wang, Y., Qing, C., Wang, W., & Yang, Y. (2017). In vitro and in vivo efficacy studies of Lavender angustifolia essential oil and its active constituents on the proliferation of human prostate cancer. Integrative cancer therapies, 16(2), 215-226.
47. Zheljazkov, V. D., Kacaniova, M., Dincheva, I., Radoukova, T., Semerdjieva, I. B., Astatkie, T., & Schlegel, V. (2018). Essential oil composition, antioxidant and antimicrobial activity of the galbuli of six juniper species. Industrial Crops and Products, 124, 449-458.
48. Zhou, Y., Tang, J., Du, Y., Ding, J., & Liu, J. Y. (2016). The green tea polyphenol EGCG potentiates the antiproliferative activity of sunitinib in human cancer cells. Tumor Biology, 37(7), 8555-8566.
49. Zu, Y., Yu, H., Liang, L., Fu, Y., Efferth, T., Liu, X., & Wu, N. (2010). Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules, 15(5), 3200-3210.