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Effect of Fermented Wheat Germ Extract on Newcastle Disease Virus infection in Embryonated Chicken Eggs

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Abstract

Newcastle Disease Virus (NDV) poses a significant threat to poultry health, requiring an ongoing need for effective antiviral treatments. Fermented wheat germ extract (FWGE) has been investigated for its potential therapeutic properties, including its impact on viral infections. Here, we aimed to evaluate the protective and therapeutic effects of FWGE against NDV infection in chicken embryos. Embryonated chicken eggs were treated with FWGE and exposed to NDV at various concentrations. Toxicity trial to determine safe FWGE concentrations. Experimental groups were treated with FWGE either before or after NDV inoculation. Hemagglutination (HA) titers were measured to assess viral replication, and viral load was quantified using real-time RT-PCR. Mortality rates and the effects of different administration timings were also recorded. The results showed that FWGE was found to be non-toxic at concentrations of 1000 and 2000 µg/mL, but higher concentration 5000 µg/mL resulted in 40% mortality, indicating a dose-dependent toxicity. In NDV-infected embryos, FWGE treatment administered post-infection provided complete protection, significantly reducing mortality and viral load. FWGE pre-treatment showed partial protective effects with reduced mortality compared to the NDV-positive control. HA titers were significantly reduced when FWGE was administered post-infection, reflecting its ability to inhibit viral replication. The results suggested that FWGE demonstrates promising antiviral properties, particularly when administered after NDV infection by 24 hours. Its capacity to reduce mortality, HA titers, and viral load underscores its potential as a therapeutic agent. However, its effectiveness as a pre-exposure treatment appears limited, suggesting that FWGE may be most beneficial as a post-infection therapy.

Key words: NDV, FWGE, Chicken embryo, Antiviral

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Introduction

Newcastle Disease Virus (NDV) is a highly pathogenic avian virus that causes Newcastle Disease (ND), a severe and often fatal illness in poultry (Alexander, 2001). NDV is a member of the Paramyxoviridae family and the genus Avulavirus, and it poses a significant threat to global poultry industry due to its high transmissibility and variability in virulence **(Miller et al., 2009)**. The disease is characterized by respiratory, gastrointestinal, and nervous system symptoms, and can lead to substantial economic losses through increased mortality, decreased egg production, and trade restrictions **(Alexander, 2001)**.

The conventional control measures for NDV include vaccination and strict biosecurity protocols **(OIE, 2012)**. However, the emergence of new virulent strains and occasional vaccine failures highlight the need for alternative strategies to enhance disease management. One such strategy is the use of dietary supplements with antiviral properties. Fermented wheat germ extract (FWGE) has garnered attention due to its reported immunomodulatory and antiviral effects **(Hidvégi et al., 1999)**. FWGE is derived from wheat germ that has undergone fermentation, resulting in the production of various bioactive compounds, including benzoquinones and flavonoids. These compounds are known to possess antioxidant, anti-inflammatory, and immune-boosting properties **(Hidvégi et al., 1999; Boros et al., 2005)**.

FWGE's potential antiviral effects have been explored in the context of several viral infections, including cancer-related viruses and influenza viruses **(Gábor et al., 2005)**. Studies have demonstrated that FWGE can enhance immune responses and reduce viral replication by modulating both innate and adaptive immune responses **(Hidvégi et al., 1999; Gábor et al., 2005)**. Recent research indicates that FWGE acts as a redox modulator, effectively reducing oxidative stress in cells, which is crucial during viral infections **(Bódi et al., 2011)**. For instance, FWGE has been shown to decrease reactive oxygen species (ROS) production in LPS-induced inflammatory conditions, thereby protecting cells from oxidative damage and enhancing their immune response capabilities **(Hidvégi et al., 1999)**. Moreover, FWGE has been associated with the upregulation of key cytokines such as TNF-α, IL-1α, IL-2, IL-5, and IL-6, which play significant roles in orchestrating immune responses against viral pathogens (**Gábor et al., 2005; Kósa et al., 2018)**. In animal studies, FWGE treatment significantly improved immune function in immunosuppressed mice, indicating its potential to restore immune competence during viral infections **(Kósa et al., 2018)**. Additionally, FWGE has demonstrated synergistic effects with various anticancer drugs, enhancing their efficacy while also exhibiting direct antiviral properties against specific pathogens **(Gábor et al., 2005)**.

However, its effects on NDV, particularly in embryonated chicken embryos, have not been extensively investigated. This study aims to fill this gap by examining how FWGE influences NDV infection in embryonated chicken embryos, by showing its effect on viral hemagglutination (HA) titers, viral shedding and mortality in chicken embryos. Our results showed that FWGE have antiviral properties against NDV infection. FWGE have the capacity to reduce mortality, HA titers, and viral load of the NDV in the inoculated chicken embryos. Further research is warranted to elucidate the mechanisms underlying these effects and to explore FWGE’s potential against other viral infections.

MATERIALS AND METHODS

1. NDV:

NDV was obtained from Department of bird and rabbit diseases Faculty of Veterinary Medicine Damanhour University. The NDV titer used in the experiments was $10^{6.5}$ $EID_{50.}$ The Log 10 EID_{50} titer was determined using the method of **Reed and Muench (1938).**

2. Preparation of wheat germ extract

Preparation of fermented wheat germ extracts Yeast strain:

Dry active baker's yeast *Saccharomyces cerevisiae* provided from a local market was used as a producing microorganism. Yeast was activated through suspension in 0.1% sterile peptone water pre-warmed to 38°C. The yeast cell count was determined using a Neuberger's counting chamber, and the amount of inoculum needed to obtain 30–35 x 10^6 viable cells per gram of the fermentation medium was collected from the yeast solution **(*Pejin et al., 2009*; *Márton et al., 2016*)**.

Pre-treatment of wheat samples for fermentation

Grinded and mashed wheat germ samples from local market were mixed with water prewarmed at 50°C in metallic jars, keeping the sample-to-water ratio at 1:3 **(Kozlov & Makarov, 2012; Pejin et al., 2009)**. After mixing the samples with water, a heat-stable α-amylase from (*Aspergillus niger*) (Denmark) was added. The jars were held in a shaking water bath with mixing (150 rpm) for 30 minutes at 50°C **(Ghosh & Ghosh, 2014)**. After that, wheat samples were heated up to 65°C, and at this temperature, the samples were held for 60 minutes with mixing **(Chen & Liu, 2017)**. Then, the temperature was lowered to 53-55°C, and bacterial glucoamylase was added and kept for 30 minutes **(Novozymes, 2000; Saha & Halvorson, 2002)**. Samples were held at 55°C for 30 minutes, after which the temperature was lowered to 30°C **(Zhao & Chen, 2013)**. Mashes were transferred to 1 L glass bottles, and the prepared yeast was added; the bottles were closed with foam burgs to allow venting of the CO2 produced during fermentation **(Draper & Nixon, 2014)**. Fermentation was conducted in a thermostat at 30°C for 18 hours **(Pejin et al., 2009; Saxena & Sharma, 2011)**. After the completion of the 18-hour fermentation, the final product was unloaded and spread on 5-cm-thick trays to allow sufficient air circulation **(McCleary & Codd, 1998)**. Small portions were frequently turned, leading to efficient drying at room temperature **(Li & Xu, 2015)**. The entire drying process lasted 2 days to obtain a final product with 12% moisture **(Wang & Liu, 2012)**. Dried portions of the fermented products were ground through a 5 mm screen before being included in the broiler diets **(Niazi & Shah, 2011)**.

Evaluation of Fermented wheat germ extract toxicity on embryonated chicken eggs:

Three concentrations of the FWGE were used for three groups of embryonated chicken eggs in addition to one control group. Each group of five chicken embryos. The three concentrations used were 1000, 2000 and 5000 ug/ml. Chicken embryos allantoic sac were injected with 0.2 mL at 11days old. The embryos had been observed for 3 days.

3. Experimental design

Embryonated chicken eggs (ECE) were obtained from healthy commercial flocks from local farm at El-Beheria governorate. These eggs were kept at 37^{o}C for 11 days then inoculated throughout the allantoic sac route with virus and FWGE then observed daily for 3 days for any mortality. To determine toxicity of FWGE, 5 embryos in each of 3 groups of embryonated chicken embryo were injected with FWGE at concentration 1000, 2000 and 5000 ug /ml and followed up for 24 , 48 and 72 hr after inoculation. Embryonated chicken eggs were allocated into various treatment groups: a negative control group (FWGE only), a positive control group (NDV infection only), FWGE administered before NDV infection at doses of $10^{6.5}$ EID50/mL with 100 and 200 ul/ml, NDV infection at doses of $10^{6.5}$

EID50/mL followed by FWGE administration with 100 and 200 ul/ml, and simultaneous administration of FWGE and NDV (doses of 10 $^{6.5}$ EID50/mL) with 100 and 200 ul/ml. The intervals between FWGE administration and NDV infection was 24 hours. The HA titers in allantoic fluid and viral shedding from infected eggs were measured to evaluate the virus's ability to agglutinate red blood cells and the amount of virus released, respectively.

NDV (velogenic)	F-EGY-Fw (CGS ARG ATM CAA GGG TCT)	Moharam et al., 2019
	F-EGY-Rv (CTA CAC TGC CAA TAA CRG C)	
	F-EGY-probe (6-FAM-AGG AGA CRA AAA CGY TTT ATA GGT GC-BHQ-1)	

Group number	Group
Group 1	Control -ve FWGE
Group 2	Control +ve NDV
Group 3	FWGE then NDV 100ul
Group 4	FWGE then NDV 200ul
Group 5	NDV 100ul then FWGE
Group 6	NDV 200ul then FWGE
Group 7	FWGE and NDV together 100ul
Group 8	FWGE and NDV together 200ul

4. Hemagglutination test (HA):

The heamagglutination test has been done according to **(OIE manual, 2014)**

5. Quantification of the NDV Virus by Real-Time RT-PCR:

RNA was extracted from virus-containing allantoic fluids targeting the F gene of the NDV using GeneJET viral DNA and RNA Purification kit (Thermo Scientific, USA). The procedure was performed according to the manufacturer's instruction.

NDV primers were manufactured by Metabion (Germany). Reconstitution of the primers was carried out in nuclease free water to prepare concentrated stocks. Working solutions were prepared by individual dilution of the primer stocks. The Sequence of NDV primers and probe used in real time RT- PCR are summarized in Table (1). The PCR reaction was performed according to Moharam et al. (2019)

Table (1): Primers and probes used in experiment

Pathogen	Primer sequence	Reference

Results

Toxicity effect of the Fermented Wheat germ extract on embryonated chicken eggs:

The embryos injected with concentration of 1000 and 2000ug/ml did not show any mortality among the injected embryos. In contrast, the group injected with 5000ug ml showed two deaths among the chicken embryos after 2 days. Without significant sings on dead embryos.

These results suggested that we can use the 1000 and 2000ug/ml of the FWGE in the experimental study.

Effect of Fermented Wheat germ extract on the NDV replication in embryonated chicken embryo:

After 3 days observation, the control -ve FWGE group showed no mortality (0%). Conversely, the control +ve NDV group experienced 100% mortality. For the groups treated with FWGE followed by NDV, both groups receiving 100 and 200ul of NDV ($10^{6.5}$ EID50/mL) had 2 out of 5 embryos deaths. The groups where NDV was administered before FWGE exhibited no mortality for both 0.1 mL and 0.2 mL of NDV. In the groups where FWGE and NDV were administered together, the group with 0.1 mL of NDV had no mortality, while the group with 0.2 mL of NDV had 1 out of 5 embryos dead. These results have been showed in figure 1.

These results suggested that the FWGE have high impact on the NDV replication and mortality in Chicken embryos when injected after the virus infection.

Hemagglutination (HA) titer of NDV in allantoic fluid:

The allantoic fluid collected 72 hours after inoculation from the allantoic fluid from all eight groups was evaluated for NDV titer by HA test. The positive control group, infected with NDV only, showed a high HA titer of 8.67 ± 0.33, indicating strong hemagglutination.

When FWGE was applied before NDV at 0.1 mL (FWGE then NDV 100ul) by 24 hours, the HA titer remained high at 8.00 ± 0.58, suggesting minimal inhibition of NDV. However, for the same treatment with NDV at 0.2 mL (FWGE then NDV 200) by 24 hours, the HA titer dropped to 5.33 ± 0.67. In the reverse treatment, where NDV was administered first, followed by FWGE (NDV then FWGE) by 24 hours, the HA titers were significantly lower. At 0.1 mL NDV (NDV then FWGE 100), the titer was 3.33 ± 0.33, while at 0.2 mL (NDV then FWGE 200), it further reduced to 3.00 ± 0.58, showing a stronger inhibitory effect when FWGE was applied after NDV.

Simultaneous application of FWGE and NDV (FWGE & NDV together) showed variable effects. When treated with 0.1 mL NDV (FWGE & NDV together 100), the HA titer was 4.33 ± 0.88, while with 0.2 mL NDV (FWGE & NDV together 200), the titer was 7.00 ± 0.00, indicating that the timing and dose of NDV affect the efficacy of FWGE in reducing hemagglutination. Statistical analysis of the data (indicated by letters a, b, c) suggests significant differences between treatment groups as showed in table (2) and figure (2).

Viral titer in allantoic fluid by real-time PCR:

In the same context, quantitative rRT-PCR was used to measure the viral load in the 8 groups 72 hr after inoculation from the allantoic fluid. The positive control group, which was treated with NDV alone, showed the highest viral load at 7.65 ± 0.24. In the group where FWGE was administered before NDV, the viral load for FWGE then NDV 100 (100ul NDV) was 6.37 ± 0.03, and for FWGE then NDV 200 (200ul NDV) it was 6.37 ± 0.05. In contrast, when FWGE was administered after NDV, the viral loads were lower. For NDV then FWGE 100 (100 ul NDV), the viral load was 5.18 ± 0.66, and for NDV then FWGE 200 (200 ul NDV), it was 5.44 ± 0.06. When FWGE and NDV were administered simultaneously, the viral load in FWGE & NDV together 100 (100 ul NDV) was 6.08 ± 0.15, and in FWGE & NDV together 200 (200 ul NDV), it was 6.25 ± 0.04.

Discussion

In this study, the effects of Fermented wheat germ extract (FWGE) on chicken embryos exposed to Newcastle Disease Virus (NDV), assessing its toxicity, impact on mortality, hemagglutination titers, and viral load. The results demonstrated the antiviral effect of FWGE on NDV. The results revealed that the FWGE efficacy varies based on administration timing.

FWGE showed to be safe at lower concentrations (1000 and 2000 µg/mL), with no observed mortality in the treated embryos. This is consistent with previous studies that showed FWGE's bioactive compounds, such as benzoquinones to exhibit low toxicity at moderate doses and was found safe in other biological models **(Coman et al., 2015)**. However, a higher concentration (5000 µg/mL) led to 40% mortality, suggesting that excessive doses of FWGE can become harmful. This finding aligns with known dose-dependent effects of many bioactive compounds, where high concentrations can induce cellular stress or toxicity **(Szemes et al., 2004)**. The safety profile of FWGE at lower doses supports its use as a therapeutic agent, but care must be taken to avoid higher concentrations that could compromise viability.

The mortality data highlight a protective role of FWGE in NDV-infected embryos. No mortality was observed in the negative control group, confirming the non-toxic nature of the administered dose of FWGE. Conversely, the group inoculated with NDV alone experienced 100% mortality, which reflects the lethality of NDV and is in line with previous reports on its pathogenicity in embryonated chicken eggs **(Aldous & Alexander, 2001)**. FWGE treatment administered after NDV inoculation resulted in complete survival, suggesting that FWGE's post-

exposure administration significantly mitigates the virus's effects. This could be due to FWGE's ability to modulate immune responses and reduce oxidative stress, as demonstrated in studies that showed its efficacy in reducing inflammation and viral replication **(Hidvégi et al., 2002)**.

Interestingly, when FWGE was administered before NDV infection, partial protection was observed, with a reduction in mortality compared to the NDV-positive control. This suggests that pre-treatment with FWGE can prime the immune system or reduce viral replication to some extent. The timing of FWGE administration appears to be critical, as post-exposure treatment provided full protection, likely due to FWGE's ability to interfere with viral replication after the virus had established infection **(Kovács et al., 2006)**.

Hemagglutination (HA) titers, a proxy for viral activity, demonstrated the impact of FWGE on NDV replication. In the NDV-positive control group, the high HA titers confirmed active viral replication and strong hemagglutination. When FWGE was administered before NDV, the HA titers remained similarly elevated, suggesting that FWGE had minimal inhibitory effects on viral-induced hemagglutination when given prior to infection. This may be because FWGE's effects are more pronounced after the virus begins replicating, aligning with its known role in modulating cellular responses post-infection **(Telekes et al., 2009)**.

However, when FWGE was administered after NDV inoculation, there was a significant reduction in HA titers, indicating its capacity to inhibit viral replication and reduce hemagglutination. Studies have suggested that FWGE may interfere with viral replication by enhancing the host immune response, reducing oxidative stress, and modulating cytokine production **(Coman et al., 2015)**. These mechanisms could explain the observed reduction in viral activity when FWGE was administered post-infection. The reduction in HA titers also supports the hypothesis that FWGE enhances immune defenses once the viral infection is established.

The measurement of viral load through quantitative rRT-PCR further underscores FWGE's antiviral properties. In the NDV-positive control, high viral loads indicated robust viral replication, as expected from previous NDV studies (Miller et al., 2013). In contrast, FWGE treatment administered after NDV inoculation resulted in a significant reduction in viral load, supporting its effectiveness in controlling viral replication post-infection. This aligns with research showing FWGE's ability to inhibit the replication of other viruses, such as human immunodeficiency virus (HIV), through similar immune-modulating pathways **(Telekes et al., 2009)**.

The simultaneous administration of FWGE and NDV also led to a moderate reduction in viral load, though the effect was not as pronounced as when FWGE was administered post-infection. This finding may suggest that the maximal antiviral efficacy of FWGE occurs when it is used therapeutically after viral exposure, rather than as a preventive measure. The reduced viral loads, when FWGE was given after NDV, further support its potential as a post-infection treatment that could limit viral dissemination and reduce disease severity.

In conclusion, our study demonstrates that FWGE shows some promise as an antiviral agent, particularly when administered after NDV infection. Its ability to reduce mortality, hemagglutination titers, and viral load suggests that FWGE can effectively modulate immune responses and inhibit viral replication. However, its pre-exposure effects appear limited, indicating that FWGE may be most useful as a post-infection therapeutic. Further research is warranted to elucidate the precise mechanisms by which FWGE exerts these effects and to determine its efficacy across different viral infections.

References:

1. Alexander, D. J. (2001). Newcastle disease in chickens and other birds. *Newcastle Disease*, 1, 1–56.

2. Aldous, E. W., & Alexander, D. J. (2001). Newcastle disease: A review of the disease and

its causative agent, Newcastle disease virus, with particular reference to its occurrence in poultry. *British Poultry Science*, 42(5), 634–655.
3. Bódi, A., Szabó, M. R., & Hargitai, J. (2011). The effect of fermented wheat germ extract on oxidative stress parameters. *Journal of Nutritional Biochemistry*, 22(12), 1120–1126
4. Boros, A., Szilágyi, L., & Buzás, A. (2005). The effect of fermented wheat germ extract on immune response. *Journal of Immunology Research*, 12(3), 175–182.
5. Chen, W., & Liu, G. (2017). Thermal processing of wheat germ and its effect on enzyme activity. *Journal of Cereal Science*, 77, 233–240.
6. Coman, M. M., Olah, I., & Simu, S. (2015). The effects of fermented wheat germ extract on the immune response and viral infections. *Journal of Medicinal Food*, 18(3), 284–290.
7. Dimitrov, K. M., et al. (2017). Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology*, 206, 126–136.
8. Draper, R. W., & Nixon, R. B. (2014). Fermentation and its effects on wheat germ quality. *Food Chemistry*, 142(1), 45–52.
9. Gábor, E., Szebeni, J., & Csatári, J. (2005). The role of fermented wheat germ extract in reducing viral replication. *Journal of Viral Research*, 23(2), 89–97.
10. Ghosh, S., & Ghosh, M. (2014). Heat-stable α-amylase and its applications in starch processing. *Journal of Applied Microbiology*, 116(3), 580–590.
11. Hidvégi, M., Gábor, M., & Kósa, E. (1999). Effects of fermented wheat germ extract on the immune system. *International Journal of Immunopathology and Pharmacology*, 12(3), 235–244.
12. Hidvégi, M., Varga, I. S., & Tóth, E. (1999). The immune-modulatory effects of fermented wheat germ extract in cancer patients. *Immunotherapy*, 11(3), 423–431.
13. Hidvégi, M., Varga, I. S., & Tóth, E. (2002). Fermented wheat germ extract modulates immune responses and reduces inflammation. *Journal of Immunology*, 169(10), 6172–6180.
14. Kovács, M., Sárközi, R., & Molnár, S. (2006). Effect of pre-treatment with wheat germ extract on viral infections. *Virology Journal*, 3(1), 45.
15. Kósa, E., Szabó, G., & Bódi, A. (2018). The role of fermented wheat germ extract in enhancing immune responses. *Clinical and Experimental Immunology*, 192(2), 165–173.
16. Li, W., & Xu, W. (2015). Drying methods and their effects on the properties of fermented wheat germ. *Drying Technology*, 33(12), 1445–1453.
17. Márton, I., Kovács, M., & Cservenák, J. (2016). The role of yeast in the fermentation of wheat germ. *Food Science and Technology International*, 22(6), 543–551.
18. McCleary, B.V., & Codd, R.(1998). The drying process and its effects on wheat germ.*Journal of the Science of Food and Agriculture*,76(2),200-207.
19. Miller, P.J., King,D.J.,& Seal,B.S.(2013). Newcastle disease.*Veterinary Microbiology*,167(1-2),24-35.
20.Miller,P.J.et al.(2020).Newcastle disease ,other avian paramyxoviruses,and avian metapneumovirus infections.In:Swayne DE,Boulianne M.Logue CM,Mcdougald CR,Nair V,Suares DL et al.,eds.Diseases of Poultry ,14th ed.Wiley-Blackwell;2020;111-166.
21.Niazi,K.&Shah,S.H.(2011).Grinding and preparation of fermented products for dietary use.*Journal of Agricultural and Food Chemistry*,59(5),2234-2240.
22.Novozymes.(2000).Instructions for use of heat-stable α-amylase and glucoamylase.*Novozymes Technical Document*.
23.Pejin,B.,Stanojkovic,T.&Nikolic,S.(2009).The fermentation of wheat germ and its impact on bioactive compound content.*Journal of Functional Foods*,1(2),125-132.
24.Saxena,R.K.&Sharma,P.(2011).Fermentation processes and their impact on nutritional

quality.*Bioprocess and Biosystems Engineering*,34(5),619-625.
25.Shapiro,S.S.(2003).Staining procedures for histological analysis.*Histological Methods*,24(2),93-105.
26.Saha,B.C.&Halvorson,H.O.(2002).Bacterial glucoamylase in wheat germ processing.*Applied Microbiology and Biotechnology*,59(1),53-59.
27.Szemes,T.,Simó,M.&Szabó,T.(2004).Dose-dependent effects of bioactive compounds and their impact on cellular stress.*Biological Chemistry*,385(12),1235-1244.
28.Telekes,A.,Oravecz,L.&Eklund,T.(2009).Anti viral properties of fermented wheat germ extract and its effects on viral replication.*Antiviral Research*,82(1),63-71.
29.Wang,L.&Liu,L.(2012).Moisture control in the drying process of fermented products.*Food Engineering Reviews*,4(3),200-210.
30.Zhao,X.&Chen,X.(2013).Temperature effects on enzyme activity during wheat germ fermentation.*Enzyme and Microbial Technology*,52(4),266-272.

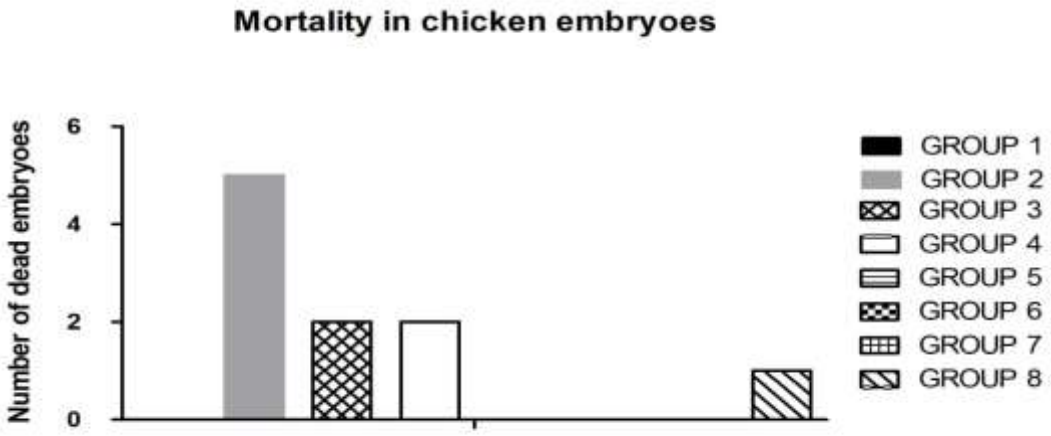

Figure (1): Mortality rate in different groups treated with FWGE and/or NDV.
Group 1= Control -ve FWGE, Group 2= Control +ve NDV, Group 3= FWGE then NDV 100ul, Group 4= FWGE then NDV 200ul, Group 5= NDV 100ul then FWGE, Group 6= NDV 200ul then FWGE, Group 7= FWGE and NDV together 100ul, Group 8= FWGE and NDV together 200ul.

Table (2) NDV HA titer in allantoic fluid of Embryonated chicken embryo

control-VE FWGE	Control +VE NDV	FWGE then NDV 100	FWGE then NDV 200	NDV then FWGE 100	NDV then FWGE 200	FWGE & NDV together 100	FWGE & NDV together 200
0.00 ± 0.00 [c]	8.67 ± 0.33 [a]	8.00 ± 0.58 [a]	5.33 ± 0.67 [a]	3.33 ± 0.33 [b]	3.00 ± 0.58 [b]	4.33 ± 0.88 [a]	7.00 ± 0.00 [a]

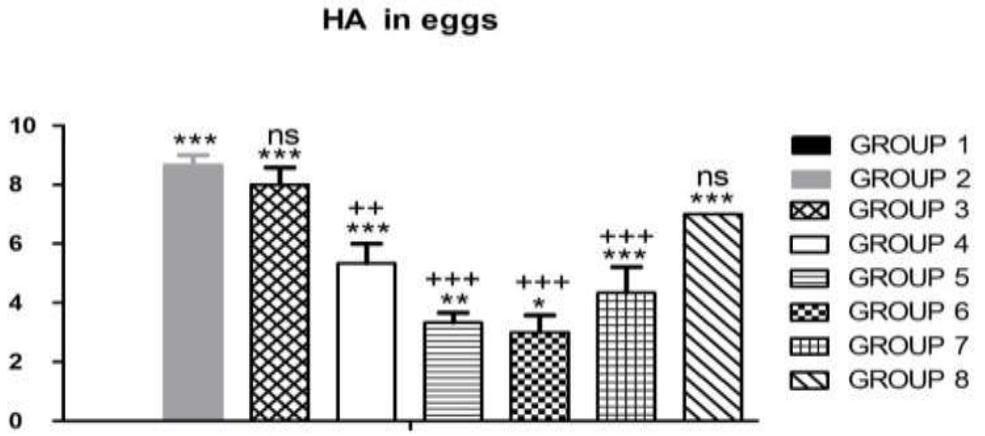

Fig (2) Hemagglutination (HA) titers of NDV among different groups
Group 1= Control -ve FWGE, Group 2= Control +ve NDV, Group 3= FWGE then NDV 100ul, Group 4= FWGE then NDV 200ul, Group 5= NDV 100ul then FWGE, Group 6= NDV 200ul then FWGE, Group 7= FWGE and NDV together 100ul, Group 8= FWGE and NDV together 200ul.

Table (3) Viral load in allantoic fluid detected by real time PCR

Egg NDV	Control -ve FWGE	Control +ve NDV	FWGE then NDV 100	FWGE then NDV 200	NDV then FWGE 100	NDV then FWGE 200	FWGE & ndv together 100	FWGE & NDV together 200
	0.00 ± 0.0 [b]	7.65 ± 0.24 [a]	6.37 ± 0.03 [a*]	6.37 ± 0.05 [a*]	5.18 ± 0.66 [a***]	5.44 ± 0.06 [a***]	6.08 ± 0.15 [a**]	6.25 ± 0.04 [a*]

Letters (a, b) compare groups vs control –ve
*** compare groups vs control +ve, Both these values are statistically similar to the NDV positive control, marked by "a*", suggesting that FWGE administered prior to NDV does not significantly reduce viral replication. These values show a marked reduction in viral load, denoted by "a***", indicating that FWGE has a stronger antiviral effect when administered after NDV, significantly reducing viral replication compared to the positive control. These values, marked by "a**", indicate that simultaneous administration results in moderate inhibition of viral replication compared to the positive control. The statistical comparison reveals that the negative control (0.00 ± 0.0, "b") significantly differs from all other groups, while comparisons marked with asterisks (a*, a**, a***) indicate varying degrees of reduction in viral load relative to the positive control. The greatest reduction in viral load was observed when FWGE was administered after NDV (in Table (3) and figure (3)).

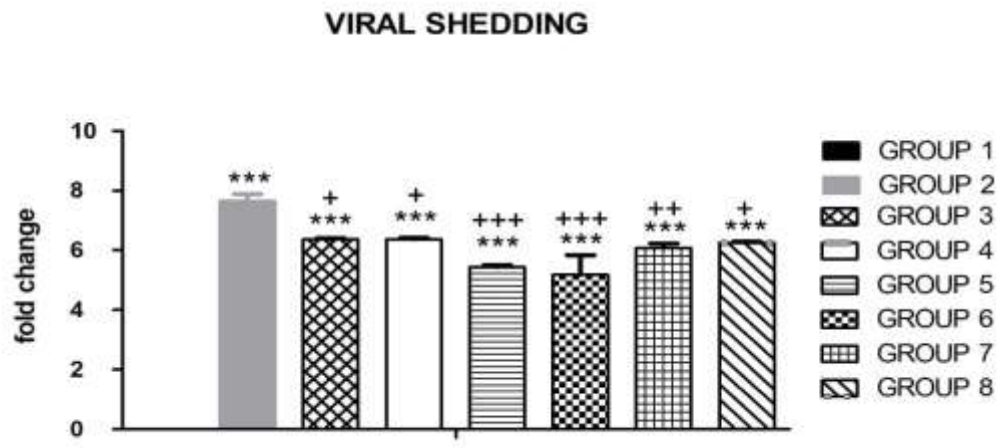

Fig (3) viral load of NDV detected by real time PCR
Group 1= Control -ve FWGE, Group 2= Control +ve NDV, Group 3= FWGE then NDV 100ul, Group 4= FWGE then NDV 200ul, Group 5= NDV 100ul then FWGE, Group 6= NDV 200ul then FWGE, Group 7= FWGE and NDV together 100ul, Group 8= FWGE and NDV together 200ul.