Trial Control of Infectious Bursal Disease Virus Isolated From Broiler Chicken Using Iron Oxide Chitosan Nanocomposite

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Research Article

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Abstract

A severe outbreak of Infectious Bursal Disease (IBD) recorded in (30) chicken flocks at different districts in Sharqia Governorate during 2019 that showed high mortalities (30%) and severe immunosuppression. Attempts to control (IBD) by antiviral iron oxide chitosan nanocomposite (characterized nano-size 35.1±5 nm with a stable state, zeta potentials16.8 ± 10.9, the PDI. 0.91 and iron concentration in nanocomposite is 2625 mg/L),

Eighty Baladi broiler chicks at the age of 21 days were divided into 4 groups. Group A (as the negative control group), group B (as the positive control group), group C (as the nanocomposite treatment group), and group D (only received nanocomposite). The used treatment was iron oxide chitosan nanocomposite with the concentration of (1 mg/ 100ml) IC50 orally administered for 3 days post infection.

The virus shedding showed marked significant decrease (P<0.05) in group C compared to group B was 0.5 to 1 log \(10\) compared to group B was 3 to 6.4 log \(10\).

Histopathological lesions of bursa, spleen, thymus, liver, and kidney in group C after 6 days PI showed hyper activity of lymphoid population compared to chicks infected group which revealed necrosis and depletion of lymphoid elements of the bursa, thymus, and spleen with blood vessels had marked congestion and hemorrhages in parenchyma.

Base on this study, iron oxide chitosan nanocomposite showed an antiviral activity that could significantly reduce viral shedding, and could decrease pathological changes in lymphoid organs which is considered to be better protection for solving health problems in infected chicks.

Introduction:

Infectious Bursal Disease (IBD) is classified as a serious disease of chicken that has profound economic effects. It is responsible of high mortalities, immunosuppression and characterized by severe lesions in Fabricius bursa [1 and 2]. It is caused by IBD virus from the Avibirmingham genus, the Birnaviridae family. The serotype 1 of IBDv is pathogenic to chickens [3].

Acute IBD is related with clinical indicators such as diarrhoea, prostration, and abrupt mortality in chickens younger than 10 weeks old. In the first four days of acute IBD cases, examinations revealed nephritis, muscle haemorrhages, and bursal lesions such as bursal oedema or haemorrhages, followed by bursal atrophy. [4].

Nanotechnology is becoming increasingly important in the diagnosis and management of animal diseases. The use of metal or metal oxide nanoparticles in virus targeting formulations is becoming more common, and it promises to improve the agents’ diagnostic or therapeutic capabilities while also increasing the chances of targeted medication delivery in a novel method. The lowered ratio of viral
suspensions after treatment with iron oxide nanoparticles was calculated in a recent study to show that the iron oxide nanoparticle has antiviral action. [5].

Iron oxide antiviral activity was reported in vitro [6], with stronger suppression at lower doses, possibly due to its small size, simple contact with the virus, and typical spatial organization of the nanoparticles attached. Nanoparticles interact with the virus by preferentially binding to the Influenza virus protein knobs, based on the center-to-center distance between nanoparticles and the fact that exposed sulfur-bearing residues on protein knobs would be optimal places for engagement with nanoparticles. Because of this interaction, nanoparticles prevent the virus from attaching to host cells.

Although the antiviral function of the composites has yet to be determined, the experimental results demonstrating a link between antiviral activity and iron oxide NP concentrations suggest that virions and composites were interacting. A interaction of iron oxide with eSH protein groups in the protein inactivating cell could be the molecular mechanism. [7].

However, clarification is still needed on whether and how nanoparticles disrupt membrane function. It is necessary to tackle the impact of nanoparticles on essential cellular pathways such as DNA synthesis, RNA, and proteins. Consequently, detailed studies of the antiviral mechanism of the iron oxides NPs could lead to the development of functional iron oxides NPs containing materials that would minimize concerns about the risk of the spread of iron oxides NPs to the infection region [8].

Chitosan is a natural polymer made from alkaline chitin hydrolysis, a chemical component present in arthropod exoskeletons, crab shells, and insect cuticles. Chitosan and its nanoparticles have gotten a lot of attention in the pharmaceutical, food, agricultural, textile, and tissue industries because of their inherent biocompatibility, biodegradability, and lack of toxicity. [9].

This study aims to investigate the effect of iron oxide chitosan nanocomposite on virus shedding and the pathologic lesion associated with experimental Infection Bursal Disease (IBD) in infection in susceptible broiler chicks.

**Material And Methods:**

**Materials:**

Ferrous Sulphate extra pure, 99.5% (FeSO4.7H2O) (SRLPvt. Ltd), Ferric chloride hexahydrate, 98% (FeCl3.6H2O) (Himedia), Ammonia 25% (v/v), 25-29% (Merck), and Chitosan 85% deacetylated (Alfa Aesar).

**Preparation of Iron Oxides Chitosan Nanocomposite and Characterization:**

Co-precipitation preparation method was used through adding 0.55g FeSO4.7H2O and 0.95g of FeCl3.6H2O to double distilled water (15mL) where 10 mins stirred in a magnetic stirrer reaching to pH 11
by adding Ammonia 7% solution. The precipitated black color appeared, and it was washed several times. After drying of iron oxide nanoparticles, chitosan solution 85% deacetylated (200 mg) was added and mixed under magnetic stirrer for 1 hr. mixture chitosan coated iron oxide particles were obtained [10].

High-resolution transmission electron microscopy (HRTEM), image JEM 2100F transmission electron microscope with accelerating voltage 200 kV, Fourier Transmittance Infrared FT/IR-6100 Spectrometer and zeta sizer Nano ZS equipment were used for characterization (Malvern Instruments, Worcestershire, UK).

**Nano-Cytotoxicity of Iron Oxide Chitosan Nanocomposite:**

Nawah Scientific Inc. provided the green monkey cell line (Mokatam, Cairo, Egypt). At 37°C, cells were cultured in DMEM media with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated foetal bovine serum in a humidified, 5% (v/v) CO₂ atmosphere. SRB) Sulfurhodamine B assay was used to determine cell viability. In 96-well plates, aliquots of 100 L cell suspension (5x10³ cells) were incubated in full medium for 24 hours. Another aliquot of 100 L medium containing medicines at various concentrations ranging from (0.01,0.1,1,10,100 ug/ml) was given to the cells. After 72 hours of exposure to the medication, the cells were fixed by replacing the medium with 150 litres of 10% TCA and incubated for 1 hour at 4°C. After removing the TCA solution, the cells were washed five times with distilled water. Aliquots of 70 L SRB solution (0.4 percent w/v) were added and incubated for 10 minutes in a dark place at room temperature. Plates were washed three times with 1% acetic acid and air-dried overnight. Then, 150 L of TRIS (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm using an OIE-compliant BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany) [4].

**Natural IBD Infected Chickens:**

Samples were collected from 40 of infected Balady broiler flocks (3wk old) in Sharqia governorate showed high mortalities incidence (30%) and severe clinical disease.

**Isolation of IBD Virus on SPF Embryos:**

PCR positive Bursa homogenate subjected for virus isolation by inoculation of centrifuged supernatant fluid on SPF ECE in the chorioallantoic membrane at 12 days then inoculated eggs were incubated at 37°C for 5 days. Daily observation of inoculated eggs and dead ones chilled at 4C overnight and after that were examined for lesion and confirmed for IBDV by PCR [11].

**PCR Amplification and Analysis:**

In a 25 µl reaction, 12.5 µl of PCR Mastermix (Emerald Amp Max PCR Master Mix, Takara, Japan), 7.5 µl of PCR grade water, 1 µl of forward and reverse primers (20 pmol conc.), and 3 µl of extracted DNA were employed. An applied biosystem 2720 thermal cycler was used to perform the PCR reaction. The primary denaturation process was carried out at 95°C for 5 minutes, followed by 30 seconds at 94°C, 30 seconds at 50°C, and 30 seconds at 72°C (for 35 cycles). A final extension step of 10 minutes at 72°C was
performed. At room temperature, PCR products were run on a 1.5% agarose gel (Applichem, Germany, GmbH). The fragment sizes were determined using a Gelpilot 100 bp DNA ladder (Qiagen, GmbH, Germany). A gel documentation system took the images of the gels (Alpha Innotech, Biometra).

**Sequence analysis of VP2 of IBDV**

Gel containing DNA band with size (620bp) were purified with the QIAquick Gel Extraction Kit (Qiagen). Sequencing of this purified product directly using 2 µl of Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster City, CA), 1 µl of each primer specific for VP2 gene were used in the reaction.

Thermal profile of the reaction: one cycle at 96°C for 1 min, 25 repeated cycles of 96°C for 10 Sec, 50°C for 5 Sec and 60°C for 2 min. Then, the sequencing reactions were purified using a spin column Centrisep® kit (Applied Biosystems, USA) to remove the extra free dNTPs bases and followed by loading the purified reactions in a sequencer plate of ABI (Applied Biosystems 3130 genetic analyzers, USA). Sequences of VP2 gene was submitted to the Gene Bank under Accession Number OK483322.

**Experimental Design:**

80 one-day old Balady chicks were raised for 4 weeks old (the experimental period), the feed and water were added ad libitum. At the age of 21 days, they were divided into 4 groups of twenty chickens per group. Group A (as the negative control group), group B (as the positive control group) with virus dose of 100ul/bird $10^3$ EID50 orally administered at 21 days old age, group C (as the treatment group) with virus dose of $10^3$ EID50 with iron oxide chitosan nanocomposite concentration (1 mg/ 100ml) IC50 orally administered for 3 days after infection, and group D iron oxide chitosan nanocomposite concentration 1 mg/ 100ml IC50 orally administered for 3 successive days. All groups were kept under strict biosecurity measures were taken for all groups. Bursa samples were collected for quantitative PCR from every group. Tissue samples were collected from the internal organ (fabricia bursa-spleen-thymus- liver-kidney) from experimentally infected, and from recently dead chickens. Serum was collected for iron determination using Biodiagnostics kits according to [12].

**Samples:**

Five bursa samples from naturally infected chickens were collected under complete aseptic condition pooled and kept at -20°C until use for virus isolation.

**Virus Titration:**

Bursa homogenate was collected from challenged chicken with IBD virus either treated with iron oxide chitosan nanocomposite or not (gr. B and gr.C). Virus concentration was calculated bon SPF ECE using Reed, Muench method [13].

**Pathological Examination:**

Samples were collected from all groups at 3 and 6 days post infection. The collected specimens from bursa of fabricious, spleen, thymus, liver, and kidney were fixed in 10% neutral buffer formalin solution
and then processed using the routine histopathological technique. Paraffin sections of 3-4 micron thickness were prepared and stained with Haematoxylin and Eosin stain for examining microscopically [14].

Results:

Iron Oxide Chitosan Nanocomposite and Characterization:

The characteristic properties of synthesized and characterization were analyzed by different analytical methods:

- The concentration of iron oxide in nanocomposite is 2625 mg/L according to atomic absorption methods.
- The magnetic nanoparticles bound with chitosan have a well-shaped spherical form with a smooth surface, and the average particle size is around 35.15 nm with a narrow size distribution, according to the typical HRTEM for the nanocomposite. As a result, composite nanoparticles are more likely to attract one another and establish a stable state as shown in Fig. (1).
- Zeta Potential: the zeta potentials iron oxide chitosan nanocomposite 16.8 ± 10.9, as a measurement of not only the conductivity 4.69, viscosity 0.887, but also the PDI. 0.91.
- In compared to pure substances, the FTIR fingerprint displays the chemical interaction, molecular fingerprint, and detector of functional groups. The highest FTIR peaks, 3144.75 and 2010.17 cm⁻¹, were attributable to phenols and alkynes' O-H and C-H stretch vibrations. Stretching of amino (-NH2), nitro compounds, alkyl groups, phenols, and carboxylic groups correlate to FTIR spectral bands at 1752.74, 1630.92, 1402.28, 1113.11, and 841.70 cm⁻¹. Other brief peaks, ranging from 637.53 to 433.18 cm⁻¹, have been attributed to the presence of Fe oxygen, as shown in Fig. (2).

- **Nano-cytotoxicity of Iron Oxide Chitosan Nanocomposite**: The SRB (Sulforhodamine B) assay is easy, fast, and responsive. It offers strong cell number linearity, allows saturating dye concentrations to be used, is less vulnerable to environmental variability, is independent of intermediate metabolism, and offers a fixed end point that does not require a time-sensitive initial reaction velocity calculation. The iron oxide chitosan nanocomposite nano-cytotoxicity results show that viability percentage is 95.73% for high concentration 100 ug/ml and 98.48% for low concentration 0.01 ug/ml, therefore the IC50 is > 100 ug/ml as shown in Fig. (3).

Experimental Study:

Virus Propagation and Titration:

The virus was isolated on 12 days of SPF ECE. Death of embryos was at 2-5 Days PI. The CAM was collected from dead embryos after 48 hours of inoculation. The most common observation was the death of the embryo at 2-5 days PI. Virus titer was 3 log₁⁰ EID50 /1 ml of CAM homogenate. Gross lesions of dead embryos showed dwarf embryo and distended abdomen with edema and congestion of internal
organs Plate.1 (Fig. a). The CAM was collected from dead embryos after 48 hours of inoculation showed petechial hemorrhages (Fig. b).

**Titration of Viral Shedding from Challenged Chicken:**

Bursa homogenate collected from groups A and D which were not infected by virus showed no viral shedding while the B group chickens showed viral shedding between 3 to 6.4 log$^{10}$. While the C group with iron oxide chitosan nanocomposite after infected with IBD virus showed a significant decrease in viral shedding about 0.5 to 1 log$^{10}$ while treated chicken with iron oxide chitosan nanocomposite without virus inoculation showed no viral shedding. (Table 1).

There were significant differences (P<0.05) in iron serum levels between groups. The C group which iron oxide chitosan nanocomposite treated, 3 and 6 days after being infected with IBD virus showed high levels as compared to the B group, while the D group chicken with nanocomposite without virus inoculation showed higher rates than the A group as shown in Table (2).

**Histopathological Findings:**

**Clinical Signs and Gross Lesions:**

The clinical signs of chicks following experimentally challenge of 3 weeks old group (B) were anorexia, sleepiness, ruffled feathers with droopy wings, prostration, and whitish mucoid diarrhea plate.1 (Fig. c); in addition, (30%) mortality rate was also recorded. The muscles of the infected chicks showed ecchymotic and paint brush hemorrhages which distributed mainly in the thigh, leg, and pectoral muscles Plate.1 (Fig. d, e). The hemorrhages were also present at the junction between proventriculus and gizzard.

Bursa of infected chicks revealed swollen and hemorrhagic cloacal bursa which was covered by gelatinous material with yellowish exudate within lumen at 3 days PI (Fig. f), at 6 days PI the bursa become atrophied (fig. g). Moreover, the liver showed the peripheral area of infarction which appears yellow in color (Fig. h). The kidney was enlarged and pale. Spleen and thymus were swollen. However, no marked clinical signs or postmortem alteration were observed in all examined organs of the infected and treated group (c). The mortality rate was 5%, as only one chick died at six days post infection. Non infected and treated group (D) exhibited no clinical signs and normal bursal, spleen and thymus macroscopically as chicks of the -ve control group (A) with no recorded mortalities.

**Histopathological findings:**

**Cloacal Bursa of Experimental Chicks**

(Plate.2) Control +ve group (B) infected by $10^3$ EID50 IBDV after 3 days of infection showed central necrosis of most bursal follicles and hemorrhages between follicles (Fig. 1). Whereas, the bursa of fabricius after 6 days of infection showed atrophied lymphoid populations and caseous necrosis of most lymphoid follicles center (Fig. 2). Iron oxide chitosan treated group
(C) at 3 days PI, the bursa showed cystic cavitations and heterophil infiltration (Fig. 3); while at 6 days PI showed lymphocytic repopulation of the lymphoid follicles (Fig. 4). Non infected and treated group (D) with iron oxide chitosan showed normal bursal structures.

**Spleen of Experimental Chicks (Plate. 3)**

The +ve control group (B) infected by $10^3$ EID50 IBDV after 3 days of infection showed necrosis of most lymphoid follicles and the perarterial lymphoid sheaths and significant lymphocytic depletion of the lymphoid follicles (Fig. 5). However, after 6 days of infection, it showed edema and necrotic cells of the red pulp (Fig. 6). Iron oxide chitosan treated group (C) at 3 days PI showed congestion of blood vessels (Fig. 7). Furthermore, treated group (C) at 6 days PI showed repopulation of lymphocyte of the lymphoid follicles (Fig. 8). Non infected and treated group (D) with iron oxide chitosan showed normal white pulp and red pulp.

**Histopathological Lesions of Thymus (Plate.4):**

The +ve control group (B) infected by $10^3$ EID50 IBDV after 3 days of infection revealed necrosis of most lymphoid lobules, hemorrhages throughout parenchyma and marked congestion of blood vessels (Fig. 9). After 6 days of infection showed a reduction of lymphoid populations (Fig. 10). Iron oxide chitosan treated group (C) after 3 days PI revealed moderate activity of cortical and medullary lymphoid contents of thymic nodules and dilated septal blood vessels (Fig. 11). Moreover, after 6 days PI showed congestion of blood vessels, apparently normal cortical and medullary lymphoid population (Fig. 12). Non infected and treated group (D) with iron oxide chitosan showed normal lymphatic structures.

**Histopathological Lesions of Liver (Plate.5):**

The +ve control group (B) infected by $10^3$ EID50 IBDV after 3 days of infection showed necrotic area admixed with inflammatory cells and congestion of hepatic blood vessels Fig. 13. After 6 days of infection showed minute inflammatory cells within sinusoids and congestion of hepatic blood vessels and sinusoids (Fig. 14). Iron oxide chitosan treated group (c) at 3 days PI showed nearly normal hepatic acini and congestion of some hepatic blood vessels (Fig. 15), as well as, treatment at 6 days PI showed apparently normal hepatic parenchyma and congestion hepatic sinusoids (Fig. 16). Non infected and treated group (D) showed normal hepatic parenchyma and vascular tree.

**Histopathological Lesions of Kidney (Plate. VII):**

The +ve control group (B) infected by $10^3$ EID50 IBDV showed the focal hemorrhagic area and severe degeneration and necrosis of some renal tubules (Fig. 17). 6 days after infection showed degenerative changes of the renal tubular epithelium (Fig. 18). Iron oxide chitosan treated group (C) at 3 days PI showed regenerative attempts of the renal tubular epithelium (Fig. 19). However, at 6 days PI showed apparently normal renal tubules and regenerative attempts of the renal tubular epithelium (Fig. 19). Non infected and treated group (D) showed normal renal tubules and glomerular structures.

**Discussion:**
Our results provide new insights into the significance of iron oxide chitosan nanocomposite as a potent inhibitor of the IBD virus and rationalize its development.

Other authors found that the optical and structural properties of iron oxide nanoparticles exhibit remarkable changes when coated with organic polymers. The nanoparticle size varied from 20 to 90 nm. Due to the presence of -NH2 groups on the surface of the FeO nanoparticle after the addition of chitosan, the surface charge was substantially changed to positive values + 12.9 mV. It shows that at typical concentrations, chitosan-coated magnetic nanoparticles are non-cytotoxic and have high biocompatibility. The existence of metal-oxygen (Fe=O) bonds was detected in the FTIR spectrum at 500–800 cm\(^{-1}\), while the presence of amino groups (-NH2) was detected at 1646 cm\(^{-1}\), confirming the presence of chitosan in the produced chitosan-FeO nanocomposite. [15–17].

Results showed that nanocomposite decrease IBD viral multiplication after infection. Results reveal that the IBD virus titer had reduced after 3-9 day exposed by cells to nanocomposite. Also, certain receptors which are important in IBD virus component and entry into the host cells are not occupied. These test findings suggest that the nanocomposite are aimed at and interfere with several stages in the IBD virus life cycle following viral adsorption and internal cell internalization.

Although the mechanism of the composites as antiviral has yet to be found, experimental evidence showing a link between antiviral activity and iron oxide nanocomposite concentrations suggests that the virions and composites interacted. A interaction of iron oxide with eSH groups of proteins in the cell could be the molecular process, inactivating the proteins. [7].

During 24 h of virus infection, the antiviral activity detected by viral RNA transcripts changes using RT-PCR, when treated with iron oxide nanoparticles lead to reduce virus (80 fold).

Also, no marked clinical signs were notice and no mortalities were recorded in nanocomposite treated group (C). This laboratory observation when compared with field investigations as morbidity reached (70%) and high mortality rate 30% based on this comparison, this experimental open a new approach for used of IP-NPs [8].

From pathological and histopathological observation of this study, iron oxide chitosan nanocomposite has ameliorative potentials at both gross and histopathological levels. Initial swelling of the primary and secondary lymphoid organs of chicks, significant haemorrhages, and acute inflammation characterised by hyperemia followed by severe atrophy of the primary lymphoid organs are the predominant gross abnormalities during the acute phase of IBDV. [18].

In this investigation, the field virus was found to have the ability to cause severe gross lesions in the bursa, thymus, and spleen. On day 6 of the infection, the principal lymphoid organs began to atrophy rapidly and noticeably. Lesions in the thymus, bursa, and spleen of 1BDV-infected chicks with severe clinical disease correlated with peak mortality and gross lesions at the acute phase of the disease, resulting in diminished immune-protective efficacies [19].
Significant depletion of lymphoid cells, reticular cell hyperplasia, oedema, haemorrhages, and congestion of blood vessels, atrophy, and fibroplasia, followed by hyperplasia of reticular cells were seen in the bursa, thymus, and spleen in the current investigation. On the sixth day of the infection, complete damage to the bursa, including marked desquamation of epithelial lining and failure of the bursa to repopulate with lymphocytes, caused premature regression of the bursa in the chickens that survived, which could affect B cell function, reduce immune function, and damage the bursa. This could explain why hens suffer from immunosuppression when they have IBD [20].

Conclusion:

Our results provide new insights into the importance of iron oxide chitosan nanocomposite as a potent inhibitor of the IBD virus and rationalize its development. We assume that the method discussed here has the ability to develop medically appropriate virucidal drugs to suppress viral infections. It should be emphasized that the proposed approach is fundamentally broad in nature, allowing for the possible prevention and treatment of multiple viral infections with a single medication, a significant benefit, mostly in virology, where rapid and often sudden infections occur.

Attempted treatment for control of (IBD) by antiviral iron oxide chitosan nanocomposite at 1mg /100ml distal water orally administered 3 successive days could significantly reduce virus shedding and ameliorate the clinical and pathological lesions induced by IBDV. Future investigations are needed to evaluate the role of iron oxide chitosan nanocomposite as preventive antiviral drug under field and laboratory condition.

Declarations:

Conflict of interest / Declaration of Competing Interest:

None

Declaration of Competing Interest:

None

Ethical approval:

The animal study in this manuscript was done according to approved protocol by the Ethical Committee of the Animal Health Research Institute, Ministry of Agriculture, Giza, Egypt.

References:

1. Tadesse B, Jenbere S. Sero-Prevalence of Infectious Bursal Disease in Backyard Chickens at Selected Woredas of Eastern Ethiopia. J Biol Agric 2014; 4: 17-2014. www.iiste.org. ISSN 2224-3208 (Paper) ISSN 2225-093X (Online). Vol. 4 (17), (2014).
2. Wahome MW, Njagi LW, Nyaga PN, Mbuthia PG, Bebora LC, Bwana MO. Occurrence of antibodies to infectious bursal disease virus in non-vaccinated indigenous chicken, ducks and turkeys in Kenya. Inter J Vet Sci. 2017; 6(3): 159-162.ijvets.com

3. Mutinda Isolation, pathological and immunological characterization of Kenyan infectious bursal disease virus strains for vaccine development. PhD thesis University of Nairobi, Kenya. 2016.

4. OIE (Office International des Epizooties) Terrestrial Manual. Chapter 3.3.12- Infectious Bursal Disease). In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2018.

5. Tejabhiram Y, and Shukla D. Role of metal and metal oxide nanoparticles as diagnostic and therapeutic tools for highly prevalent viral Nanomedicine: NBM 2016; xx:1-12. https://doi: 10.1016/j.nano.2016.08.016.

6. Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ. Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol, 2005; 3(6):1e10. https://doi.org/10.1186/1477-3155-3-6

7. Mori Y, Ono T, Miyahira Y, Nguyen VQ, Matsui T, Ishiha Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. Nanoscale Research Letters. 2013; 8:93. doi: 10.1186/1556-276X-8-93

8. Kumar R, NayakK, Sahoo G C, Pandey K, Sarkar MC, Ansari Y, Das VNR, Topno RK, Bhawna MM, Pradeep D. Iron oxide nanoparticles based antiviral activity of H1N1 influenza A virus. J Infect Chemother. 2019; 25: 325-329. DOI: 10.1016/j.jiac.2018.12.006

9. Dutta PK, Dutta J, Tripathi VS. Chitin and chitosan. Chemistry, properties and applications. J Sci Ind Res. 2004; 63:20–31. http:// nopr. niscair. res.in /handle /123456789/5397

10. Rabe A M, Jayanthi V, Nixon Raj N, Ramachandran D, Brijitta J. Synthesis and Characterization of Chitosan-Coated Iron Oxide Nanoparticles. Proceedings of the “International Conference on Advanced Nanomaterials & Emerging Engineering Technologies” (ICANMEET-2013)organized by Sathyabama University, Chennai, India in association with DRDO, New Delhi, India, 24th -26th, July, 2013.

11. Ammerman NC,Beier-Sexton M, Abdu F Azad. Growth and maintenance of Vero cell lines. Curr Protoc Microbiol. November, DOI: 10.1002/9780471729259.mca04es11.

12. Dreux C. Colorimetric determination of iron using thio-glycolic acid. Ann. Biol. Clin. 35: 275.

13. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. The American Journal of Hygiene. 1938; 27: 493-497.

14. Suvarana SK, Layton C, Bancroft JD. Bancroft’s theory and practice of Histological techniques. 8th, Churchill Livingstone. Elsevier, England. 2018.

15. Bharathi D, Ranjithkumar R, Vasantharaj S, Chandarshekar B, Bhuvaneshwari V. Synthesis and characterization of chitosan/iron oxide nanocomposite for biomedical applications. Inter. J. Biol. Macromolecules. doi:10.1016/j.ijbiomac.2019.03.233
16. Bharathi D, Ranjithkumar R, Chandarshekar B, Bhuvaneshwari V. Preparation of chitosan coated zinc oxide nanocomposite for enhanced antibacterial and photocatalytic activity: as a bionanocomposite, International Journal of Biological Macromolecules. 2019; 129: 989–996. doi: 10.1016/j.ijbiomac.2019.02.061.

17. Pham XN, Nguyen TP, Pham TN, Tran TTN, Tran TVT Synthesis and characterization of chitosan-coated magnetite nanoparticles and their application in curcumin drug delivery. Adv. Nat. Sci. Nanosci. Nanotechnol. 2016; 7 045010 (9pp) doi:10.1088/2043-6262/7/4/045010

18. Sliva MS, Rissi, DR,Swayne DE. Very virulent infectious bursal disease virus duces mors severed disease and lesion in specific-pathogen-free (SPF) leghoms than in SPF broiler chickens Avian Diseases. 2016; 60(1):63-66. DOI: 10.1637/11230-070615-ResNote.1

19. Aliyu HB, Saidu L, Jamilu A, Andamin AD, Akpavie SO. Outbreaks of virulent infectious bursal disease in flocks of battery cage brooding system of commercial chickens. J. Vet. Med. 2016; 7 pages. https://doi.org/10.1155/2016/8182160

20. Etetradossi N, Saif YM. Infectious Bursal Disease. In: Diseases of Poultry ( DE Swayne JR Glisson , LR McDougald, Lk Nolan, DL Suarez, N Venugopal editors) thirteenth edition. John Wiley and Sons Inc., Ames, Iowa, USA. 2013; Pp 219-246.

Tables:

**Table (1):** Result of Isolation and Titration of IBD Virus from Collected Bursa at 3, 6 and 9 Days Post-infection.

| Group | EID$_{50}$ log$^{10}$ |
|-------|---------------------|
|       | 3-day PI | 6-day PI | 9-day PI |
| A     | zero      | zero     | zero     |
| B     | 3 log$^{10}$ | 4.5 log$^{10}$ | 6.4 log$^{10}$ |
| C     | 1.4 log$^{10}$ | 0.5 log$^{10}$ | zero     |
| D     | zero      | zero     | zero     |

**Table (2):** Result of Iron Serum Level at 3, 6, and 9 Days Post-infection.
| Group | Iron serum level (μg/dl) | 3-day PI   | 6-day PI   | 9-day PI   |
|-------|-------------------------|------------|------------|------------|
|       |                         |            |            |            |
| A     | 102.66± 8.03<sup>ab</sup> | 109.66± 7.9<sup>ab</sup> | 111.67± 10.41<sup>a</sup> |
| B     | 68.33±5.68<sup>c</sup> | 58.67±4.16<sup>c</sup> | 45.09±13.74<sup>b</sup> |
| C     | 93±6.08<sup>b</sup> | 101.33±3.06<sup>b</sup> | 105.05±5.01<sup>a</sup> |
| D     | 111.6±2.89<sup>a</sup> | 117.3±2.52<sup>a</sup> | 137.76±28.11<sup>a</sup> |

N.B. the different litters of columns denote significant variations between means (at P ≤ 0.05)

**Plate:**

Plates 1 to 6 are available in the Supplemental Files section.

**Figures**

**Figure 1**

HERTEM magnetic nanoparticles bonded with chitosan have a well-shaped spherical form with a smooth surface, and their average particle size is approximately 35.1±5 nm
Figure 2

FT-IR spectra of iron oxide chitosan nanocomposite

Figure 3

Cell viability % of iron oxide chitosan nanocomposite effect on Vero cells

Supplementary Files

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