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Highlight on Propolis-Pollen Nanoemulsion Effect on Some Avian Viruses on Chicken Embryo Eggs

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Abstract

THIS STUDY investigates the antiviral potential of Propolis-pollen nanoemulsion (PP-NE) against four major poultry viruses: H5N8 avian influenza (H5), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV). PP-Ne were prepared using a phase titration approach and registered under Egyptian Academy of Scientific Research number EG/P/2024/653. The nanoparticles were characterized by LC-MS/MS and transmission electron microscopy (TEM), revealing a spherical shape with an average size of 13.34±2.102 nm. The cytotoxicity assessment using Vero cells and Sulforhodamine B (SRB) stain indicated an IC50 of 23.19 µg/mL. Phenolic compounds, including curcumadiol and methyl oleate, were identified as key components.To assess the antiviral efficacy, virus-nanoparticle mixtures were incubated at 37°C for 2, 4, 8, and 24 hours before inoculating specific pathogen-free embryonated chicken eggs (SPF-ECE). Viral activity was monitored using real-time quantitative reverse transcription PCR (qRT-PCR) tests. The results demonstrated significant inhibition of viral replication for all tested viruses. H5N8 after 8 hours ($p = 0.009$), IBDV after 24 hours ($p = 0.002192$), NDV after 4 hours ($p = 0.004106$), and IBV showed complete inhibition after 24 hours ($p = 0.000255$) .These findings highlight the potential of PP-NE as an effective antiviral agent in veterinary medicine, warranting further research into their application for controlling viral infections in poultry.

Keywords: Propolis-pollen nanoemulsion (PP-NE); Avian influenza (H5N8); Newcastle disease virus (NDV); Infectious bronchitis virus (IBV); Infectious bursal disease virus (IBDV); Phase titration; LC-MS/MS.

Introduction

Viruses are ubiquitous pathogens that pose significant threats to both human and animal health. The preparation, propagation, and genetic characterization of virus reference strains are critical steps in virological research, diagnostic assay

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development, and vaccine production [1].This study focuses on four significant viruses that pose major threats to poultry health and productivity: H5N8 avian influenza (AI), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV). Each of these viruses has distinct characteristics and impacts on the poultry industry, making their study crucial for disease control and prevention [2].

H5N8 (AI) is a highly pathogenic strain of the influenza virus that has caused severe outbreaks in poultry populations in Egypt [3] and worldwide. It is characterized by high mortality rates in affected flocks and can lead to significant economic losses due to culling and trade restrictions. The virus can also pose a zoonotic risk, although human infections are rare [4]. NDV is another highly contagious virus that affects a wide range of bird species. It can cause a variety of clinical signs, from mild respiratory symptoms to severe neurological disease and high mortality. NDV is classified into different genotypes, with Genotype 7 (G7) being one of the more recent and virulent strains that have emerged, causing significant concerns for poultry health [5].

The IBV is a coronavirus that primarily targets the respiratory tract of chickens but can also affect the reproductive and renal systems. It is highly contagious and can spread rapidly within flocks, leading to reduced egg production, poor egg quality, and increased susceptibility to secondary infections. The virus exists in numerous serotypes, which complicates vaccine development and disease control efforts [6].

IBDV is an immunosuppressive virus that targets the bursa of Fabricius, an essential organ for the development of the chicken's immune system. Infection with IBDV can lead to immunosuppression, making birds more susceptible to other diseases. The virus is particularly devastating in young chickens and can cause high mortality rates [7].

Pollen, the male reproductive unit of seed plants, is a microscopic marvel essential for plant propagation [8].Produced within the anther of a flower, pollen grains exhibit an astounding diversity in size, shape, and surface ornamentation, reflecting the evolutionary adaptations of different plant species. Encased in a robust outer layer composed primarily of sporopollenin, pollen grains are remarkably resilient structures [9].

Beyond its pivotal role in fertilization, pollen has captured the fascination of scientists across disciplines. The intricate details of pollen morphology offer invaluable insights into plant evolution, taxonomy, and ecology [10]. Additionally, the analysis of pollen grains, known as palynology, has become a cornerstone in fields such as archaeology, climatology, and forensic science. From its fundamental role in plant life cycles to its far-reaching applications in various scientific endeavors, pollen continues to be a subject of enduring interest and exploration [11].

In recent years, the emergence of nanotechnology has opened new avenues for developing innovative antiviral strategies. One such innovation is the preparation and characterization of Propolis-based nanoparticles (PP-NPs). Propolis, a resinous substance collected by honeybees from various plant sources, has been extensively studied for its wide range of biological activities, including antimicrobial [12] , anti-inflammatory, and antioxidant properties [13].In recent years, the focus has shifted towards enhancing the bioavailability and efficacy of propolis through nanotechnology [14]. PP-NPs represent a novel approach in the field of antiviral research, combining the natural therapeutic properties of propolis with the advantages of nanotechnology [15].

Nanoparticles have unique physicochemical properties, such as a high surface area-to-volume ratio, that can enhance the delivery and effectiveness of bioactive compounds [16].When incorporated into nanoparticles by pollen, propolis can achieve improved stability, controlled release, and targeted delivery to infected cells. These properties make PP-NE a promising candidate for antiviral applications.

Understanding these viruses' molecular biology, pathogenicity, and epidemiology is essential for developing effective vaccines, diagnostic tools, and control measures. This study aims to advance our knowledge of these pathogens and explore novel antiviral strategies, such as the application of propolis-based nanoparticles, to mitigate their impact on poultry health.

Material and Methods

Viruses' preparation and designation:

Reference strains of the viruses under investigation have been isolated, propagated, and tittered in Reference laboratory for veterinary quality control of poultry production, according to OIE manual of each virus under investigation [17].The reference strains have been genetically characterized and designated on Gene bank (NCBI), under the following designation: H_5N_8 (AI) A/ chicken /Egypt / A2/2021 (with accession no. OK160062, NDV (Egypt/NDV/RlQP/2021) c MZ409479, IBV (IBV/EGY/RLQP /CH/CV41/2021) with accession no. OM621909 and IBDV (EGYPT-IBD-RLQP-2021) with accession no. MZ409478

Preparation and Characterization of PP-NE:

The phase titration approach was used in the Nanomaterials Research and Synthesis Unit to prepare PP-NE, according to the changes described by Sorour [18]. (Patient registering with the Egyptian Academy of Scientific Research under number EG/P/2024/653).

PP-NE was characterized using LC–MS/MS in Nawah Company and the JEOL JSM-1400 transmission electron microscopy (TEM) model.

African green monkey kidney cells (Vero cells) were used to test the cytotoxicity of PP-NE. These cells were acquired from the source and cytotoxicity test evaluation by using Sulforhodamine B (SRB) assay [19].

Cytotoxicity assay of PP-NE in SPF ECE

Two-fold serial dilution (3 dilutions) of PP-NE using filtrated phosphate buffer saline. Inoculation of origin and each dilution in 5 SPF ECE. Incubation at 37^{o}C for 5 days with daily checks and recording of daily mortalities. Evaluation of cytotoxicity and dilution selection will be used in the experimental trial.

Assessment of the Antiviral effect of the PP-NE:

The 4 viruses under investigation have been incubated invitro with the PP-NE material as the protocol that was illustrated in Fig. (1). Briefly, 100ul of EID50=104 of each virus has been mixed with 100ul of the prepared PP-NE with concentration (20%) and incubated at 37°C for four-time points as 2, 4, 8, and 24 hours. The incubated mixtures at each time point were inoculated in 5 specific pathogen-free embryonated chicken eggs (SPF-ECE), then incubated at 37°C for 5 days with daily monitoring. Reference strains of the viruses have been inoculated in 5 eggs as positive controls; however, the negative control groups have been inoculated with sterile PBS 1%.

Real-time RT-qPCR for evaluation of virus replication:

The harvested allantoic fluid and the chick embryo chorioallantoic membrane (CAM) from the inoculated eggs have been prepared for viral RNA extraction following the work instruction of Patho Gene-spin™ DNA/RNA Extraction Kit – iNtron – cat no. 17154, then the extracted RNAs were examined for viruses by real-time RT-qPCR, following the work instruction of TransScript® Probe One-Step RT-qPCR SuperMix – Trans – cat. No. AQ221-01. The specific primers and probes used for the virus detection were referred to [20-23].

Statistical analysis:

The significance of the virus dynamic replication has been determined according to the p-value of the ANOVA-single-way test.

Results

Preparation and Characterization of PP-NE:

The size distribution of PP-NE was limited, measuring 13.34±2.102 nm. The results of the HRTEM investigation verified the spherical shape, size homogeneity, and lack of aggregation.

Viability Assessment (SRB Assay):

Viability Assessment (SRB Assay): The impact of PP-NE on Vero cell viability varied with concentration. The value of IC_{50} was found to be 23.19 µg/mL, as shown in supplementary fig.1

Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) analysis of nanoemulsion revealed common presence of phenolic compounds such as curcumadiol (11.5%), Methyl oleate (27.11%), Phenolic acids (3.39%), 10,12-Pentacosadiynoic acid (3.95%), Eupatorin (1.93%) and Quinindoline (1.57%).

Cytotoxicity assay of PP-NE in SPF ECE

We used a chicken embryo model to evaluate the developmental toxicity of four dosages of PP-NE. An untreated group receiving an injection of PBS solution served as the negative control group. Except for the first dose of 1/5, which resulted in a mortality rate of chicken embryos following exposure on the second and third days, no death or malformed embryos were detected in any of the doses (Table 1).

Antiviral efficacy of the PP-NE:

The study investigated the antiviral effect of PP-NE on four significant poultry viruses IBV, HPAI-H5N8, IBD, and NDV. The number of positive embryonated chicken eggs (ECE) after inoculation with the virus-nanoparticle mixtures was assessed at four different incubation times: 2 hours, 4 hours, 8 hours, and 24 hours. The viral activity in the allantoic fluid was confirmed using real-time quantitative reverse transcription PCR (qRT-PCR).

-On IBV:

The nanomaterial revealed a strong effect on IBV, which started after 4 incubation hours at 37°C. The longer incubation time revealed reduced virus replication ability for the inoculated eggs. All the inoculated eggs after 24 hr invitro incubation for virus / PP-NE mixture were negative for virus amplification, showing a significant reduction in virus replication with p-value = 0.000255, as shown in Fig.(4).

A long incubation time reduced the number of positive inoculated treated eggs, as shown in Table (2). The treated eggs showed no positivity after 24 hours of incubation, as shown in Fig. (2).

Comparing the cytopathic effect of the IBV on the treated and non-treated infected eggs, we found that the treated group showed normal size and postured embryo, however, the non-treated infected eggs showed the characteristic cytopathic effect of the IBV which is curling and dwarfing embryo, as shown in supplementary fig.1

On H5N8:

The results of the real-time RT-qPCR revealed a remarkably significant reduction in virus copy log after 8 hours of incubation at 37c, with P-value=0.009. Accordingly, these results indicate that the nanoparticle material has hindered the virus replication after 8 hours of treatment. Although there is a reduction in the concentration of the treated eggs, there is no complete hindrance to the virus replication in the treated eggs through time, as shown in Table (2) and Fig. (3)

On IBD:

The effect of the PP-NE started after 4 hours of incubation at 37c and revealed a remarkable reduction after 24 hours at 37c, with a significant p-value = 0.002192, as shown in Fig (4). The virus replication in the treated eggs has been reduced and stopped after 8 hours of incubation with 60% of the total treated eggs, as shown in Table (2).

The cytopathic effect of IBDV was compatible with the results of the RT-qPCR, as the non-treated infected eggs showed haemorrhage on embryos with greenish liver comparable with the treated group that showed less lesion severity, as shown in supplementary Fig. 3

On NDV:

The real-time RT-PCR revealed a remarkable reduction in virus log titer after 4 hours of in-vitro incubation at 37°C. The same as 4 hours of incubation, the virus log titer reduced after 8 hours of incubation. As expected, the incubation time of 24 hours resulted in a significant reduction in virus replication, with P-value= 0.004106, as shown in Fig. 5 and Table 2.

Effect of PP-NE on different viruses by time

At the 2-hour mark, all tested viruses maintained their infectivity, as evidenced by the positive results in all inoculated eggs. This indicates that the PP-NE did not exhibit immediate antiviral activity under these conditions.

After 4 hours of incubation, a slight reduction in positive eggs was observed for IBV, IBD, and NDV, with one egg showing no signs of infection for each virus. However, all eggs remained positive for H5, indicating a potential resistance to the nanoparticle treatment at this incubation period.

At 8 hours, a notable decline in the number of positive eggs was observed, especially for IBV, where only two eggs remained positive. H5 remained unaffected, suggesting its relative stability and resistance to PP-NE treatment even during this extended incubation period. The reduction in positive eggs for IBD and NDV indicates a partial antiviral effect of the nanoparticles.

After 24 hours of incubation, the PP-NE showed a complete antiviral effect against IBV, with no positive eggs detected. For H5, four out of five eggs remained positive, indicating some degree of resistance to the nanoparticle treatment. The number of positive eggs for both IBD and NDV was reduced to two, demonstrating the nanoparticles' partial effectiveness against these viruses at prolonged incubation times.

Discussion

The PP-NE was prepared using a phase titration approach, resulting in a highly consistent and narrowly distributed particle size. The average size of the PP-NE was measured to be 13.34±2.102 nm. HRTEM confirmed the spherical shape, size homogeneity, and absence of aggregation, indicating a well-dispersed nanoparticle formulation. The small size and uniform distribution are critical factors that likely enhance the bioavailability and cellular uptake of the nanoemulsion, which can contribute to its biological efficacy [24].

The cytotoxicity of PP-NE was evaluated using the Sulforhodamine B (SRB) assay, a standard method for assessing cell viability. The assay results showed that the impact of PP-NE on Vero cell viability was concentration-dependent, with an IC_{50} value of 23.19 µg/mL. This IC_{50} value indicates the concentration at which 50% of the cells remain viable and suggests that PP-NE has moderate cytotoxicity. This level of cytotoxicity is important for potential therapeutic applications [25], as it suggests that PP-NE can be used at concentrations that are effective against viruses without causing significant harm to host cells.

LC-MS/MS Analysis

The LC-MS/MS analysis of the nanoemulsion provided a detailed composition profile, revealing the presence of various phenolic compounds. The major components identified included: Curcumadiol (11.5%): A bioactive compound known for its antioxidant and anti-inflammatory properties [26].Methyl oleate (27.11%): A fatty acid ester with potential antimicrobial activity. Phenolic acids (3.39%): Known for their antiviral and antioxidant properties [27].10,12-Pentacosadiynoic acid (3.95%): A compound with potential antiviral properties [28].Eupatorin (1.93%): A flavonoid with reported anti-inflammatory and anticancer effects [29].Quinindoline (1.57%): An alkaloid with potential pharmacological properties [30].

The diverse range of phenolic compounds present in the PP-NE contributes to its potential antiviral activity. These compounds can interact with viral proteins and host cell receptors, thereby inhibiting various stages of the viral life cycle, such as viral entry, replication, and assembly [31].

The study demonstrated a significant antiviral effect of PP-NE on IBV. The efficacy of PP-NE was evident as early as 4 hours after incubation at 37°C, with a notable reduction in viral replication. The longer incubation periods further enhanced this effect, culminating in a complete inhibition of IBV replication after 24 hours, as evidenced by the absence of viral amplification in all inoculated eggs. At the 4-hour mark, the PP-NE showed a strong inhibitory effect on IBV replication. The presence of viral activity was significantly reduced, indicating that the nanoparticles began exerting their antiviral properties early during the incubation period. This early reduction in viral replication suggests that PP-NE can rapidly interact with viral particles or interfere with the virus's ability to infect host cells. After 24 hours of incubation, all the inoculated eggs tested negative for IBV amplification. This complete inhibition indicates that PP-NE effectively neutralized the virus, preventing its replication. The significant reduction in virus replication was statistically confirmed, with a p-value of 0.000255, underscoring the robustness of the antiviral activity of PP-NE. This result suggests that the longer the virus is exposed to the nanoparticles, the more effective the nanoparticles are at reducing viral infectivity.

The significant reduction in IBV replication highlights the potential of PP-NE as an effective antiviral agent. This finding is particularly important for the poultry industry, where IBV poses a significant threat to chicken health and production. The ability of PP-NE to completely inhibit IBV replication after 24 hours of exposure suggests a promising alternative or complementary treatment to existing antiviral drugs and vaccines.

The study's results indicate that PP-NE exhibits significant antiviral activity against the H_5N_8 strain of avian influenza virus.

This conclusion is supported by real-time quantitative reverse transcription PCR (RT-qPCR) data, which showed a marked reduction in viral RNA copies after 8 hours of incubation at 37°C. The observed decrease in viral load, as evidenced by the increased cycle threshold (Ct) values, highlights the nanoparticles' effectiveness in inhibiting viral replication. The reduction in viral load may be returned to Ct value increase in RT-qPCR is inversely proportional to the amount of target nucleic acid in the sample. A higher Ct value indicates a lower quantity of viral RNA, suggesting that the viral replication was inhibited. The study reported a significant reduction in virus copy log after 8 hours of treatment with PP-NE, with a P-value of 0.009. This statistical significance confirms that the observed reduction was not due to random chance and highlights the effectiveness of the nanoparticles in reducing viral load.

Time-Dependent Efficacy: The fact that a significant reduction was observed specifically after 8 hours suggests that the antiviral effects of PP-NE may be time-dependent. The nanoparticles likely require a certain period to exert their maximum antiviral activity, either by directly interacting with the virus or interfering with the viral replication cycle.

Clinical and Practical Implications, the ability of PP-NE to significantly reduce H_5N_8 viral load after 8 hours of incubation suggests a promising therapeutic potential. Given the public health threat posed by avian influenza viruses, especially those with zoonotic potential, developing new antiviral agents like PP-NE is crucial. These nanoparticles could potentially serve as an adjunctive treatment alongside existing antiviral drugs and vaccines, providing a complementary mechanism of action that could help mitigate the spread and severity of influenza outbreaks [32].

That may be returned to direct Inactivation: The nanoparticles may interact directly with viral particles, disrupting the viral envelope or capsid and thereby inactivating the virus [33].Inhibition of Viral Entry: PP-NPs might block the attachment of the virus to host cell receptors, preventing the initial stage of infection [34]. Interference with Viral Replication: The bioactive compounds in propolis, such as phenolic acids and flavonoids, may interfere with viral replication machinery, reducing the synthesis of viral RNA and proteins.

The effect of PP-NE on IBDV replication became noticeable after 4 hours of incubation at 37°C. The Ct values in real-time PCR indicated a further and more significant reduction after 24 hours of incubation, demonstrating a time-dependent antiviral effect. The significant reduction in viral load, with a p-value of 0.002192, suggests that the nanoparticles effectively inhibit the virus's ability to replicate over time [35].

The delay in the noticeable reduction of viral RNA until after 4 hours might indicate that the PP-NE requires time to accumulate around or interact with the virus, potentially disrupting its structure or function. The observed reduction after 24 hours could result from the nanoparticles interfering with the virus's replication machinery, possibly by binding to viral proteins or RNA, thereby preventing the synthesis of new viral particles [36].

The ability of PP-NE to significantly reduce IBDV replication could be of great benefit in controlling this disease, which is known to cause immunosuppression in poultry, leading to secondary infections and economic losses. The nanoparticles' efficacy suggests they could be used as a therapeutic agent to manage IBDV infections.

The study showed that PP-NE significantly reduced NDV replication within 4 hours of incubation, as evidenced by the decrease in virus log titer. This effect persisted and intensified after 8 hours, and by 24 hours, a notable reduction in viral replication was observed, with a p-value of 0.004106. The rapid onset of the antiviral effect suggests that PP-NPs may act quickly to inhibit viral processes [37].

The rapid reduction in viral load could be due to PP-NE directly interacting with the NDV particles, potentially destabilizing the viral envelope or interfering with receptor-binding sites, thus preventing viral entry into host cells. The continued reduction in viral replication over time suggests that PP-NPs might also impact the virus's ability to replicate inside host cells, possibly by interfering with the viral RNA synthesis or assembly of new virions [38-39].

NDV is a highly contagious virus with significant implications for the poultry industry [40].The demonstrated efficacy of PP-NE in reducing NDV replication suggests they could serve as a novel antiviral strategy, either as a standalone treatment or in combination with existing vaccines and antiviral agents. This could help mitigate the spread of the virus and reduce the severity of outbreaks.

Conclusion

The study successfully demonstrated the antiviral efficacy of PP-NE against four significant poultry viruses: H_5N_8 avian influenza (H_5), NDV, IBV, and IBDV. In vitro experiments showed that PP-NE significantly inhibited viral replication across all tested viruses, with a marked reduction in viral load observed through qRT-PCR. The treatment time required for virus inhibition varied among the viruses, with the replication of IBV showing significant suppression after 24 hours, H_5N_8 after 8 hours, IBDV after 24 hours, and NDV after 4 hours of incubation with the nanoparticles. These findings indicate that PP-NE possesses broad-spectrum antiviral properties, making them a promising candidate for controlling viral infections in poultry. Furthermore, the cytotoxicity assessment confirmed that PP-NE has an acceptable safety profile, with an IC_{50} of 23.19 µg/mL in Vero cells.

This study could be considered preliminary for the prominence of the promising role of PP-NE as a natural broad-spectrum antiviral material.

Article Information

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Conflicts of Interest: The authors have declared that they do not have any conflicting interests.

Authors’ contribution: All authors were responsible for designing the experiment. DMAE conducted the synthesis and characterization of nanomaterials, and DS and DO performed the experiment and collected the data. While DME and AA worked and verified the numerical results by PCR. AA was responsible for the primers selected and the analysis of the results. All authors writing of the manuscript. ZR, EMAH, and MS aided in interpreting the results. All authors discussed the results and commented on the manuscript. Each author contributed to their unique section of the work. All authors have reviewed and endorsed the final manuscript.

Ethical of approval:

According to the World Organization for Animal Health (OIE) and the Eighth Edition of the Guide for the Care and Use of Laboratory Animals (2011). The ethical committee approved via an authorized veterinarian with applying minimum constrain to the animals and using approved sample collection methods by the AHRI director.

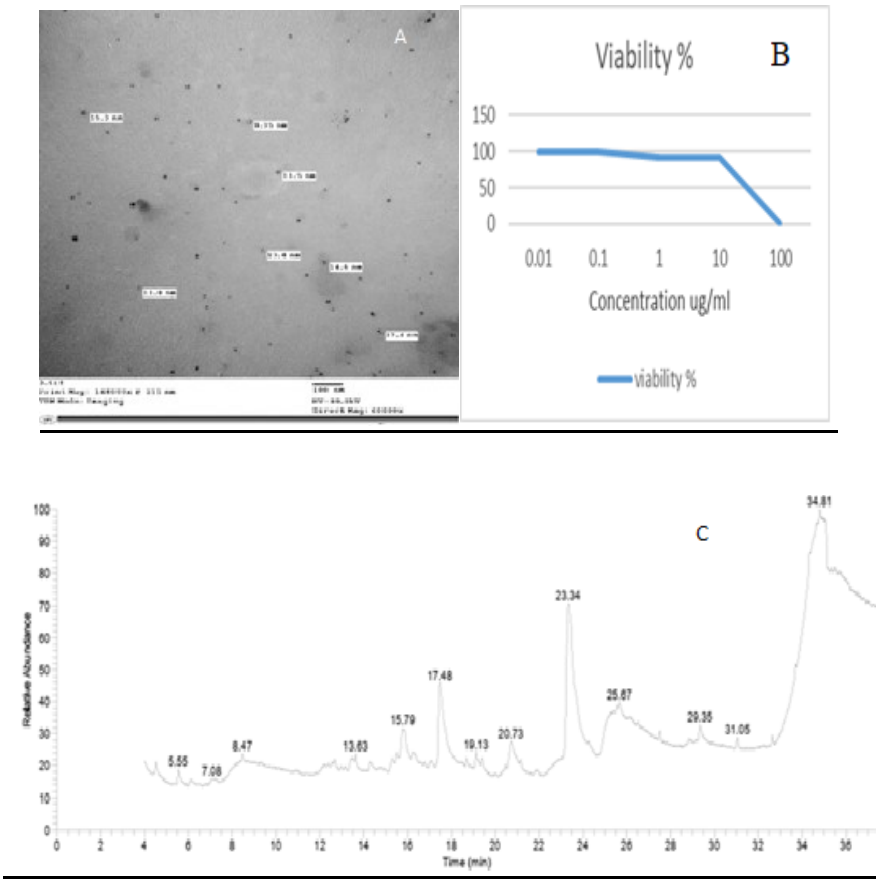

Fig. 1 (A). The transmission electron microscopy (TEM) study indicated that the specimen had a spherical form, with an average size of 13.34±2.102 nm. (B): The cell viability percentage of the nanoemulsion was evaluated using the SRB test. (C): LC–MS/MS analysis of nanoemulsion

TABLE 1.Mortality Rate of PP-NE in SPF ECE at four Doses (20%, 10%, 5% and 2.5%)

Dilution / days	1	2	3	4	5
Origin 20% PP-NE	0/5	1/5	1/5	0/5	0/5
1st dilution 10%	0/5	0/5	0/5	0/5	0/5
2nd dilution 5%	0/5	0/5	0/5	0/5	0/5
3rd dilution 2.5%	0/5	0/5	0/5	0/5	0/5

TABLE 2. The number of virus-positive eggs that have been treated with PP-NE:

Invitro incubation time	IBV	H_5	IBD	NDV
2hr	5/5	5/5	5/5	5/5
4hr	4/5	5/5	4/5	4/5
8hr	2/5	5/5	3/5	4/5
24hr	0/5	4/5	2/5	2/5

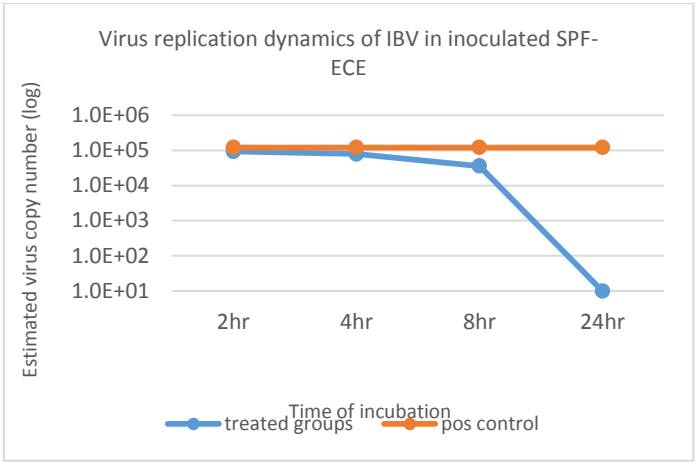

Fig. 2. The antiviral effect of the PP-NE on IBV virus replication. The estimated copy number of virus particles for the treated eggs has been compared with the positive controls with a significant reduction in virus replication with p-value = 0.000255.

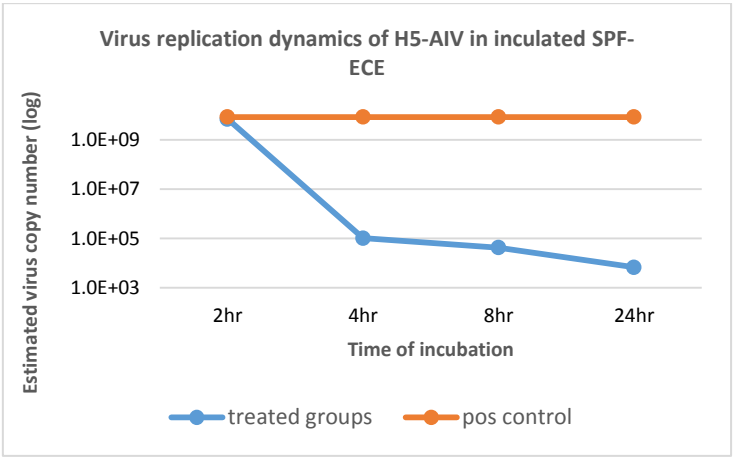

Fig. 3. The antiviral effect of the PP-NE on H5N8 virus replication. The estimated copy number of virus particles for the treated eggs has been compared with the positive controls with a significant reduction in virus replication with p-value = 0.009.

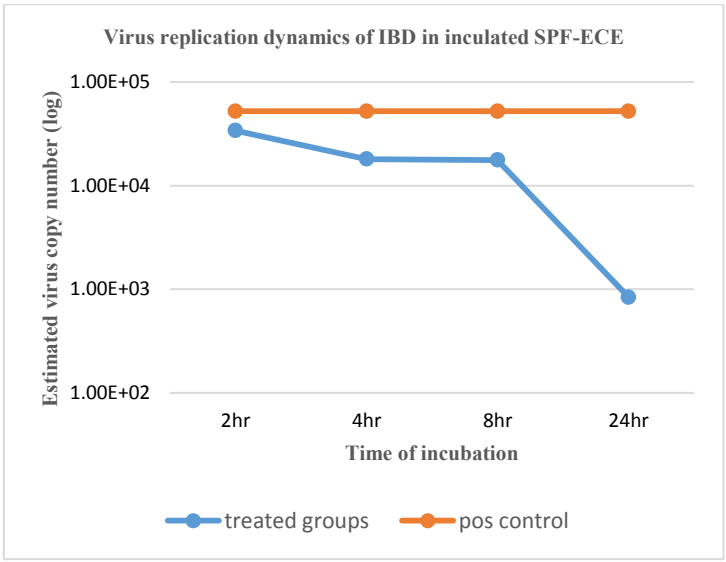

Fig. 4. The antiviral effect of the PP-NE on IBD virus replication. The estimated copy number of virus particles for the treated eggs has been compared with the positive controls with a significant reduction in virus replication with p-value =0.002192

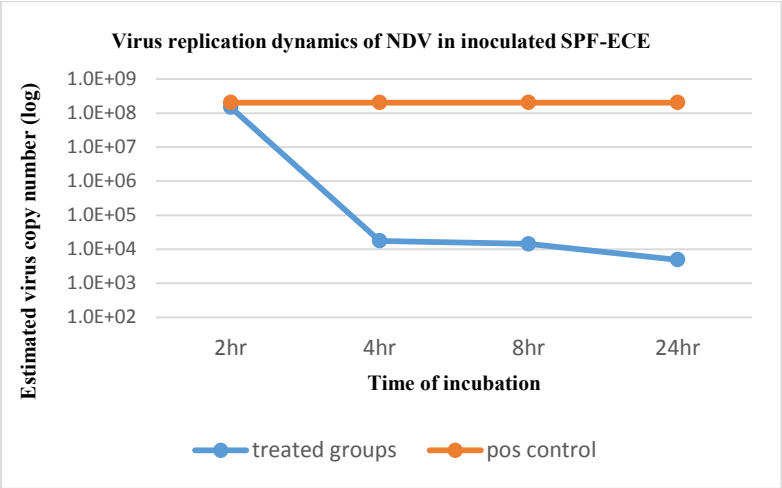

Fig. 5. The antiviral effect of the PP-NE on NDV virus replication. The estimated copy number of virus particles for the treated eggs has been compared with the positive controls with a significant reduction in virus replication with p-value = 0.004106

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تسليط الضوء على تأثير مستحلب البروبوليس وحبوب اللقاح النانوي على بعض الفيروسات الموجودة على بيض أجنة الدجاج

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الملخص

تبحث هذه الدراسة في الإمكانات المضادة للفيروسات لجسيمات النانو القائمة على البروبوليس وصمغ النحل (PP-NE) ضد أربعة فيروسات رئيسية للدواجن: إنفلونزا الطيور (H5) H5N8، وفيروس مرض نيوكاسل (NDV)، وفيروس التهاب الشعب الهوائية المعدي (IBV)، وفيروس مرض الجراب المعدي (IBDV). تم تحضير جزيئات النانو القائمة على البروبوليس ، وفقًا للطريقة التي وصفها سرور وآخرون (2021)، وتم تسجيلها تحت رقم الأكاديمية المصرية للبحث العلمي EG/P/2024/653. تم تحديد خصائص الجسيمات النانوية باستخدام LC-MS/MS والمجهر الإلكتروني النافذ (TEM)، وكشفت عن شكل كروي بحجم متوسط 13.34±2.102 نانومتر. أشار تقييم السمية الخلوية باستخدام خلايا فيرو وصبغة سلفورودامين ب (SRB) إلى IC50 يبلغ 23.19 ميكروجرام/مل. تم تحديد المركبات الفينولية، بما في ذلك الكركماديول والميثيل أوليت، كمكونات رئيسية.

لتقييم فعالية مضادات الفيروسات، تم تحضين مخاليط الجسيمات النانوية الفيروسية عند 37 درجة مئوية لمدة 2 و4 و8 و24 ساعة قبل تلقيح بيض دجاج مخصب خالٍ من مسببات الأمراض (SPF-ECE). تمت مراقبة النشاط الفيروسي باستخدام تفاعل البوليميراز المتسلسل العكسي الكمي في الوقت الحقيقي (qRT-PCR) واختبارات التراص الدموي. أظهرت النتائج تثبيطًا كبيرًا لتكاثر الفيروس لجميع الفيروسات المختبرة. أظهر IBV تثبيطًا كاملاً بعد 24 ساعة ($p = 0.000255$)، وH5N8 بعد 8 ساعات ($p = 0.009$)، وIBDV بعد 24 ساعة ($p = 0.002192$)، وNDV بعد 4 ساعات ($p = 0.004106$).

تسلط هذه النتائج الضوء على إمكانات المستحلب النانوي كعامل مضاد للفيروسات فعال في مجال الطب البيطري، مما يستدعي إجراء المزيد من البحوث في تطبيقها للسيطرة على العدوى الفيروسية في الدواجن.

الكلمات الدالة: جسيمات نانوية من البروبوليس-بولين (PP-NE)؛ إنفلونزا الطيور (H5N8)؛ فيروس مرض نيوكاسل (NDV)؛ فيروس التهاب الشعب الهوائية المعدي (IBV)؛ فيروس مرض الجراب المعدي (IBDV).