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Effect of argentum colloid on Hemagglutinating property of Newcastle disease virus (NDV)

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Abstract
Newcastle disease virus (NDV) is a non-segmented, single-stranded, negative sense RNA virus belonging to order *Mononegavirales*, family Paramyxoviridae, subfamily Paramyxovirinae, genus Avulavirus. It has the capacity to agglutinate the red blood cell (RBC) of mammalian and other birds due to presence of HN protein on its surface. In current study four different concentrations of silver colloid were used in the form of three treatments- pre, co and post and their effect on HA property of NDV was observed. It was found that silver Nanoparticles show a concentration dependent inhibition on HA activity of Newcastle disease virus**.** The decrease in the log HA titer was significant in case of co-treatment and post-treatment at all concentrations of AgNPs except 0.84 µg/ ml and no significant difference was seen when the two treatments were compared with each other.

Keywords: Parthenogenetic embryos, *Mononegavirales*, *Paramyxoviridae*, hemagglutinating

Introduction
Newcastle disease virus (NDV) is a non-segmented, single-stranded, negative sense RNA virus. Thevirus is enveloped, pleomorphic, and measure around 150 to 300 nanometers in diameter when observed in an electron micrograph. NDV is the causal agent of acute infectious disease of domestic poultry and few other species of birds -the Newcastle disease. The virus is able to infect all orders of avian species, and virulent strains can cause significant clinical signs. Due to the wide range of susceptible hosts, the virus has been able to establish itself worldwide. This disease can have upsetting effects on the poultry industry due to the high morbidity and mortality linked with virulent strains of the virus (Kim *et al*., 2008; Lee *et al*., 2008 and Miller *et al*., 2010) [6, 8, 9].

Newcastle disease virus (NDV) is classified in the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Avulavirus* (Lamb *et al*., 2005 and Cattoli *et al*., 2011) [7, 1]. On The basis of haemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays, genus *Avulavirus* has been grouped into ten serotypes (Waheed *et al*., 2013) [13] and all strains of NDV, regardless of virulence, belong to avian paramyxovirus type 1 (APMV-1).

NDV is an enveloped virus having two membrane proteins, the hemagglutinin–neuraminidase (HN) protein associated with cell attachment and release, and the fusion (F) protein mediating fusion of the viral envelope with cellular membranes (de Leeuw *et al*., 2005 and Uddin *et al*., 2017) [2, 12]. For infectivity and pathogenicity of NDV, both HN and F glycoproteins are important for virus for the production of protective antigens and virus neutralizing antibody responses (Karaca *et al*., 1998 and Sun *et al*., 2008) [5, 11].

NDV has the capacity to agglutinate the red blood cell (RBC) of mammalian and other birds. The HN protein is also responsible for different NDV strains to agglutinate type of RBCs (Ezeibe *et al*., 2014) [4]. All NDV strains agglutinate chicken RBC (Ezeibe *et al.*, 2006) [3], but additionally, the lentogenic strains agglutinate mammalian RBCs. Therefore, the study was aimed to see the effect of SNPs on hemagglutinating (HA) activity of NDV.

Materials and Methods
Silver nanoparticles were synthesized by borohydride reduction of silver nitrate as described by Sally D Solomon.

Virus was grown in chicken embryo fibroblast primary culture and1.5×10^{07} $TCID_{50}$/ ml of NDV was used for this study as determined by $TCID_{50}$ in 96 well microtiter plates.

Chicken embryo fibroblast culture (CEF)

Nine day old embryonated eggs were sterilized by using a cotton swab. With the help of forceps and blunt scissors embryo was taken out from the broad end of the egg after breaking open the egg shell. The appendages of the embryo were amputated and evisceration was done. The body was then minced using a sharp scissor and 5 ml of 0.25% of trypsin was added to it in a flask. The flask was put on magnetic stirrer for 10 minutes. The resulting trypsinized homogenate was then filtered through cell culture filter. The filtrate was centrifuged at 5000 rpm for 5 min and subsequently 2 washings were given to cell pallet in HBSS. The pallet was then dissolved in M-199 cell culture media along with 5% Foetal calf serum and was distributed into 96 wellcell culture plates. The plates were incubated at 37 ºC in CO_2 incubator for 24 hours and confluency was checked.

Infection of CEF monolayer and Silver nanoparticle treatment

Four concentrations of SNPs (6.75, 3.37, 1.68 and 0.84 µg/ml) were made in HBSS and 1.5×10^{07} $TCID_{50}$/ ml of NDV was used for this study as determined by $TCID_{50}$ in 96 well microtiter plates.

Cell pre-treatment

Confluent monolayer was washed twice with PBS and then treated with 50 µl of four concentrations of SNP (12 wells per concentrations) and incubated at 37ºC for 30 min. One row is left untreated to act as blank. After 30 min. the wells are washed twice with PBS and then 50 µl of NDV was added to the wells treated with SNP and then incubated for 30 min. at 37ºC. The wells were then washed with HBSS and 1 ml of complete media containing M-199 media and 5% FCS was added to each well. The plate was then incubated at 37 ºC for 3 days and was observed for cytopathic effect. The plate was given three cycles of freezing and thawing and cell culture media for each well was stored separately for different concentrations for HA assay.

Cell Co-treatment

In this treatment assay monolayers were exposed to SNP treatment and NDV infection at the same time and then incubated in the same way as in case of cell pre-treatment assay. The media from the wells was checked for HA titre after three cycles of freezing and thawing.

Cell-Post treatment

In this assay monolayers were first exposed to NDV infection and then treated with SNP following the same procedure as in case of cell pre-treatment assay. The cell culture media was then collected and after three cycles of freezing and thawing was checked for HA titre.

Haemagglutination Assay

In a V-bottom microtiter plate tenfold Serial dilution of the viral antigen was carried out up to the 9thth well (1/10-1/2560) and 10th well contained NSS only and served as NSS control whereas, the 11th and 12th wells contained NSS+RBCs and served as RBC control. 50 µl of 1% suspension of chicken RBCs in NSS was added to all the wells in the row and the plates were shaken by gently tapping against the palm and left undisturbed for 30 min at 4 ˚C. A positive result consisted of a layer of irregular matt of agglutinated RBCs at the bottom of plate and a negative result consisted of a compact, sharply demarcated disc or button of sedimented RBCs, identical to appearance of RBCs in control wells. Each test was done in duplicates.

The reciprocal of highest dilution that showed Hem agglutination was taken as HA titre of the virus.

Results and Discussion

The mean ± S.E. values of log inverse HA titer of cell culture media collected from NDV infected monolayers treated with varying concentration of silver nanoparticles is given in table 1 Statistically the decrease in mean± S.E. log HA titer of pre-treatment at different concentrations of AgNPs was insignificant compared to the mean ± S.E. of control. The decrease in the log HA titer is significant in case of co-treatment and post-treatment at all concentrations of AgNPs except 0.84 µg/ ml and no significant difference was seen when the two treatments were compared with each other. There is concentration dependent decrease in the log HA titer which can be due to decrease in the concentration of virus particles or due to binding of silver nanoparticles to the HA ligands on virions and their blockade or due to conformational change brought in them due to interaction with silver nanoparticles. Fig 1 is the plot of log inverse HA titer of NDV at various concentrations of AgNP concentration.

Table 1: Mean ± S.E. values of log inverse HA titer of NDV

	Log Inverse HA Titer		
Treatment/Agnp Conc.	**Pre Treatment**	**Co Treatment**	**Post Treatment**
6.75 µg/ ml	2.7058 ± 0.100^{aA}	1.7024 ± 0.100^{bB}	1.8027 ± 0.100^{bB}
3.37 µg/ ml	2.9065 ± 0.100^{aA}	1.8027 ± 0.100^{bB}	1.8027 ± 0.100^{bB}
1.68 µg/ ml	3.0069 ± 0.100^{aA}	1.9031 ± 0.173^{bB}	2.0034 ± 0.100^{bB}
0.84 µg/ ml	3.0069 ± 0.100^{aA}	2.7058 ± 0.265^{aA}	2.9065 ± 0.100^{aA}
0.00 µg/ ml	3.0069 ± 0.100^{aA}	3.0069 ± 0.100^{aA}	3.0069 ± 0.100^{aA}

n= 3, small letters denote significance within columns whereas capital letters denote significance within rows.

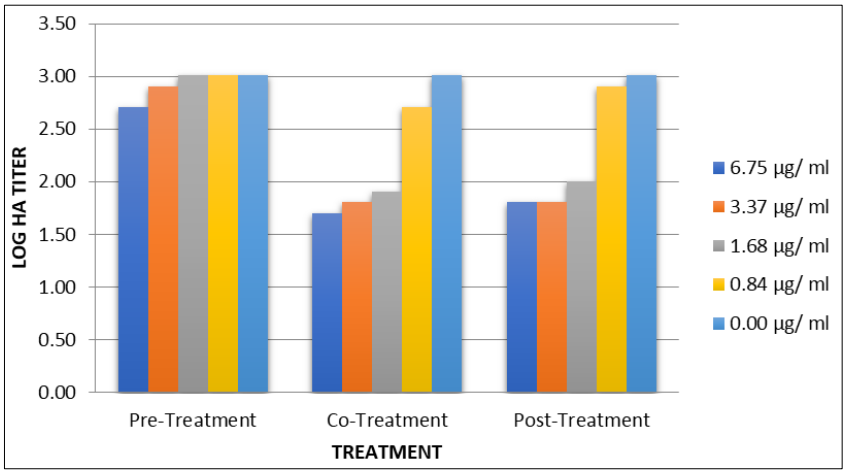

Fig 1: Plot of AgNP concentration Vs log inverse HA titer of NDV

In current study AgNPs showed a dose dependent inhibitory effect on HA property NDV which is in conformity with the current literature. Yin *et al.* (2013) [14] in their study on *in vitro* inhibition of NDV by silver nanoparticles found AgNPs to be inhibitory to NDV. They proposed the effect to be due to binding of AgNPs to the cell surface receptors and changing their confirmation and there by changing the number of virus particles binding to cell surface. They also proposed that AgNPs may also interact with the virus bound on its surface, damage its capsid and prevent the adsorption and entry of virus into the cell. Respiratory Syncytial Virus (RSV) belongs to the family *Paramyxoviridae* and is similar in structure to NDV.

Conclusion

Silver Nanoparticles show a concentration dependent inhibition on HA activity of Newcastle disease virus**.** The decrease in the log HA titer is significant in case of co-treatment and post-treatment at all concentrations of AgNPs except 0.84 µg/ ml and no significant difference was seen when the two treatments were compared with each other.

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