

Olea europaea leaves extract downregulate Newcastle disease virus gene expression in cancer cells

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Abstract:

Many researches have been made to find antiviral agents from natural sources. Leaf of *Olea europaea* (OLE) is one of the most important medicinal plants, as it is a well-known source of different phytochemicals. This study was carried out to investigate the effect of using *Olea europaea* leaves extract on the level of viral gene expression (M and F) in Hela cell line by using the qRT-PCR. Also, we examined the adding of interferon-beta (IFN- β) as an antiviral factor and analyzed the expression of viral and IFN- β genes to identify viral targets for OLE extract. First, we propagated the virus in embryonated chicken eggs and showed to kill embryos in different times intervals (less than 72 hours) with marked severe hemorrhage in infected embryos, and agglutination activity reflects titer (1024) and $1 \times 10^{6.4}$ (TCID₅₀). Hela cells were cultured and different concentration of OLE extract was added; cell viability was measured by MTT assay. Gene expression showed that the expression of the viral gene was downregulated in the presence of OLE extract. Pretreatment with IFN- β downregulated viral gene expression higher than the extract, also evaluated the IFN- β concentration by ELISA assay. OLE can be considered as antiviral agent and used this property to help in control Newcastle disease.

Introduction:

Newcastle disease virus (NDV) remains one of the most important viral infections (32). The etiological agent of Newcastle disease is a participant of the order Mononegavirales, family Paramyxoviridae and genus Avulavirus (27). The genome of the NDV encoded for at least eight proteins: NP, P, M, F, HN, L, S, and M2. M protein is the most numerous necessary protein in the virion (7). The F protein is the key to determine the virulence of the viral strain (43). The developments of viral resistance towards antiviral agents improve the need for new active compounds against viral infections. Natural products have historically and continually been examined for promising new leads in pharmacological development (49). Plants used as a source of medicinal value is an ancient concept because of their advantages, safety, efficacy, and availability throughout the world (1). Many of the plant materials are used in traditional medicine because they are readily available and cheaper compared to modern therapeutic drugs (29). Moreover, many studies have used known purified plant chemicals, very few screening programs have been initiated on crude plant materials. Natural products from plants

Traditionally have provided the drug industry with one of the most important sources of 'lead' compounds (19). The World Health Organization (WHO) has published that about 80% of the world population depends on traditional medicine for their primary health care (24). The olive leaves have a rich history of medicinal uses (45). This plant is cultivated widely in Mediterranean region, Arabian Peninsula, the Indian subcontinent and Asia (34). The chemical composition of the olive leaf is characterized by the presence of several secoiridoids (the main component – oleuropein) and unconjugated secoiridoid-type-aldehydes (oleacin), Triterpenes and flavonoids, namely rutin and glycosides of apigenin and luteolin (6). The phenolic compound was also present in the olive which belonging to the iridoid group. (40) Tested the effects of the calcium elenolate on a range of viruses in-vitro and found that it destroyed all the viruses it was tested against, These included herpes, vaccinia, pseudorabies, influenza A (PR8), Newcastle disease, parainfluenza 3, Coxsackie A 21, encephalomyocarditis, polio 1, 2 and 3, vesicular stomatitis, Sindbis and Reovirus 3 (Deering) viruses. Besides, OLE has also exhibited significant antiviral activity against respiratory syncytial virus and parainfluenza type 3 virus (26). (20) Found that OLE inhibits the in vitro infectivity of ILT virus. In another study done by (46), it revealed that OLE has an anti-HIV-1 activity. The protective action of the plant taking place with several mechanisms, but the exact

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mechanism by which this action is elicited is not known. So our study was designed to evaluate the effect of OLE aqueous leaf extract on Newcastle disease virus gene expression (M and F) and cellular IFN- β gene, also comparing this effect with the addition of IFN- β in Hela cell line.

Materials and Methods:

Reagents

Roswell Park Memorial Institute medium (RPMI 1640), Fetal bovine serum (FBS) (Invitrogen Corporation, USA), Methyl thiazolyl tetrazolium 1gm.(MTT) (Bioworld, USA), Human Interferon Beta (IFN- β) ELISA kit BioAssay (USBIOLOGICAL). Automated total RNA extraction (Anatolia, Turkey), KAPA Sybergreen One-step qRT-PCR (KAPA Biosystems USA), DNase Enzyme kit (USBIOLOGICAL, US

Method

Cell culture

Hela cell line (Human epithelial carcinoma cell line)

Hela cell line was provided by the cell Bank Unit, Experimental Therapy Department, Iraqi Center for Cancer and Medical Genetic Research (ICCMGR).

Virus propagation and identification

Newcastle Disease Virus Isolate

The isolate kindly provided by Iraqi center for cancer and medical genetic research ICCMGR (Najaf APMV1/ Chicken/ Iraq-Najaf/ ICCMGR /2012).

Propagation of NDV in embryonated chicken eggs

Ten days old chicken eggs were used for virus propagation the egg was rinsed with 70% ethanol to sterilize outer surface then (0.1ml) of the prepared virus was inoculated in the allantoic fluid with a fine needle (allantoic route), melted paraffin sealed the puncture hole in the egg. Eggs were incubated at 37 °C and checked daily for embryo viability. After 24h dead embryos if any were discarded. Embryos which died subsequently were transferred to the refrigerator (6-8hrs). Allantoic fluid was collected by sterile syringe, purified from debris and erythrocytes by centrifuge, dispensed into small tubes and stored at -80°C. The virus was further passaged in embryonated eggs two passages in local eggs and one passage in SPF eggs in which high titer of the virus can be obtained and reached (9-10) HAU/ ml (2)

Hemagglutination (HA) test

The chicken RBC was prepared by washed three times with PBS and centrifugation at 1000rpm 4°C for 10 minutes and prepared (1%) RBC solution to be used in hemagglutination test which carried out as (10,4)

Tissue Culture Infection Dose 50 (TCID50) test

Hela cells were seeded at 1cells/well in 96well microliter plates, virus suspension was serially tenfold dilution, Cells were inoculated into four wells for each dilution (50 μ l per well), control cells were inoculated with serum-free media.

The plate was incubated for two h at room temperature, then washed with PBS and 200 μ l serum-free medium was added, the plates were covered again and incubated at 37°C, the inoculated plate was examined daily for three days, and NDV

titer was calculated as described by (15). The titer can also be calculated as described by (37).

Preparation of crude aqueous extract

Olea europaea leaves were selected from the local olive trees during June-July, plants leaves were washed and dried at room temperature about (25- 30°C) until be dried about (1-3) days. The dried leaves were crushed to a fine powder by an electrical grinder and extracted by boiling distilled water (35) at a ratio of(150 g /3000ml w/v). Stirring for 15min, then it mixed (15-20) minutes away from heat, filtered through a filter paper (twice) , concentrated by evaporating, reconstituted with water to 0.1gm./ml as starting material, sterilized by Millipore filtration with a 0.45micron filter, and stored at (-20 °C) until use (17).

Cytotoxicity assay in Hela cell line

It was done to determine the highest tolerated dose (extract concentration) causing minimal cytopathological changes (CPE) compared with control Hela culture to be used further in the antiviral assays. The protocol was described by (41).

The cells were diluted with complete medium to approximately 5Xcells/ ml. (counted and calculated by hemocytometer), then 200 μ l / well of cell suspension were dispensed into 96 wells plate. The plates were covered and Incubated in 5 % CO₂, humidified, 37°C incubator till confluent sheet is formed. Fresh medium containing different concentrations of the tested extract (2000,1000,500,250,125,62.5 μ g/ml) was added, there were three replicates for each tested concentration, the control left with no treatment. The cells were incubated at 37°C under a humidified atmosphere containing 5% CO₂. Cell viability was measured after 72 hr. of infection by removing the medium, adding 100 μ l of MTT solution and incubating for two h at 37°C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100 μ l of Dimethyl Sulphoxide (DMSO) followed by incubation at room temperature for 15 minutes with shaking. The absorbency was measured on a microplate reader at 492 nm (9). The inhibiting rate of cell growth (the percentage of cytotoxicity was calculated as (G.I) = (A-B)/Ax100, Where A is the mean optical density of untreated wells and B is the optical density of treated wells (16, 5).

Antiviral screening.

The concentrations of the extract were determined (1000,500) g/ml, plates that had cultured of Hela cell line were prepared, NDV at 0.1 MOI (TCID50/ml) in sterile tube using cold serum-free medium was prepared., Cells infection with NDV by removing the medium from the microtitration plate, 100 μ l of free serum media with virus was added to each well, while the control cell was treated with serum-free media only, after 2hr. of adsorption at room temperature, Virus solution was removed, plant extract with different concentration diluted in warm serum-free media were added (three replicates for each concentration). The plate was covered again with a new sterile adhesive and Incubated at 37°C in5% CO₂ incubator for 72 hrs. This procedure was accomplished according to (31). Cell viability was measured by MTT stain and calculation the inhibition rate of cell growth.

Sample collection for PCR and ELISA

The complete monolayer HeLa cell line was prepared in 16 tissue culture flasks (25 cm²) at a concentration of 1 cell and incubated at 37°C for 24h, the medium was removed, and eight flasks were inoculated with (0.01MOI) of NDV, incubated at room temperature for 2 hrs. The plant extract of OLE was added for 4 flasks (1000ug/ml), and free serum media for the 4 flasks as positive virus control, The negative control 4 flasks were treated with Serum Free Media only, and the last 4 flasks treated with interferon beta (IFN-β) for 15 hr. (500IU/ml) before infection with NDV (20). All flasks were re-incubated at 37°C for 12, 24, 36 and 48 h. (each time harvested four flasks, for NDV, OLE extract, interferon beta and one for control). In each time point, the cells were harvested with 1 ml of PBS by cell scraper after collected the culture media for detection of interferon beta by ELISA assay, the suspension of the cells were harvested from each flask into Eppendorf tube and centrifuged for 10 min. at 1000rpm 4°C, then the supernatant was discarded, and the cells pellet was re-suspended in 200 μl RB buffer (supply with the RNA extraction Kit) and mix by vor-

tex, stored at -80°C till use for genetic detection of the mRNA level.

Detection of interferon beta concentrations by ELISA Bio-Assay

Using ready to use ELISA kit (RayBiotech, USA). The absorbance was determined immediately at 450nm using a microplate reader. The standard curve is used to determine the concentration of samples.

RNA extraction:

Total RNA was extracted from the infected and negative control HeLa cell line which harvested at each time point using the automated extraction RNA kit, each sample was treated with DNase (15unit/sample) to remove genomic DNA and the concentration of RNA was measured by using the Nanodrop (Thermo scientific USA).

Quantitative real-time polymerase chain reaction (qRT-PCR):

The qRT-PCR method was in a final volume of 20 uL using a QuantiFast SYBR Green PCR Master Mix Kit (KAPA Kit) with specific primers (table 1)

Table-1 Primers Design

Gene	Sequence	References
M gene F	5'- AGTGATGTGCTCGGACCTTC-3'	Al-Habeeb et al.,2013
R	5'-CCTGAGGAGAGGCATTTGCTA-3'	
F gene F	5'-TCCGGCTTAAAGAGAGCATTG-3'	ApE software
R	5'-ACTGCCACTGATAGTTGTGATAAT-3'	
rRNA 18s F	5'-GGAGTATGGTTGCAAAGCTGA-3'	Aursnes, et al.,2011
(Human) R	5'-ATCTGTCAATCCTGTCCGTGT-3'	

The RT-PCR reactions were performed in 20 μl (Agilent 8800 gradient PCR). Containing Nuclease-free water 6.6 μl, RT 0.4 μl, Master Mix 10 μl, forward primer 1 μl and reverse primer 1 μl, template 1 μl Reverse transcription was carried out at 42°C for 5 min. PCR reactions were subjected to 40 cycles containing of denaturation for 30 seconds at 95°C, annealing 52°C for 20sec., extension for 20 seconds at 72°C, and one final extension cycle at 95°C for 1 min.

Real-Time qRT-PCR analysis

□CT (test) = CT gene of interest (test) – CT internal control
 □CT (calibrator) = CT gene of interest (calibrator) – CT internal control. The calibrator was chosen from the control samples.

The □CT of the test samples was normalized to the □CT of the calibrator:

□□CT was calculated according to the following equation:
 □□CT = □CT (test) - □CT (calibrator)

Finally, the expression ratio was calculated according to the formula

$2^{-\square\square Ct}$ = Normalized expression ratio.

Statistical Analysis

The Statistical Analysis System (39) program was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results:

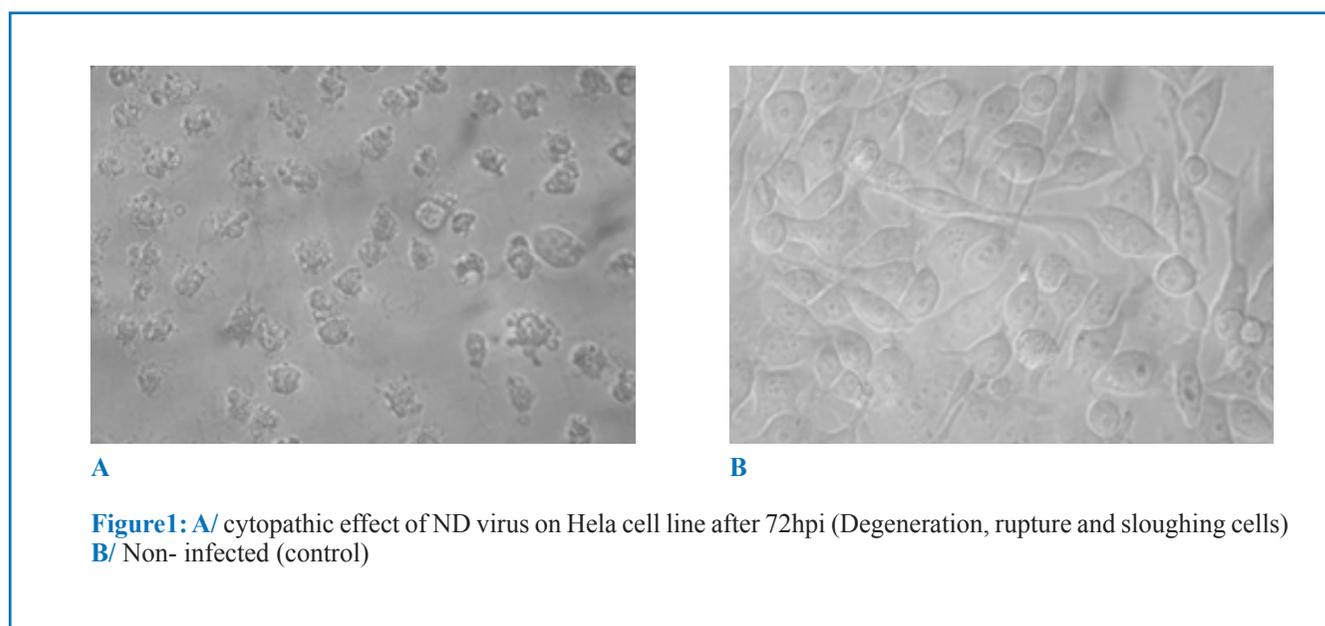
Virus propagation in embryonated chicken eggs:

The isolate was inoculated into the allantoic sac. Isolated viruses killed embryos in certain times (more than 30h and less than (72 h.) with marked sever hemorrhage in infected embryos in contrast to control uninfected embryos that were not inoculated remained alive for more than 96 h. post infection. Embryonic death within 24 hr. post inoculation was considered non-specific while death after that and up to 4 days must be harvested.

Cytopathic effect of NDV in HeLa cell line

HeLa cells were infected with NDV from allantoic fluid, and the yield of progeny virus was high at the beginning of the in-

fection, also the cytopathic changes were more extensive after 72h of infection than those in control non-infected Hela cells as shown in Figure (1).



Titration of the virus in cell culture

The allantoic fluid collected from the infected fertilized eggs and filtered in Millipore filter(0.45um), then virus titer measured by TCID50. The result showed that the titer of the isolated virus in Hela cell line was $2 \times 10^{6.4}$ TCID50 / ml.,

Cytotoxicity on Hela cell line

Hela tumor cell line was seeded as 1×10^4 cells / well in 96 well plats and after 24h, when the cells become con-

fluent monolayer, they were exposed to the extracts at (2000,1000,500,250,125,62.5) ug/ml and incubated at 37°C for 72h., then stained by MTT stain and calculate the inhibition %.

The results show that Olea extract had the highest inhibition rate ($54.6\% \pm 2.71$) in the concentration of 2000 ug/ ml, and decreased to ($18.7\% \pm 0.92$) in the concentration of 62.5 as shown in the table (2).

Table (2) growth inhibition% of *O. europaea* leaves extract on Hela cell line after 72 hr. of exposure

Conc. (µg/ml)	<i>Olea europaea</i>
	62.5
125	25.5 ± 1.78
250	36.6 ± 2.09
500	41.1 ± 1.73
1000	48.2 ± 2.66
2000	54.6 ± 2.71
LSD value	6.922 *

Antiviral activity of the plant extract

Two concentration (1000 and 500) / ml were used to determine the effect of leave plant extract on the ND virus. Viability of Hela cell line was measured by MTT assay (inhibition %) as anti-viral effect. after 72 h. post-infection) the plates were read by ELISA reader (492 nm) . The results were shown decla

ine of the cytopathic effect of the virus on Hela cells when combined with extract (extract+ virus) as showed in table 3 The result decline from (80.6%) in positive control (NDV infected cells) to (70.4 and 73.0) for Olea extract concentration 1000 and 500g respectively.

Table (3) Percentage of growth inhibition of the combination (Virus + extract) in HeLa cell line

Extract	Virus control In HeLa cells	Combination
g / ml		HeLa cells
Olea 1000	80.6%	70.4%
extract 500	80.6%	73.0%

Effect of the extract on gene expression of ND virus in HeLa cell line

Effect of extracts on M gene expression

One concentration 1000 µg/ml was used to evaluate the antiviral effects of the plant extract on the expression of the Newcastle virus genes (M and F) in HeLa cells which successfully detected by Real-time PCR (qRT-PCR). The result showed that the virus-infected cell (positive control) gave the highest ex-

pression of M gene at 24 h postinfection (36.73), while the gene expression of the extract group was lower than the virus alone (25.63). The virus group decline at 48hpi. to give fold expression of (8.0) and the extract also decline at this time to give fold of gene expression (3.11) as in table(4.)

The IFN- β affected the M gene expression of NDV from 24h which give (7.51) and increase with time to reach (18.12) at 36h then decline to (3.2) at 48h.

(Table 4) M gene expression in HeLa cells after infected with NDV

Groups	Fold /Time				LSD value
	12 hr.	24 hr.	36 hr.	48 hr.	
Virus	18.76 ± 1.42	36.73 ± 1.29	12.73 ± 0.84	8.0 ± 0.57	5.712 *
INF-β	7.51 ± 0.66	11.95 ± 0.84	18.12 ± 1.26	3.2 ± 0.04	3.965 *
Olea extract	17.87 ± 0.82	25.63 ± 1.52	7.41 ± 0.64	3.11 ± 0.12	3.837 *
LSD value	3.914 *	5.633 *	3.852 *	3.043 *	----

* (P<0.05), NS: Non-significant

Effect of extract on F gene expression

The extract downregulated F gene expression in comparison with virus

control which give (14.82) fold at 12hr. while the extract groups decline the gene fold to (12.78), at 24hpi the virus group

reach (29.04) but Olea had (22.62), at 48hpi the expression also downregulate on the virus group and the extract (12.29, 5.31) to the virus and extract respectively as in table (5). The INF- β group was at first decreased the F gene expression to (6.58), but then the fold increased to (8.81, 11.47) then decline to (4.37).

Table (5) F gene expression in Hela cells after infected with NDV

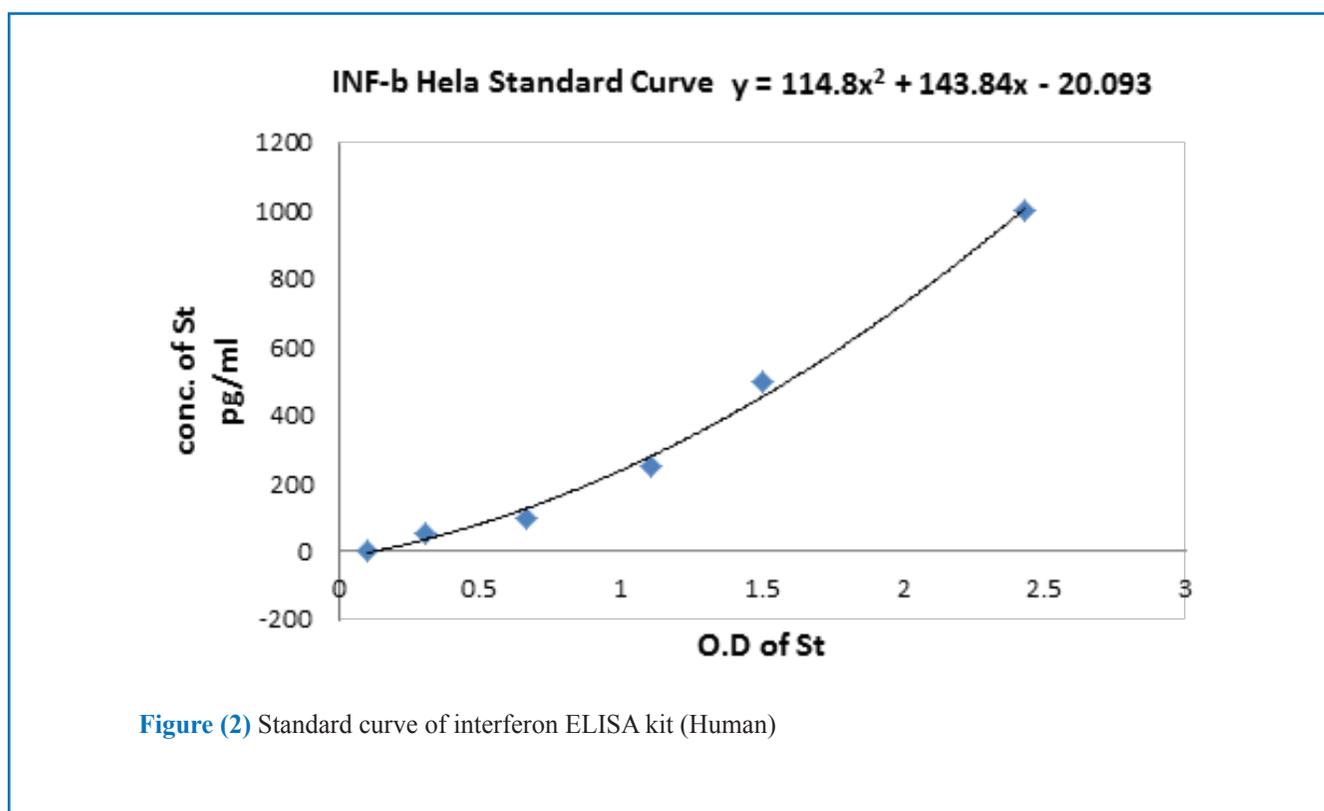
Groups	Fold /Time				LSD value
	12 hr.	24 hr.	36 hr.	48 hr.	
Virus	14.82 ± 0.84	29.04 ± 1.68	12.29 ± 0.85	7.46 ± 0.36	5.784 *
INF-b	6.58 ± 0.27	8.81 ± 0.73	11.47 ± 0.72	4.37 ± 0.15	2.941 *
Olea extract	12.78 ± 0.46	22.62 ± 1.09	5.31 ± 0.37	3.31 ± 0.07	4.025 *
LSD value	4.923 *	4.085 *	3.266 *	2.946 *	---

* (P<0.05), NS: Non-significant

Human IFN-b Quantification

Hela cell line was infected with Newcastle virus (0.01 MOI), and after 72hr., the media of the infected cells (three groups) were used for the detection of interferon-b by ELISA kit in dif-

ferent time of the study (12, 24, 36, 48 hr.). The standard curve was plotted using Excel program, with standard optical density (O.D.) on the (x-axis) and concentration on the (y-axis). A standard curve run for this assay is shown in figure (2).



The virus group (positive control) had the lowest concentration of IFN-b among the other groups in the study at 12hpi, which increase after 24h and return to decrease at 36, 48 h, the

same as the interferon group (the cells exposed to 500 IU of INF-b for 15hr. before infected with ND virus) and the Olea extract, as in (table 6)

Table (6) Result of ELISA test for detection of interferon-b (mean) in Hela cell line after infected with ND virus

Groups	Concentration / Time				LSD value
	12 hr.	24 hr.	36 hr.	48 hr.	
Virus	320 ± 15.73	491 ± 20.74	402 ± 17.04	314 ± 14.79	46.95 *
INF-b	383 ± 15.22	460 ± 17.36	447 ± 15.91	345 ± 17.42	41.28 *
Olea extract	372 ± 15.96	410 ± 13.68	380 ± 13.79	330 ± 13.95	33.71 *
LSD value	35.722 *	41.261 *	38.073 *	38.406 NS	----

* (P<0.05)), NS: Non-significant.

Discussion:

The Iraqi strain of Newcastle disease virus (NDV) has the ability to kill the chicken embryos from 48 to 72 hr., after the inoculation of the chicken embryonated eggs. This indicates of the virulence level of the strain which was the velogenic type that able to kill embryos after 40-72hr. This result agrees with (2) and (3) who isolated the Iraqi local strain that was used in this study. The results confirm that the strain is still virulent and hold virulent characteristics. Hemorrhage was observed in the infected embryos when compared with the control. The virus was purified and quantified by hemagglutination test showed a positive result as a typical hemagglutination mesh pattern of a chicken red blood cell with titer reached to (1024 HAU) in the third passage. These results agree with (38, 10). The titer of NDV virus was measured on Hela cells for the determination of TCID₅₀. The hallmark of NDV infection in host cells was the formation of syncytia by the isolated virus, enhanced oncolytic properties as mentioned by (3, 10) whom reported that syncytium is a conspicuous feature of infection of cell monolayer by NDV, it results from the fusion of an infected cells with neighboring infected or uninfected cell.

The results showed that the titer was ($10^{6.4}$ TCID₅₀/ml) in Hela cells, this result shows an extremely high yield of the virus after low MOI infection of tumor cells resulted in rapid and excessive cytopathology and death of these cells. APMV-1 strains can replicate in a diversity of cell cultures of avian and non-avian origin, cell line as referred by (38) mentioned that NDV could replicate 10,000 times higher in human cancer cells than in normal human cell and plaque assays were used to determine the cytolytic activity of NDV. NDV formed plaques on all tested tumor cells as well as on chick embryo cells (CEC), the native host for NDV.

The Newcastle disease virus (NDV)-Hela system was chosen

to study the multiplicity of infection. It was observed that with much virulence, which was sufficient to infect all cells in the culture, the time at which antigen first appeared and the amount present at subsequent intervals were dependent on the multiplicity of infection (50). It seemed probable that the time at which alterations in cell structure and functions would appear might also depend on the multiplicity(25). When HeLa cells were infected with NDV at low multiplicity of infection, they yield of progeny virus was higher, and the cytopathic changes were extensive. The Uninfected cells surround the plaques as reported by (13).

Olea extract in a concentration of 2000 µg/ml had inhibition% in Hela cell line reach (54.6 ± 2.71) and decreased to (18.7 ± 0.92) in the concentration 62.5µg/ml, One thousand µg /ml is the IC₅₀ of the extract, this agrees with the finding of (28) found that the (CC₅₀) after cell treatment with O. Europaea extract was 700µg/ml. (50) Estimated that the 1000µg/ml were used for antiviral effect ,(14) demonstrated that the(IC₅₀ µg/ml) for Olea extract was >1000µg/ml. The cytotoxic effect may due to the presence of polyphenols, which have been proved to have anticancer action by many researchers. (33) demonstrated of free radicals. (12) conducted a recent data on the ability of 3, 4-dihydroxy-phenylethanol (DOPET) to arrest cell proliferation and induce apoptosis in cultured human cells, which support the view that cancer prevention exerted by olive oil could be ascribed to its high content of DOPET and its precursor oleuropein aglycone. (47) demonstrated that the variation in cytotoxic effects of the extract depending on the type, concentration, and duration of exposure. This is in agreement with many in vitro studies which are conducted to assess the cytotoxic effect of different plant extracts and their purified component on various normal and cancer cell line (36).

When 1000µg/ml used as anti-viral the results showed that the used of extracts lowering the inhibition% of the cells, In

Hela cells the control virus had 80.6 inhibition and the inhibition in Olea was (70.4 ,73.0), but its still high may be due to cytotoxicity of the extract as mention (28).

The antiviral effect of the extract studied by qRT-PCR to study the expression change of Newcastle virus mRNA of Fusion (F gene) and Matrix (M gene) in a term hour post infection (12, 24, 36, 48 hr.) because Newcastle disease viral replication is the most rapid among the paramyxoviruses,.The virus able to overtake host cell protein synthesis within six hours (22),

There were significant differences at 12,24,36,48 h. among the three groups of the study (virus as a positive control, IFN-b and Olea extract) in fold expression of M gene in Hela cell line,

We tested the addition of IFN-b to the cells 15h before infection and saw that there were downregulate in gene expression fold throughout the study period in comper to virus positive control, these results agree with (21) who showed that the NDV growth and replication were suppressed by pretreatment with exogenous IFN-b in normal cells. (39) Revealed that IFN capable of controlling most, if not all virus infection in the absence of adaptive immunity. However, viruses can still replicate because they have some strategy for at least partially circumventing the IFN response. In addition, the pretreatment of cells with interferon-b can sometimes enhance the IFN yield (an effect called priming) (18).

. In Hela cells, we saw that the maximum yield of M gene expression at 24hr.post infection except for the IFN group which had high level at 36hr. may be due to effect of it on virus gene and the inhibition of replication, and the extract reach the same level as IFN group (3.2, 3.11, 3.27) at 48hpi for IFN, Olea, Ocimum respectively.

We can observe the same results in ELISA test for detection of IFN-b, there are slightly different between the virus and IFN group and the Olea extract had the lowest concentration. The detection of IFN-b by ELISA test showed that all groups reached the highest concentration at 24hpi, then start to decline up to 48h.(30) performed an ELISA and looked at the levels of Interferon-beta in the supernatant. They found that the HPV-positive cervical cancer cell lines (Human Papillomavirus in Hela cell line) showed decreased levels of Interferon-beta, indicating that the presence of HPV can lead to suppressed antiviral responses. (21) reviled that the level of phosphorylated STAT1 and STAT2 and that of the ISGF3 complex was markedly reduced in IFN-b treated tumor cells.

Conclusion

The results indicate that *O. europaea* crude extract can be considered as a potential source of anti -Newcastle virus agent by its effect on viral gene expression and has an important role in compared with the action of interferon –Beta.

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