Saudi Journal of Biological Sciences 29 (2022) 1239–1245

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# The natural antiviral and immune stimulant effects of *Allium cepa* essential oil onion extract against virulent Newcastle disease virus

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ARTICLE INFO

*Article history:*
Received 7 August 2021
Revised 11 September 2021
Accepted 13 September 2021
Available online 21 September 2021

*Keyword:*
NDV
*Allium cepa* essential oil
Onion extract
Antiviral effect
Vaccine

ABSTRACT

Fifty broiler chicks were divided into five groups to study the antiviral and immune-stimulant effect of *Allium cepa* essential oils (ACEO). The effect of *Allium cepa* essential oils administration single or combined with NVD vaccine in broilers, more than one parameter was studied in this study i.e., the clinical symptoms that appeared on the chicks after the experimental infection with velogenic Newcastle disease virus, postmortem lesions, pathological lesions scoring, mortality rate (MR), and viral shedding, birds immunity was assessed by HI test and protection percent post-challenge with vNDV. Our result showed that mild clinical signs, lesion scoring, decreased viral shedding in ACEO treated groups 3 (G 3) more than control groups post-challenge with vNDV. Delayed onset of mild clinical signs in G3 followed by complete recovery 7th-day post-challenge (DPC). Low MR (40 and 0%) and high protection percent (100 and 60%) in ACEO treated G3 and G5, respectively. spleen, thymus, cecal tonsil, proventriculus, and cerebrum lesions scoring in G3 and G5 were significantly ($(p \leq 0.05)$.) lower than the control group, proving a decrease in NDV replication and effective antiviral activity of ACEO. HI titer significantly increased ($p \leq 0.05$) In G3, G4 and G5 compared with control groups. There is no significant difference in HI titer in ACEO treated groups and vaccinated groups. In conclusion, oral administration of ACEO combined with NDV vaccines significantly reduces or eliminates lethal clinical signs, lesions, viral shedding, and enhances immune response and protection percent after vNDV challenge proving the natural antiviral and immune stimulant effect of ACEO onion extract. Implementing such a regime might aid NDV control in broiler flocks in endemic areas and reduce the epidemiological load of NDV in the environment.


## 1. Introduction

Newcastle disease is one of the most dangerous diseases that affect poultry and one of the most widespread and it also results in heavy economic losses. The cause of this disease is the Newcastle virus, which joins the Paramyxoviridae family, avian orthoavulavirus 1 genus. From the time of 1st isolation of NDV in Egypt in 1948′s has become a problem facing the poultry sector in Egypt (Rima et al., 2019). Assessment of the commercial vaccines and raising the immunity of birds by some natural materials were recommended. Recently, there has been a great interest in biologically active plant extracts especially, in Europe, Japan, and USA (Jafari et al., 2011). The onion (*Allium cepa L.*) is a well-known and grown

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.09.033
1319-562X/

all over the globe. In traditional medicine, onions have been used to cure and prevent a variety of illnesses and disorders since ancient times (Ebesunun et al., 2007). Onion is a worldwide cultivated bulbous plant whose bulbs have many organic sulphur compounds i.e., S-methylcysteine sulfoxide, Trans-S-(1-propenyl) cysteine sulfoxide, Spropylcycteine sulfoxides and flavonoids, cycloallicin, phenolic acids, sterols as stigmasterol, cholesterol, b-sitosterol and saponins, these compounds have antiviral, antibacterial, antifungal, antiparasitic, antioxidant and anti-inflammatory (Goodarzi et al., 2013). And it has been proven that onion extract has the ability to inhibit the action of the Newcastle virus, as it is possible to inactivate the activity of NDV haemagglutination of LaSota strains and Hitchner-B1 (Abo-Zeid, 1989). Onion extract may reduce the mortality rate in birds infected with the Newcastle virus, also it has an effect on the rate of weight gain of birds (Nadia et al., 1991). Onion and garlic flavonoids have a significant inhibitory effect on virus replication, phytochemicals used these plants have been found to prevent the virus from forming protein and genetic material (Zandi et al., 2011). The primary flavonoids found in onions are kaempferol and quercetin. Many viruses have been shown to be affected by these compounds (Kumar and Pandey, 2013). Onion extracts appeared useful in reducing Newcastle Disease virus infection by preventing the virus from attaching to the cell (Harazem et al., 2019). Onion's antiviral properties have been related to ribavirin, allicin, zalcitabine, and quercetin (Chen et al., 2011).

This study aims to find out the effect of ACEO administration single or combined with NVD vaccine in broilers.

## 2. Materials and methods

### 2.1. Ethics statement

All national and institutional applicable instructions for the care and use of animals were followed. The protocol and procedures employed were reviewed and approved by the ZU-IACUC committee with the approval number of ZU-IACUC/2/F/27/2021.

### 2.2. Birds, vaccines and antiviral agent

A total of 50 one-day-old commercial Cobb chicks purchased from a local hatchery in Egypt, were used. Two commercial Newcastle disease virus vaccines (MEFLUVAC-H9ND-16, and LaSota strain) were used according to the manufacturer's recommendations. ACEO natural antiviral agent supplied by Dr. Abdel Basset El-Gamal, Pharmaceutical Division, National Research Center, Egypt. *Allium cepa* mature bulb, fresh green bulb and green stalk 1Kg (each) were subjected to hydro-distillation by using Linkersson apparatus for 12hrs (Harazem et al., 2019) . For extraction of essential oil**,** the distilled steam of each part was extracted twice with petroleum ether (2 × 150 ml). The organic layer was dried over anhydrous sodium sulfate, which on the removal of the solvent afforded pale-colored oil. Dried oil was stored in an air-tight amber-colored bottle at 4$^{0}$C in the refrigerator (Shahzad et al., 2009; Armenta et al., 2015).

### 2.3. Challenge virus

All groups were challenged at 36 days old with virulent NDV (accession no. MZ029052) strain recently isolated from chicken flocks via nasal cavity (0.2 ml) containing $10^{6.5}$ EID50.

### 2.4. Experiment design

This study was designed to find out the effect of ACEO administration single or combined with NVD vaccine in broilers by comparing the effect of each of them alone or together via evaluation parameters as HI test, virus shedding assay, and pathological scoring system. Therefore, 50 Cobb chicks purchased from local hatchery were divided into 5 groups, starting from the age of one day, where group 1 (G1) was negative control (non-vaccinated, non-treated with ACEO), G2 positive control (non-vaccinated, non-treated, and experimentally infected), G 3 (vaccinated and treated with ACEO), G4 (vaccinated only), and G5 (treated with ACEO only) all groups experimentally infected except G1.

### 2.5. Vaccine regime

Inactivated vaccine at 1 day old, 0.5 ml via s/c rout, followed by lasota in drinking water at 9 day old and 15 day old all groups challenged at 36 day old via nasal rout.

### 2.6. Sampling

Birds were monitored daily; blood samples were collected weekly. After experimental infection, clinical symptoms were recorded and three tracheal swabs were collected from each group at 3, 5, and 7th DPC for evaluation of viral shedding. At the end of experiment three birds were slaughtered from each group for postmortem examination and tissue samples were collected and placed in formalin for histopathological examination.

### 2.7. Hemagglutination inhibition (HI) Test

HI test was used to monitor humoral immune response for each experimental group according to the Office International des Epizooties (OIE) manual (OIE., 2015).

### 2.8. qRT-PCR for virus shedding

The viral genomic RNAs of the selected Tracheal swabs were extracted using QiAamp Viral RNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions,rRT-PCR was carried out by a commercial Quantitect Probe rRT-PCR kit (Qiagen, Inc., Valencia CA). The forward, reverse Primers and probe were used after (Wise et al., 2004). Real-time rRT-PCR was performed in the Stratagene 3005P MXpro Real-Time PCR System (Stratagene, USA). A triplicate of 8.5–10-fold dilutions of challenge NDV were used to generate a standard curve using stock virus dilutions. The point at which the curve crosses the horizontal threshold line is defined as Ct, we plotted virus log10 titers of a sample against the Ct value, where the best fit line was constructed. ND virus quantity in unknown samples was derived by planning the Ct of an unknown against the standard curve and were expressed in log10 EID50/mL equivalents.

### 2.9. Histopathological examination

Different Tissue samples from different birds were collected during postmortem examination and fixed in 10 % formalin; after that is processed according to (Bancroft J. D and Layton C, 2013).

### 2.10. Statistical analysis

Shapiro-Willk test was used to test the data normal distribution. Compare between means for HI data were conducted by one-way ANOVA and subsequent Duncan's multiple range test (Duncan, 1955). A non-parametric test called Kruskal Wallis Test

was used to determine the statistical differences between histopathological lesion scores. Probability values of<5% ($p < 0.05$) were considered significant, differences between groups were analyzed using one-way ANOVA with Tukey's post hoc test. A probability (p) value $\leq 0.05$ was considered statically significant (Amer et al., 2020).

## 3. Results

### 3.1. Clinical signs and postmortem examination

Birds in group 1 at 3rd, 5th, and 7th day post challenge (DPC) exhibit no clinical signs, which reflect negative control status. In group 2, experimentally infected birds showed depression, foamy conjunctivitis and some birds show diarrhea at 3rd DPC. The clinical signs begin to increase in severity by five and 7th DPC with greenish diarrhea, nervous manifestation with decrease of feed intake, and high MR% (100 %). No clinical signs appeared in G3 at the 3rd DPC followed by slight depression, mild respiratory distress and mild transient diarrhea at the 5$^{th}$DPC that quickly disappeared with complete recovery and mortality rate (MR) zero % at 7th DPC. In G4 mild clinical signs appeared early at 3$^{rd}$DPC, then eye lesions disappeared at 5th day, while mild respiratory distress and diarrhea still recorded at7$^{th}$ DPC. Group 5 revealed mild depression, mild eye lesions, and mild respiratory distress at the 3rd DPC, which became moderate at the 5th-day post-challenge and still recorded till the 7th day DPC with 40 % MR with high protection percent than control group**.**

Postmortem examination of G2 revealed that presence of heamorrhage at proventriculus tips (Fig. 1A), inflammation and heamorrhage in cecal tonsils (Fig. 1B), as well as necrosis and ulcer in intestine (Fig. 1C), this lesion indicated virulent Newcastle disease virus infection agreed with (Capua and Alexander, 2009), this lesion not found in G3, G4 but found in mild form in G5.

### 3.2. Virus shedding following challenge with NDV at 35 days of Life

Birds in experimental groups (G3, G 4 and G5) showed undetected virus shedding at 3rd DPC. Viral shedding not detected in vaccinated birds treated with ACEO at 3rd, 5th and 7th DPC with vNDV. Treatment of unvaccinated chicks (G5) with ACEO onion extract led to the significant decrease of viral shedding ($p \leq 0.05$) compared with the control group, as shown in (Table 1).

### 3.3. Histopathological examination

Birds in group 1 (control –ve) showed standard structure and had no histopathological lesions from collected organs until the experiment end (Fig. 2A-Fd). While other examined groups (G1 to G5) showed various pathological lesions recorded as following:

#### 3.3.1. Spleen

Depletion of lymphocytes with multifocal coagulative necrosis (Fig. 3-a, Fig. 4-a and Fig. 5-a) were observed in G 2, G 3 and G 4, in addition to mild to moderately congested blood vessels and multifocal hemorrhage, especially in G 4. In contrast, G 5 showed typical architecture.

#### 3.3.2. Thymus

The thymus of G 2,3 and 5 exhibited moderate to severely congested blood vessels and hemorrhage in cortex and medulla (Fig. 3-b, Fig. 4-b and Fig. 6-b), while G3 showed mild depletion of thymocytes in medulla Figure (4-b).

#### 3.3.3. Proventriculus

The proventriculus of G 2, 3, 4, and 5 showed hemorrhage, congestion, edema and inflammatory cells infiltration in mucosa and submucosa with severe interglandular congestion and hemorrhage in villi (Fig. 3-c, Fig. 5-c and Fig. 6-c). The proventriculus of G 3 showed lymphocytes infiltration in the glandular layer (Fig. 4-c). Moreover, endotheliosis of blood vessels was shown in G 4 (Fig. 5-c).

#### 3.3.4. Cecal tonsil

Cecal tonsil of G 2 and G 5 showing hyperplasia of lining epithelium with activation of goblet cells, and hemorrhage in some villi, accompanied with cystic crypt containing necrotic tissue, and depletion of lymphocytes with granulocytes infiltration (Fig. 3-d, Fig. 6-d). G3 showed depletion of lymphocytes in lamina propria with severe focal necrosis (Fig. 4-d). Cecal tonsils of G 4 showing severe hemorrhage in lamina propria (Fig. 5-d).

#### 3.3.5. Trachea

The trachea of G 2, G 3, G 4 and G 5 showed hyperplasia of lining epithelium with the proliferation of mucous glands, and thickening of mucosa due to edema, severe congestion, especially in G 2 and lymphocytes infiltration as showed in (Fig. 3-e, Fig. 4-e, Fig. 5-e and 6-e).

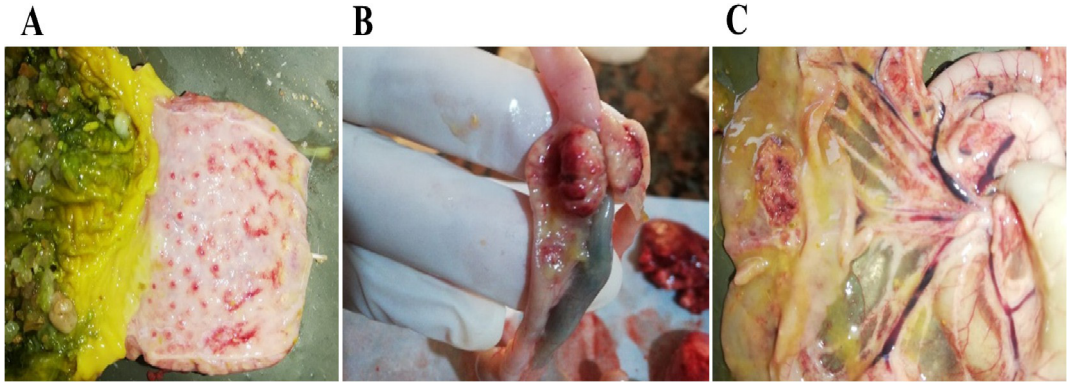


**Fig. 1.** Postmortem lesions in different organs of Cobb chicken after experimental infection with virulent NDv; A: showing heamorrhage at proventriculus glands tips at 7th dpc in challenged bird from group 2 (G2); B: showing inflammation and heamorrhage in cecal tonsils at 7th dpc in challenged bird from group 2 (G2); C: showing intestinal ulcer at 7th dpc in challenged bird from group 2 (G2).

**Table 1**
Virus shedding of experiment groups after challenged with NDV genotype VII accession no., MZ029052 **at 3rd, 5th and 7th DPC (Mean + SEM).**

| Group/ DPC | 3rd day post challenge (DPC) | | | 5th DPC | | | 7th DPC | | |
|---|---|---|---|---|---|---|---|---|---|
| | TS | % | CT value | TS | % | CT value | TS | % | CT value |
| G1 | 0/3 | 0% | 0.00 | 0/3 | 0% | 0.00 | 0/3 | 0% | 0.00 |
| G2 | 3/3 | 100% | 23.6 ± 0.48c | 3/3 | 100% | 20.0 ± 0.48c | 3/3 | 100% | 16.9 ± 0.40b |
| G3 | 0/3 | 0% | ND | 0/3 | 0% | ND | 0/3 | 0% | ND |
| G4 | 0/3 | 0% | ND | 2/3 | 66% | 38.0 ± 0.00a | 2/3 | 66% | 37.0 ± 0.58a |
| G5 | 0/3 | 0% | ND | 2/3 | 66% | 29.4 ± 0.00b | 2/3 | 66% | 35.5 ± 0.29a |

[a–b-] Mean values within the same row with different superscript letter are a statistically significant difference at ($p \leq 0.05$). **#** there is an inverse relationship between the CT value and the virus titer in the samples, the higher the CT, the lower the virus titer.

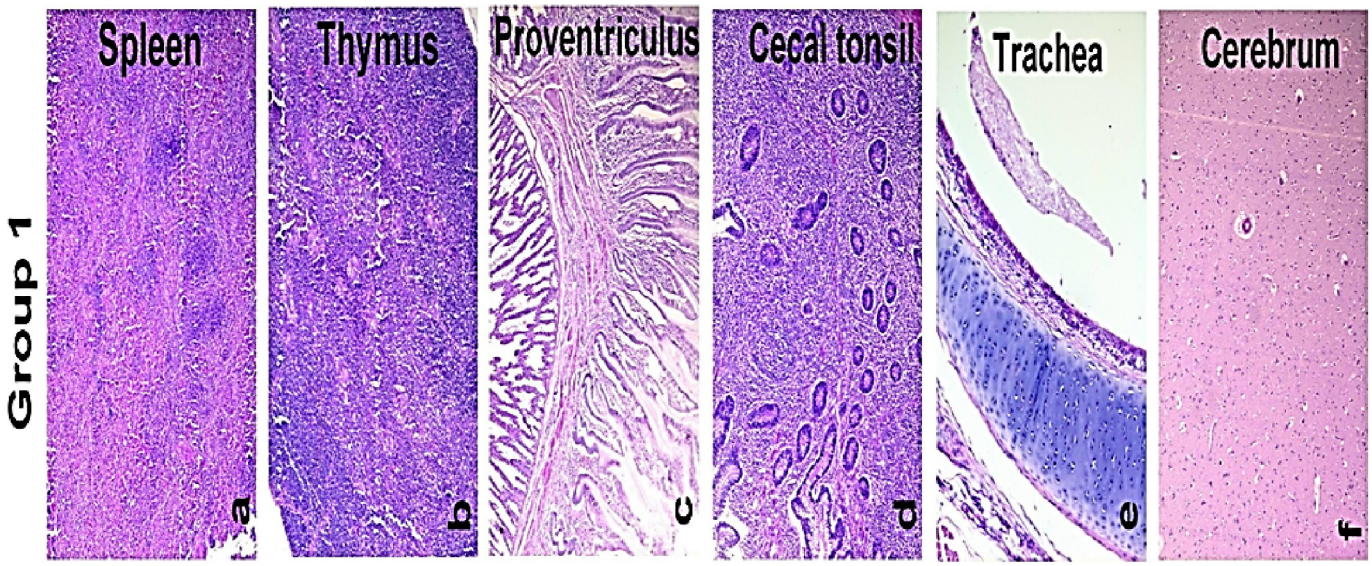


**Fig. 2.** Histopathological lesion of group 1; 1-a: Spleen showing apparently normal structures H&E X100. 1-b: Thymus showing apparently normal structures H&E X200; 1-c: Cecal tonsil showing apparently normal structures H&E X100. 1-d: Proventriculus showing apparently normal structures H&E X100. 1-e. trachea showing apparently normal structures H&E X200; 1-f Cerebrum showing apparently normal structures H&E X200.

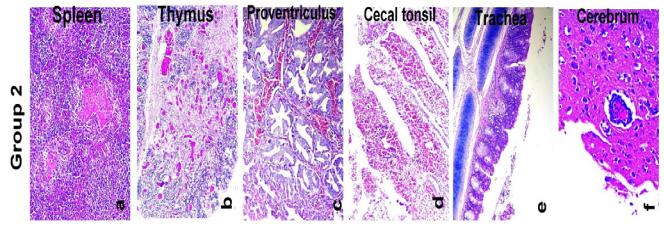


**Fig. 3.** Showed pathological lesions in G2 2-a: Spleen showing focal coagulative necrosis H&E X200. 2-b: Thymus showing severe congested blood vessels in cortex and medulla H&E X1002-c: Cecal tonsil showing hemorrhage in villi H&E X200. 2-d: Proventriculus showing severe intraglandular congestion H&E X200. 2-e: Trachea showing hyperplasia of lining epithelium with proliferation of mucous glands, and thickening of mucosa due to edema and lymphocytes infiltration H&E X200. 2-f: Cerebrum showing perivascular cuff, lymphocytes aggregation in Virchow's space bordered the blood vessels H&E X400.

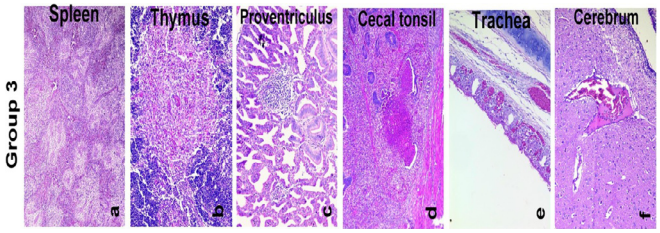


**Fig. 4.** Showed pathological lesions in G3 3-a: Spleen showing multifocal depletion of lymphocytes H&E X200. 3-b: Thymus showing depletion of thymocytes in medulla, congestion and granulocytes infiltration H&E X200. 3-c: cecal tonsil showing severe depletion of lymphocytes, hemorrhage with extensive necrosis H&E X100. 3-d: Proventriculus showing lymphocytic nodules in glandular layer H&E X200. 3-e: Trachea showing thickening of mucosa due to edema, congestion and mononuclear cells infiltration H&E X200. 3-f: Cerebrum showing congested blood vessels H&E X200.

### 3.3.6. Cerebrum

The cerebrum of G 2 showing perivascular cuff, lymphocytes aggregation in Virchow's space bordered the blood vessels (Fig. 3-f). Cerebrum G 4 and G 5 showed mild perivascular edema (Fig. 5-f, Fig. 6-f) with mild focal degeneration of neutrophils. Mildly congested blood vessels were shown in G 3 (Fig. 4-f).

### 3.4. Pathological lesion scores of infected groups at 7th DPC

There is a significant decrease ($p \leq 0.05$) in pathological lesion scoring in ACEO treated groups G3 and G5 compared with the control group (G2), especially lymphoid organs thymus, spleen, cecal tonsils and proventriculus beside brain tissue (cerebrum), indicating lowered NDV multiplication, effective antiviral and immune stimulant influences of ACEO onion extract against NDV experimental infection (Table 2).

### 3.5. Humeral immune response result (HI test)

In 1 day old, all groups HI assay revealed no significant ($p \leq 0.05$) difference between each other as they have the same

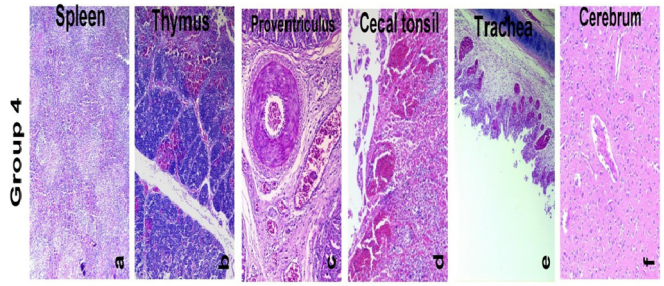


**Fig. 5.** Showed pathological lesions in G4. 4-a: Spleen showing multifocal hemorrhage and depletion of lymphocytes H&E X100. 4-b: Thymus showing multifocal hemorrhage in cortex and medulla H&E X100. 4-c: Cecal tonsils showing hemorrhage in lamina propria H&E X200. 4-d: Proventriculus showing congested blood vessels, endotheliosis and edema in submucosa H&E X200. 4-e: Trachea showing sloughed epithelium with congested blood vessels and edema and few inflammatory cells in lamina propria H&E X200. 4-f: Cerebrum showing mild congested blood vessels with perivascular edema H&E X200.

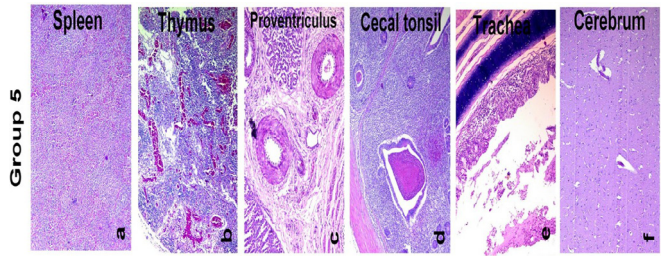


**Fig. 6.** Showed pathological lesions in G5. 5-a: Spleen showing apparently normal structures H&E X100. 5-b: Thymus showing severe congested blood vessels and hemorrhage with interstitial edema and pronounced depletion of thymocytes H&E X100. 5-c : Cecal tonsil showing showed hyperplasia of lining epithelium with proliferation of goblet cells, and dilatation of few crypts containing necrotic tissue H&E X100. 5-d: Proventriculus showing edema, congested blood vessels in submucosa H&E X200. 5-e: Trachea showing hyperplasia of lining epithelium with edema and mononuclear cells infiltration in lamina propria, with desquamated epithelium and inflammatory cells in the lumen H&E X100. 5-f: Cerebrum showing mild congested blood vessels with perivascular edema H&E X100.

maternal immunity, which begins to decrease till 2nd and 3rd week of age. From the 3rd to 5th weeks of age, the HI titer significantly increased ($p \leq 0.05$) In G3, G4 and G5 compared with control groups. There is no significant difference in HI titer in ACEO treated groups (G3 and G 5) compared with vaccinated groups (G4) at the 6th week of age (Table 3).

## 4. Discussion

Some viral diseases that infect animals cause great losses in production and economies of some countries (Swelum et al., 2020). The ND VII is the most prevalent in poultry farms in Egypt, causing heavy losses, despite of the intensity of the vaccine's programs, which forced the need for continuous evaluation and permanent search for natural plant alternatives to enhance bird's immunity and diseases resistance (El-Saadony et al., 2021). Onion is one of the most important of these plants and widely produced in Egypt which is used for this purpose. Economic losses resulted from NDV field infection due to clinical signs, pathological lesions and high mortalities after field challenge with NDV. In this study, vaccinated group (G4) and vaccinated treat group (G3) have mild clinical signs, postmortem lesions, and no mortalities (zero %). Miller et al. (2013) showed no symptoms appeared in the vaccinated birds except for some mild conjunctivitis. And these findings contradicted Landman et al. (2017) results which indicated that post vaccinal reactions that led to an increase in deaths and the emergence of respiratory symptoms. G5 and G3 treated with ACEO showed mild clinical signs, pathological lesions with low mortalities post NDV challenge compared with the untreated control group (G2) this may explained by a few of the health benefits of *Allium cepa* as it has anti-toxic, antibacterial effects, anticarcinogenic characteristics, anti-asthmatic, antithrombotic, antiplatelet activity, and the capacity to control detoxification systems (Griffiths et al., 2002; Armenta et al., 2015). Delayed mild clinical symptoms (as it appeared after 5th dpc when compared with G2) that quickly disappeared with complete recovery recorded in ACEO treated G3 indicating the antiviral, anti-inflammatory, and immune-modulatory effect of ACEO onion extract. Many studies previously recorded those essential oils contain potent anti-inflammatory properties that inhibit the metabolism of inflammatory prostaglandins (Krishan and Narang, 2014). These anti-inflammatory properties may explain the delayed onset and rapid disappearance of mild clinical signs and lesions post-NDV challenge in ACEO treated chickens. Also Peana et al. (2003) showed that essential oils have anti-inflammatory, pain-relieving, or edema-reducing properties, also Dorrigiv et al. (2021) showed that *Allum cepa* is a chelating agent with antioxidant effect, anti-inflammatory, apoptosis suppression effect and signaling pathways modulation activity, which may explain the significant decrease ($p \leq 0.05$) in pathological lesions scoring in ACEO treated groups G3 and G5 compared with the control group (G2), especially in lymphoid organs thymus, spleen, cecal tonsils and proventriculus beside brain tissue (cerebrum), indicating lowered NDV multiplication, antiviral and immune stimulant influence of ACEO onion extract against NDV experimental infection, these results

**Table 2**
Pathological lesion scores of infected group at 7th dpi. (Mean + SEM).

| Group/ organ | Spleen | Thymus | Trachea | Cecal tonsil | Proventriculus | Cerebrum |
|---|---|---|---|---|---|---|
| G1 | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{b}$ |
| G2 | 2.00 ± 0.00 $^{a}$ | 2.33 ± 0.33 $^{a}$ | 2.00 ± 0.00 $^{a}$ | 3.00 ± 0.00 $^{a}$ | 2.33 ± 0.33 $^{a}$ | 2.00 ± 0.00 $^{a}$ |
| G3 | 2.00 ± 0.00 $^{a}$ | 0.33 ± 0.33$^{b}$ | 1.67 ± 0.33 $^{a}$ | 0.00 ± 0.00$^{b}$ | 1.00 ± 0.00 $^{a,b,c}$ | 0.33 ± 0.33$^{b}$ |
| G4 | 1.00 ± 0.00$^{b}$ | 0.00 ± 0.00$^{b}$ | 1.67 ± 0.33 $^{a}$ | 0.00 ± 0.00$^{b}$ | 1.67 ± 0.33 $^{a,b}$ | 1.00 ± 0.58 $^{a,b}$ |
| G5 | 0.67 ± 0.33$^{b}$ | 0.00 ± 0.00$^{b}$ | 1.67 ± 0.33 $^{a}$ | 1.00 ± 0.58$^{b}$ | 0.67 ± 0.67$^{b,c}$ | 0.33 ± 0.33$^{b}$ |
| **Test Statistics** $^{a,b}$ | | | | | | |
| Chi-Square | 13.236 | 11.894 | 9.333 | 12.246 | 9.965 | 9.061 |
| Df | 4 | 4 | 4 | 4 | 4 | 4 |
| Asymp. Sig. | 0.010 | 0.018 | 0.053 | 0.016 | 0.041 | 0.060 |

$^{a–b-}$ Mean values within the same row with different superscript letter are a statistically significant difference at ($p \leq 0.05$). Kruskal Wallis test revealed a statistically significant difference (Asymp. Sig. = 0.010, 0.018, 0.053, 0.016, 0.041 for Spleen, Thymus, Trachea, Cecal Tonsils, and Proventriculus, respectively). The test revealed insignificant difference (Asymp. Sig. = 0.060) for Cerebrum histopathological scores. a. Kruskal Wallis Test; b. Grouping Variable: group

**Table 3**
HI antibody titer of infected group from 1 day old to 6 weeks old (Mean + SEM).

| G / days | 1 day | 1w | 2w | 3w | 4w | 5w | 6w |
|---|---|---|---|---|---|---|---|
| G1(-ve) | 7 ± 0.58$^{a}$ | 4.7 ± 0.33$^{a}$ | 1.7 ± 0.33$^{b}$ | 0.67 ± 0.33$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{b}$ | 0. 0 ± 0.00$^{d}$ |
| G2(+ve) | 7 ± 0.58$^{a}$ | 4.7 ± 0.33$^{a}$ | 1.7 ± 0.33$^{b}$ | 0.67 ± 0.33$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{b}$ | 8. 0 ± 0.58$^{a}$ |
| G3 | 7 ± 0.58$^{a}$ | 5 ± 0.00$^{a}$ | 3.7 ± 0.33$^{a}$ | 4.7 ± 0.88$^{a}$ | 6.0 ± 0.58$^{a}$ | 6. 0 ± 1.00$^{a}$ | 4. 0 ± 1.00$^{c}$ |
| G 4 | 7 ± 0.58$^{a}$ | 5 ± 0.00$^{a}$ | 3.7 ± 0.33$^{a}$ | 4.7 ± 0.88$^{a}$ | 5.3 ± 0.67$^{a}$ | 5. 7 ± 0.67$^{a}$ | 4.3 ± 0.33$^{b,c}$ |
| G5 | 7 ± 0.58$^{a}$ | 4.7 ± 0.33$^{a}$ | 1.7 ± 0.33$^{b}$ | 1.8 ± 0.33$^{b}$ | 2.3 ± 0.33$^{b}$ | 1. 0 ± 0.58$^{b}$ | 6. 0 ± 0.58$^{b}$ |

$^{a–b–c}$ Mean values within the same row with different superscript letter are a statistically significant difference at ($p \leq 0.05$). **SEM**: Standard Error of Mean.

agreed with the result of Harazem et al. (2019) reported Onion extracts were effective in decreasing infection of Newcastle Disease virus by blocking of the receptors and prevent the attachment of the virus with the host cell. Administration of ACEO orally in G3 and G5 promote chicken immune response with a significant increase ($p < 0.05$) in HI titer more than the control group. Rahman et al. (2019) showed that dietary supplementation of *Allium cepa* L, had a significant positive effect ($p < 0.05$) on antibodies titer against NDV virus, Infectious Bronchitis (IB), and Infectious Bursal Disease (IBD) in all treated groups. Onion extract ACEO treatment increase the HI titer in G3 and G5 compared with control groups with low MR and high protection percent (100 % and 60 %), respectively, after vNDV challenge. In the last decade, many studies highlighted the use of essential oils in conjunction with vaccination programs, including infectious bronchitis (IB), Newcastle disease, and infectious bursal disease. These studies show that essential oils plant extract promotes antibodies production and enhances bird immune response and efficacy of vaccination regimen (Faramarzi et al., 2013). The phenolic-rich onion extract (*Allium cepa L.*) can improve the birds' welfare, antioxidant enzymes activity, and immune status (Omar et al., 2020). Some essential oils positively influence the avian immune system since they promote the production of immunoglobulins (Alagawany et al., 2021; El-Tarabily et al., 2021), enhance lymphocytic activity, and activate interferon-γ release (Gopi et al., 2014; Krishan and Narang, 2014). The results of Raza et al. (2015) explained that herbal medicine plants might combat viral infections due to their immunostimulatory properties; numerous plants enhance the host's immune system increase their effective antiviral defenses.

## 5. Conclusion

Oral administration of ACEO onion extract alone and/or sequentially with NDv vaccines can reduce or eliminate the lethal clinical signs, pathological lesions, enhance chicken immune response and protection percent, proving the natural antiviral and immune stimulant effects. Administration of onion extract orally removes or reduces the destructive NDv symptoms and lesions, especially in lymphoid organs and brain tissue, with low mortalities. Implementing ACEO consecutively with NDV vaccines significantly decreases NDV shedding, reducing the epidemiological ND viral load in the environment and effective disease control in endemic areas.

## CRediT authorship contribution statement

**Mohamed Lebdah:** Conceptualization, Data curation, Writing – review & editing. **Laila Tantawy:** Conceptualization, Data curation, Formal analysis, Resources. **Abdelbaset M. Elgamal:** Data curation, Formal analysis, Methodology. **Adel M. Abdelaziz:** Data curation, Methodology. **Nahed Yehia:** Conceptualization, Data curation, Methodology, Resources. **Amal A. Alyamani:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Ameina S. ALmoshadak:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Mai Elsayed Mohamed:** Conceptualization, Data curation, Methodology, Resources.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors gratefully acknowledge the Department of Avian and Rabbit Medicine, Faulty of Veterinary Medicine, Zagazig University, Egypt, Poultry Diseases Department, Animal Health Research Institute (AHRI) and, Quality control on Poultry Production (RLQP), Agriculture Research Center (ARC), Giza 12618, Egypt, for technical and scientific support.

## References

Abo-Zeid, 1989. Effect of some Egyptian native plant extracts on Newcastle disease virus vaccine. Ph.D. thesis; Assuit University. Egypt.
Alagawany, M., El-Saadony, M.T., Elnesr, S.S., Farahat, M., Attia, G., Madkour, M., Reda, F.M., 2021. Use of lemongrass essential oil as a feed additive in quail's nutrition: its effect on growth, carcass, blood biochemistry, antioxidant and immunological indices, digestive enzymes and intestinal microbiota. Poult. Sci. 100 (6), 1011172.
Amer, S., Maatouq, A., Ahmed, H., Hassan, E., 2020. Evaluation for efficacy of commercially available vaccines against challenge with Newcastle disease virus genotype VII in broilers. Egyptian J. Vet. Sci. 51 (1), 35–41.
Armenta, V. F., Valenzuela- cruz, R.M., Ayala-Zavala, F. J., 2015. Onion (Allium cepa) Essential Oils. http:///doi.org/10.1016/B978-0-12-416641-7.00070-5
Bancroft, J. D, Layton, C., 2013. The Hematoxylin and eosin. In: Suvarna S. K, Layton C, Bancroft J. D, editors. Theory Practice of histological techniques. 7th ed. Ch. 10 and 11. Philadelphia: Churchill Livingstone of El Sevier, pp. 179– 220. http://dx.doi.org/10.1016/b978-0-7020-4226-3.00010-x.
Capua, I., Alexander, D.J. (Eds.), 2009. Avian Influenza and Newcastle Disease. Springer Milan, Milano.
Chen, C.H., Chou, T.W., Cheng, L.H, Ho, C.W., 2011. In vitro anti-adenoviral activity of five Allium plants. Journal of the Taiwan Institute of Chemical Engineers 42 (2), 228–232.
Dorrigiv, M., Zareiyan, A., Hosseinzadeh, H., 2021. Onion (*Allium cepa*) and its Main Constituents as Antidotes or Protective Agents against Natural or Chemical Toxicities: A Comprehensive Review. Iran. J. Pharm. Sci. IJPR 20 (1), 3–26. https://doi.org/10.22037/ijpr.2020.112773.13940.
Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics 11 (1), 1. https://doi.org/10.2307/3001478.
Ebesunun, M.O., Popoola, O.O., Agbedana, E.O., Olisekodiaka, J.M., Onuegbu, J.A., Onyeagala, A.A., 2007. The effect of garlic on plasma l ipids and lipoproteins in rats fed on high cholesterol enriched diet. Biokemistri 19 (2), 53–58.
El-Saadony, M.T, Zabermawi, N.M., Burollus, M.A., Shafi, M.E., Alagawany, M., Abd El-Hack, M.E., 2021. Nutritional aspects and health benefits of bioactive plant compounds against infectious diseases: A review. Food Rev. Int., 1–23
El-Tarabily, K.A., El-Saadony, M.T., Alagawany, M., Arif, M., Batiha, G.E., Khafaga, A.F., Abd El-Hack, M.E., 2021. Using essential oils to overcome bacterial biofilm formation and their antimicrobial resistance. Saudi J. Biol. Sci. 9 (28), 5145–5156.
Faramarzi, S., Bozorgmehrifard, M., Khaki, A., Moomivand, H., Ezati, M., Rasoulinezhad, S., Bahnamiri, A., Dizaji, B.R., 2013. Study on the effect of *Thymus vulgaris* essential oil on humoral immunity and performance of broiler chickens after La Sota vaccination. Ann. Biol. Res. 4, 290–294.

Goodarzi, M., Landy, N., Nanekarani, S., 2013. Effect of onion (*Allium cepa* L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks. Health 5, 1210.

Gopi, M., Karthik, K., Manjunathachar, H.V., Tamilmahan, P., Kesavan, M., Dashprakash, M., Balaraju, B.L., Purushothaman, M., 2014. Essential oils as a feed additive in poultry nutrition. Adv. Anim. Vet. Sci. 2, 1–7.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., Smith, B., 2002. Onions–a global benefit to health. Phytother. Res. PTR 16 (7), 603–615. https://doi.org/10.1002/(ISSN)1099-157310.1002/ptr.v16:710.1002/ptr.1222.

Harazem, R., Rahman, S., Kenawy, A., 2019. Evaluation of antiviral activity of *Allium Cepa* and *Allium Sativum* extracts against Newcastle disease virus. Alex. J. Vet. Sci. 61 (1), 108. https://doi.org/10.5455/ajvs.10.5455/ajvs.29663.

Jafari, B., Rezaie, A., Ebadi, A., Ghiamirad, M., Ahmadizadeh, C., 2011. Evaluation of medicinal plant oil (*Teucrium polium*) in diets of broilers. J. Appl. Environ. Biol. Sci 1, 583–586.

Krishan, G., Narang, A., 2014. Use of essential oils in poultry nutrition: A new approach. J. Adv. Vet. Anim. Res. 1 (4), 156. https://doi.org/10.5455/javar.10.5455/javar.2014.a36.

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. Sci. World J. 2013, 162750.

Landman, W.J.M., Vervaet, C., Remon, J.P., Huyge, K., van Eck, J.H.H., 2017. Primary Newcastle disease vaccination of broilers: comparison of the antibody seroresponse and adverse vaccinal reaction after eye–nose drop or coarse spray application, and implication of the results for a previously developed coarse dry powder vaccine. Avian Pathol. 46 (4), 451–461.

Miller, P.J., Afonso, C.L., El Attrache, J., Dorsey, K.M., Courtney, S.C., Guo, Z., Kapczynski, D.R., 2013. Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. Dev. Comp. Immunol. 41 (4), 505–513.

Nadia, M., El-Sabbagh, A., El-Assily, S., Khashaba, E., El-Bordini, F., El-Ebiary, A., Taha, M., 1991. Virucidal effect of Garlic and Onion oily extracts on different strains of Newcastle disease virus. Beni-Suef Vet. Med. 1, 1.

OIE, 2015. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals-Avian Influenza. In OIE Terrestrial Manual 2015; OIE: Rome, Italy, 2015.

Omar, A.E., Al-Khalaifah, H.S., Mohamed, W.A., Gharib, H.S., Osman, A., Al-Gabri, N. A., Amer, S.A., 2020. Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behavior, intestinal histology, amino acid digestibility, antioxidant activity, and the immune status of broiler chickens. Front. Vet. Sci. 7, 728.

Peana, A.T., D'Aquila, P.S., Chessa, M.L., Moretti, M.D.L., Serra, G., Pippia, P., 2003. Linalool produces antinociception in two experimental models of pain. Eur. J. Pharmacol. 460 (1), 37–41.

Rahman, S., Khan, S., Imtiaz, N., Siddique, U., Sultan, A., Rahim, F., ur Rahman, F., 2019. Phytomedicine (Allium cepa L.) effect on broiler immunity against infectious diseases. Biomed. Lett. 5, 1–7.

Raza, A., Muhammad, F., Bashir, S., Anwar, M.I., Awais, M.M., Akhtar, M., Aslam, B., Khaliq, T., Naseer, M.U., 2015. Antiviral and immune boosting activities of different medicinal plants against Newcastle disease virus in poultry. World's Poult. Sci. J. 71 (3), 523–532.

Rima, B., Balkema-Buschmann, A., Dundon, W., Duprex, P., Easton, A., Fouchier, R., Kurath, G., Lamb, R., Lee, B., Rota, P., 2019. ICTV Report Consortium. 2019. ICTV virus taxonomy profile: Paramyxoviridae. J. Gen. Virol. 100, 1593–1594.

Shahzad, K., Nawaz, S., Ahmad, R., Mahmud, S., Iqbal, Z., Saeed, K., 2009. Evaluation of antioxidant and antimicrobial activity of essential oil of Tangerine fruit peel. Pak. J. Biochem. Mol. Bio. 42, 4–7.

Swelum, A.A., Shafi, M.E., Albaqami, N.M., El-Saadony, M.T., Elsify, A., Abdo, M., Mohamed, E., 2020. COVID-19 in human, animal, and environment: a review. Front. Vet. Sci. 7, 578.

Wise, M.G., Suarez, D.L., Seal, B.S., Pedersen, J.C., Senne, D.A., King, D.J., Kapczynski, D.R., Spackman, E., 2004. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. J. Clin. Microbiol. 42 (1), 329–338.

Zandi, K., Teoh, B.-T., Sam, S.-S., Wong, P.-F., Mustafa, M.R., AbuBakar, S., 2011. Antiviral activity of four types of bioflavonoid against dengue virus type-2. Virol. J. 8, 1–11.