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Original Article

HAEMATO-BIOCHEMCIAL STUDIES OF NANOPARTICLE-BASED VACCINE AGAINST R2B STRAIN OF NEW CASTLE DISEASE IN CHICKS

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ABSTRACT

Objective: The goal of the current study is to create a Newcastle disease vaccine based on green synthesised metal oxide nanoparticles and to study the haematological and biochemical effects of this vaccine in chicks.

Methods: Copper Oxide Nanoparticles (CuONPs) from *Momordica charantia* were synthesised biologically. These copper oxide nanoparticles were combined with a commercially available freeze-dried Newcastle Di0sease (ND) vaccination of the live R2B strain to use it as a vaccine delivery method in the current work. Haematological and biochemical parameters were investigated in pre-challenged and post-challenged chicks.

Results: After the injection of copper nanoparticles-based vaccines, it was found that the pre-challenged animals and post challeneged animals showed highly significant difference ($P<0.05$) in their total White Blood Cells (WBC) counts, hemoglobin concentration, hematocrit value, and Erythrocyte Sedimentation Rate (ESR) in comparison to control and live vaccinated groups. It was also investigated that for biochemical parameters After the injection of copper nanoparticles-based vaccines, both pre-challenged animals and post challeneged animals showed highly significant difference ($P<0.05$) in their blood glucose level, serum total protein, creatinine, serum alkaline phosphatase, Aspartate Amino Transferase (ALT) and Alanine Amino Transferasse (AST) in comparison to control and live vaccinated groups.

Conclusion: The vaccine not only makes chicks healthier, but also shields them from the virus that causes Newcastle disease

Keywords: Pre-challenged, Post-challenged, R2B virus, Copper oxide nanoparticle

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INTRODUCTION

Chickens are prone to a variety of infectious diseases that not only negatively damage their productivity and general health but also have serious consequences for human health when ingested.

ND, which is brought on by the Newcastle disease virus (NDV), is an infectious illness that affects poultry and results in high mortality rates and reduced egg output. It was found for the first time in Java, Indonesia, in 1926, and Newcastle-upon-Tyne, England, in 1927, where it also earned its name. Both the host and the virus strain affect the disease's severity [1].

Avian paramyxovirus serotype 1 [APMV-1] virus, which is a member of the genus Avulavirus and the families Paramyxoviridae and Paramyxovirinae of the order Mononegavirales, is the culprit behind Newcastle disease [2, 3]. It is a negatively sense, single-stranded, and non-segmented RNA virus [4]. About 15.2 kb of its genome codes for six structural and two non-structural proteins, including Nucleoprotein (NP), Large RNA polymerase (L), Fusion (F), Hemagglutinin Neuraminidase (HN), Matrix (M), and Phosphor Protein (P) [5-7]. The guanine insertion during transcription of mRNA at the editing site results in the creation of the proteins W and V inside the P gene [8-10]. The primary determinant of viral virulence is the cleavability of protein F, but other proteins, including HN and V, may also have an impact on pathogenicity [11, 12]. The most prevalent protein overall, nucleoprotein gives the Newcastle disease virus (NDV) score helical nucleocapsid structure, and is the primary regulator in viral genome replication [13]. The inclusion of 372 Newcastle Disease Virus (NDV) full genome sequences into GenBank, along with the F and HN sequences, has aided in the phylogenetic characterisation of more virulent genotypes from 1990 to 2016 [14].

Despite the fact that DNA or RNA vaccines have a number of benefits, including affordability, low risk of infection, and the capacity to elicit an immune response against a particular pathogen, there are a number of difficulties associated with their effective delivery to the target sites and the necessity of prime-boost vaccination regimens with other immunogenic agents [15]. This causes molecules to degrade prematurely and prevents the translation of those molecules into effective immunogens [16]. Due to these restrictions, our focus has shifted to a new generation of vaccinations known as subunit vaccines, which are concentrated on a particular part of the pathogen. Subunit vaccines are chosen over other vaccines because they are thoroughly defined and purified, and they have better safety profiles. They have a couple of limitations, however, as an antigen is only weakly immunogenic on its own, necessitating the addition of an adjuvant in the formulation [17].

Therefore, a nanovaccine has been created. A wide variety of nanoparticles are used in the prophylactic and therapeutic development of vaccines by the modern science of nanovaccinology [18]. These nanoparticles work as an adjuvant and are intended to stimulate the immune system to produce more antibodies and longer-lasting immunity. A chemical used in combination with a particular antigen to trigger an immune response that is more potent than the antigen alone is known as an adjuvant [19].

CuONPs have been used as a vaccine delivery method in the current work in an animal model (chickens). CuONPs are now being used for the first time in the delivery of vaccines. CuONPs can be achieved in a variety of ways, but green chemistry principles are becoming more popular because of their ease of use, environmental friendliness, affordability, and capacity to influence several biological pathways. When compared to chemical approaches, green synthesis gives nanoparticles a capping that increases their stability, biocompatibility, and biological activity.

MATERIALS AND METHODS

Selection of host

White Leghorn chicks (*Gallus gallus domesticus*) were chosen as the experimental animals for the current investigation because they were consistently accessible throughout the year and could easily survive and acclimate to laboratory conditions. From the nearby

hatchery in Meerut, Uttar Pradesh we got about 50 White Leghorn chicks that were one day old. For 15 d, they were acclimated in the animal cages under normal circumstances. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India) Animal Ethical Committee (Registration number-384/PO/Re/S/01/CPCSEA) has approved all experimental methods and the facilities used to house the experimental animals. Before experimentation, the chicks were housed in clean wood and steel cages in the animal house and acclimatised to laboratory condition (Temp. 36±2, light 14 h, Darkness 10h period). They were fed on formulated chicks feed. (Hindustan Poultry feed LTD. Meerut India) and provided water daily.

Source of r2b virus

The Indian Veterinary Research Institute, IVRI, Izatnagar, Bareilly provided the R2B NDV strain for research purposes.

Selection of experimental plant

The plant *Momordica charantia* was used as a test subject for the creation of CuONPs. The experimental plant was taken from botany department, Chaudhary Charan Singh University, Meerut, U. P. The identification of plant was also done from the botany department, Chaudhary Charan Singh University, Meerut, U. P. The plant was identified as *Momordica charantia* (Ref no. Bot/PB/351) plant of family Cucurbitaceae.

Chemicals and reagents

$CuSO_4.5H_2O$ (Copper (II) sulfate pentahydrate), distilled water, Sodium Hydroxide (NaOH) and Whattman no. 1 filter paper were used in the current study. All the chemicals were available from Sigma-Aldrich.

Preparation of plant extract

Fresh *M. charantia* fruits were taken from botany department of Chaudhary Charan Singh University, Meerut, U. P. To obtain crude aqueous extract of *Momordica charantia* (CAE-MC), the fruit was thoroughly washed, pulverized to a smaller particle size, and then extracted using the reflux method and a soxhlet device. A 1:10 w/v mixture of the mixer in distilled water was utilised to prepare the aqueous extract, which was then boiled at 50 °C for 40 min. When not in use, the extract was stored at 4 °C after being filtered using Whattman no. 1 filter paper [20].

Green synthesis of copper nanoparticles from *Momordica charantia*

CuONPs were created by adding 0.1M of CuSO4.5H2O solution to crude aqueous extract (CAE-MC), in a 1:3 (v/v) ratio, followed by the addition of NaOH to raise the pH to 11. At 50 °C, the solution was heated until the colour changed to brown, signifying the synthesis of CuONPs. The solution containing the produced CuONPs was centrifuged three times at 5000 rpm after each washing cycle. The pellet was then gathered, dried, and kept at 4 °C until needed [21].

Synthesis of nanoparticle-based vaccine

The CuONPs were combined with a commercially available freeze-dried ND vaccination of the live R2B strain at a viral titer of 105.0 EID50/ml in 5 ml Phosphate-buffered saline (PBS). The solution was then shaken at 4 °C for a whole night before being vortexed and sieved using a 0.45 m syringe filter. The filtrate was then put to use in vaccination tests on lab animals.

Collection of blood and separation of serum

Five chicks from each group were slaughtered for the blood collection after receiving the vaccine for 21 d and being challenged for 14 d. With the use of a sterilized disposable syringe equipped with a (22SWG) hypodermic needle and a cardiac puncture, blood samples were immediately drawn from the heart and collected in a container for later use.

Blood sample centrifuge tubes were centrifuged at 3000 rpm for 15 min after standing in a slanting posture at room temperature for roughly an hour. With the use of a pipette, the supernatant serum was then carefully transferred to sterile plain glass vials for the assessment of the subsequent parameters.

Haematological analysis

Haematological parameters of chicks in each group were altered after 14 d of virus exposure (post-challenged chicks) and after 21 d of post-vaccination (i.e., in pre-challenged chicks). The measurement of Haemoglobin Percentage, Haematocrit Value/Packed Cell Volume (PCV), Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Indices, Total Erythrocyte/Red Blood Cells (RBC) Count, Total Leucocyte/WBC Count, and ESR are all included in the haematological research.

Biochemical analysis

For biochemical profiling, changes in biochemical parameters of chicks in each group were assessed after 14 d of virus exposure (post-challenged chicks) and after 21 d post-vaccination (i.e., in pre-challenged chicks). Blood sugar, serum total protein, cholesterol, uric acid, creatinine, serum acid phosphatase, serum alkaline phosphatase, serum glutamic oxaloacetate transaminase (SGOT), AST, and serum glutamic pyruvic transaminase (SGPT)/ALT are among the biochemical parameters examined.

Statistical analysis

Graph-paid software was used to statistically evaluate the experiment using the one-way ANOVA test. $P<0.05$ were considered significant.

RESULTS

Haematological analysis

When compared to control, it was found that in pre-challenged animals, total RBC and total WBC counts rise while haemoglobin concentration, haematocrit value, and RBC indices fall in both pre-challenged and post-challenged live vaccinated groups. Additionally, when compared to the control group, ESR rises in the live vaccine pre-challenged and post-challenged groups, but marginally falls in the CuONPs based group. In addition, after 14 d of virus exposure in the unvaccinated control group, all animals die from viral infection. However, virus challenge was found to raise the total WBC count and ESR in both the live vaccine group and the CuONPs based vaccine group. It was observed that in post challenged group (Group exposed to R2B virus), control chicks who were not given any vaccine were all dead after the exposure to R2B virus.

Additionally, in both the live vaccine and CuONPs-based vaccine groups, the hemoglobin concentration, haematocrit value, MCV, and MCH decrease. Additionally, when compared to the control, group C's RBC count increased while it stayed constant in the live vaccine group. Parallel to this, MCHC rises in the live vaccine group while slightly falling in the CuONPs-based vaccine group.

Table 1: The table shows the haematological parameters in control, live vaccinated and CuO NP based vaccinated groups in pre-challenged chicks

Pre-challenged parameters (Before virus exposure)	Control	Live vaccine	CuONP based vaccine
Hemoglobin Conc. (g/dl)	9.1±0.13	8±0.92	8.5±0.64**
Hematocrit value	26.99±0.67	22.96±0.17	23.98±0.43**
RBCs (10^6/µl)	1.9±0.08	2±0.13	2.2±0.16
MCV (fl)	141.32±8.6	125.66±5.3	125.58±14.73
MCH (pg)	46.97±2.7	42.74±1.4	43.36±3.9
MCHC (g/dl)	35.13±0.13	33.81±0.9	32.63±0.18
WBC(10^3/µl)	11.64±0.21	13.42±0.2	13.83±0.19**
ESR	2.17±0.17	2.4±0.16	2.04±0.18**

The data are available in the mean±SE of triplet samples. (**represents highly significant difference i.e. $P<0.05$)

Table 2: This table shows the haematological parameters in control, live vaccinated and CuO NP based vaccinated groups in post-challenged chicks

Post-challenged parameters (After virus exposure)	Control+virus	Live vaccine+virus	CuONP based vaccine+virus
Hemoglobin Conc. (g/dl)	0	8.07±0.1	8.76±0.41**
Hematocrit value	0	22.04±1.3	26.11±4.1**
RBCs (10^6/µl)	0	1.9±0.07	2.2±0.065
MCV (fl)	0	117.4±11.1	121.89±9.87
MCH (pg)	0	43.91±2.7	42.23±2.65
MCHC (g/dl)	0	38.01±1.12	34.75±1.43
WBC(10^3/µl)	0	13.89±0.32	14.9±0.27**
ESR	0	2.89±0.8	3.1±0.1**

The data are available in the mean±SE of triplet samples. (** represents highly significant difference i.e. P<0.05)

Biochemical analysis

After 21 d following vaccination, biochemical markers were examined. It has been reported that as compared to control animals, vaccinated animals exhibit raised levels of blood sugar, uric acid, total serum proteins, serum acid phosphatase, ALT, and AST. When compared to the control group, cholesterol levels rise in the live vaccine group while falling in the CuONPs-based vaccine group. When compared to the control group, serum alkaline phosphatase and creatinine levels in the live vaccine group fall while they rise in the CuONPs-based vaccine group. When compared to the control group, both the live vaccination group and the CuONPs-based vaccination group showed a substantial change in biochemical markers. However, it was shown that when animals were post-challenged, the vaccinated animals' blood glucose, total serum proteins, cholesterol, uric acid, creatinine, serum acid phosphatase, and AST levels increased while their serum alkaline phosphatase and ALT levels decreased. It was observed that in post challenged group (Group exposed to R2B virus), control chicks who were not given any vaccine were all dead after the exposure to R2B virus.

Therefore, as compared to control, a substantial change in haematological, and biochemical parameters was seen in both live and CuONPs-based vaccine-treated mice. It has been established that the newly developed vaccine would have the potential to increase chicken sector infectivity and production.

Table 3: This table shows the biochemical parameters in control, live vaccinated and CuO NP based vaccinated group in pre-challenged chick

Pre-challenged parameters (Before virus exposure)	Control	Live Vaccine	CuONP based vaccine
Blood Glucose (mg/dl)	198.14±1.54	244.78±1.32	241.78±0.68**
Serum Total Protein (mg/dl)	3.56±0.09	5.12±0.37	5.02±0.54**
Cholesterol (mg/dl)	155.34±0.91	161.78±0.31	154.4±0.47
Uric acid (mg/dl)	4.89±0.12	5.62±0.26	5.49±0.45
Creatinine (mg/dl)	0.61±0.17	0.49±0.01	0.81±0.04**
Serum Acid Phosphatase (IU/l)	5.62±1.1	7.56±0.99	4.98±0.789
Serum Alkaline Phosphatase (IU/l)	79.11±3.3	32.89±1.04	49.81±0.3**
ALT	9.94±0.59	11.81±0.71	12.02±0.34**
AST	113.01±1.63	124.01±0.23	123.92±0.47 **

The data are available in the mean±SE of triplet samples. (**represents highly significant difference i.e. P<0.05)

Table 4: This table shows the biochemical parameters in control, live vaccinated and CuO NP based vaccinated group in post-challenged chicks

Post-challenged parameters (After virus exposure)	Control+virus	Live vaccine+virus	CuONP Based vaccine+virus
Blood glucose (mg/dl)	0	241.98±3.12	233.81±1.32**
Serum total protein (mg/dl)	0	3.42±0.21	4.1±0.43**
Cholesterol (mg/dl)	0	159.978±1.34	156.54±1.67
Uric acid (mg/dl)	0	6.62±0.09	6.49±0.98
Creatinine (mg/dl)	0	1.49±0.265	1.91±0.237**
Serum acid phosphatase (IU/l)	0	5.516±0.389	5.498±0.254
Serum alkaline phosphatase (IU/l)	0	32.919±2.09	36.01±2.56**
ALT	0	9.16±0.43	9.02±0.097**
AST	0	182.07±1.43	123.82±0.913**

The data are available in the mean±SE of triplet samples. (**represents highly significant difference i.e. P<0.05)

DISCUSSION

ND significantly reduces the value of the chicken business on a global scale. NDV vaccinations may cause mild respiratory illnesses that enhance susceptibility to secondary bacterial diseases [22]. Therefore, the primary need of the poultry industry is the creation of safe and affordable vaccine. CuONPs have served as a medication carrier, vaccine adjuvant, and vaccine delivery method in the current work in an animal model (chickens). *Momordica charantia* fruit extract was used as a cost-and environmentally-friendly source for the extraction of Copper Oxide nanorods (CuO NRs). The colour shift from colourless to light yellow and finally to brownish has been used to validate the production of CuO NPs [23] When the diameters of nanoparticles were studied using Transmission Electron Microscopy (TEM) examination, it was discovered that the CuO NRs had a diameter of 61.48 2 nm and a length of 400–500 nm. Through X-Ray Diffraction (XRD) and Selected Area Diffraction (SAED) patterns, single-crystalline and evenly distributed structures have been observed. The XRD pattern demonstrated the development of monoclinic CuO crystals whish matched the previous study conducted by Muhaimin *et al.* [24]. Results from the UltraViolet Visible (UV-Vis) spectrum, Fourier Transform Infrared Spectroscopy (FTIR), and Energy-dispersive X-ray (EDX) analysis verified the presence of nanoparticles [25].

In the present study, following vaccination with the ND vaccine, groups of chickens were intramuscularly challenged with a narrow virulent strain of the ND virus that contained at least 106 embryo lethal doses (ELD50)/bird [26].

According to the study, CuONPs are competent against NDV and work as an antiviral agent. According to numerous studies, Cu is essential for the generation of erythrocytes, iron metabolism, and haemoglobin. According to studies, within 24 h following the injection of copper nanoparticles, serum haemoglobin concentration, RBC count, and copper-associated protein concentration increase [27]. Depending on earlier research, vaccination did not change blood sugar, total protein, total lipids, cholesterol, or triglyceride levels. ALT activity was unaffected, either. However, when compared to control unvaccinated chicks, a significant decrease in the level of albumin, albumin/globulin ratio, and alkaline phosphatase (ALP) activity was accompanied by an increase in the level of globulin and activities of AST, and lactate dehydrogenase (LDH).

Within six days of the challenge, every chick that showed clinical signs and symptoms of the illness passed away without making a full recovery. While commercially available live vaccines only demonstrated a 60% protective efficacy after immunization, CuONPs from *Momordica charantia* based Nano vaccines demonstrated an 80% protective efficacy [28].

In the current study, vaccinated groups' levels of uric acid and creatinine only marginally rise, but when they are exposed to viruses, their levels rise significantly. This demonstrates the virus's harmful impact on kidney cells, which was consistent with studies that were described. The kidney function test did not reveal any negative effects of vaccination, though. As a result, using the vaccine is safe.

CONCLUSION

In summary, a live vaccination sold commercially does not have an advantage over a vaccine based on CuONPs from *Momordica charantia*. The cause of major haematological and biochemical changes may be under a variety of possible uses, not just in poultry but also in other industries. Here, the use of CuONPs from *Momordica charantia* in conjunction with a live vaccination could solve two issues at once. As a result, the vaccine not only makes chicks healthier, but also shields them from the virus that causes ND. The newly developed vaccine would eventually discover a platform to increase the infectivity and output of the poultry sector. To further improve its commercial application, more research must be made in the future.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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