Direct Research Journal of Veterinary Medicine and Animal Science
Vol. 9(1), Pp. 1-8, July 2024
ISSN:2734-2166
DOI: https://doi.org/10.26765/DRJVMAS13891195
Article Number: DRJVMAS13891195

https://journals.directresearchpublisher.org/index.php/drjafs

Effect of Ethanol Bark Extract of Mahogany (Sweitenia macrophylla) on Viral Shedding in Cockerels Experimentally Infected with Newcastle Disease Virus Kudu 113 Strain

Hauwa'u Umar Mungadi[1]*, Samiru Ladan Argungu[2], Yunusa Alhaji Adamu[1], and Usman Musa[3]

[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.
[2]Zonal Veterinary Clinic Argungu, Kebbi State, Nigeria.
[3]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.
Corresponding Author E-mail: hauwaumarmng@gmail.com

Received11June 2024; Accepted 13 July 2024; Published 18 July 2024

ABSTRACT: Newcastle disease (ND) is a viral disease that affects both domestic and wild birds. Despite routine vaccination, outbreaks are frequently reported in the field. Studies have indicated that traditional medicinal plants provide rich sources of antiviral agents. Effect of ethanol bark extract of Mahogany (*Sweitenia macrophylla*) on ND virus shedding in cockerels experimentally infected with NDV Kudu 113 velogenic strain was determined in this study. Phytochemical analysis revealed that the extract contained some antiviral components and LD_{50} determination indicated that it is safe for consumption at the highest dose given (5500mg/kg body weight). Day old commercial cockerels (150 in number) were obtained and divided into five groups namely; A, B, C, D and E. Group A served as negative control where the birds were given normal feed and water, group B (positive control) were given only the extract, group C were only challenged with velogenic NDV, group D were given the extract after challenge with the virus, and group E were given the extract before challenge. Haemagglutination assay was carried out before experimental infection in groups C, D and E to check antibody titer of NDV. Result revealed that all the groups had unprotective titers. Real-time polymerase chain reaction was carried out to quantify ND viral shedding. The result in copies/ml was highest in group C at day 3 followed by day 5 then day 7 and lowest at day 1. Group D was second in viral shedding; highest at day 3, followed by day 1, day 5 and lowest at day 7. Group E was the lowest in viral shedding where the viral shedding was highest at day 1, followed by day 3, then day 5 and lowest at day 7. Using repeated measure ANOVA within groups C, D and E, difference in viral shedding was statistically significant with $P<0.05$. It was concluded that ethanol bark extract of Mahogany (*Sweitenia macrophylla*) has the potential of reducing Newcastle disease viral shedding in chickens when administered as both prophylactic and treatment agent with lowest shedding when given as prophylaxis. It is recommended that ethanol bark extract of Mahogany (*Sweitenia macrophylla*) should be considered as an alternative to symptomatic therapy done in the management of Newcastle disease.

Keywords: Newcastle disease, *Sweitenia macrophylla*, viral shedding
Citation: Mungadi, H.U., Argungu, S.L., Adamu, Y.A., Musa, U. (2024). Effect of Ethanol Bark Extract of Mahogany (*Sweitenia macrophylla*) on Viral Shedding in Cockerels Experimentally Infected with Newcastle Disease Virus Kudu 113 Strain.Direct Res. J. Vet. Med. Anim. Sci. Vol. 9(1), Pp. 1-8. https://doi.org/10.26765/DRJVMAS13891195. This article is published under the terms of the Creative Commons Attribution License 4.0.

INTRODUCTION

Newcastle disease (ND) is one of the most important viral diseases of birds (Orsi *et al.,* 2010) and is listed as a reportable disease by Office International des Epizooties (OIE) (Aldous and Alexander, 2001).

Notification is required by OIE of any outbreak of ND when it meets certain criteria of virulence (Cattoli *et al.,* 2022). ND is a systemic respiratory disease that is acute, easily transmitted and affects various types of poultry

especially chickens, caused by Newcastle disease virus of the family *Paramyxoviridae* (Tabbu, 2000). The disease is characterized by respiratory and nervous impairment as well as gastrointestinal and reproductive problems. Incubation period of the disease of the natural exposure has been reported to be between 2 to 15 days (Tiwari *et al.,* 2004). Newcastle disease virus (NDV) has been grouped into five (5) pathotypes with respect to tissue tropism and clinical signs viz: Viscerotropic velogenic pathotype also called (Doyle's form) characterized by severe intestine and visceral haemorrhages, Neurotropic velogenic pathotype (Beach's form) characterized by respiratory and neurological signs, Mesogenic pathotype (Beaudette's form) characterized by respiratory disease with mortality in young birds, Lentogenic pathotype (Hitchner's form) characterized by mild respiratory infections and the asymptomatic enteric pathotype with inapparent intestinal infection in chicks or replicates in the intestine without causing any sign and symptoms (Aldous and Alexander, 2001). The capacity of the virus to cause such wide variation in severity of disease has been attributed to a number of factors including health status, environmental condition, host, age and other concurrent infections. Most importantly, variation in virulence means that the detection of ND virus or evidence of infection is insufficient for adequate diagnosis and control measures for avirulent viruses (Aldous and Alexander, 2001; Yassmin *et al.,* 2021).

Despite the use of vaccines to limit the devastating effects of ND virus, ND still occurs in poultry industry worldwide, causing epidemics and significant economic losses (Hu, *et al.,* 2022). Even though current vaccines when administered properly can prevent the outbreak and death from the disease, the virus can infect vaccinated animals and replicate leading to its spread via feces and saliva to other birds (Miller and Koch, 2013; Bello *et al.,* 2018; Mahamud *et al.,* 2022). In recent years, there is a renewed interest in research on the virus due to its pathogenic potential (Naz *et al.,* 2022; Jamil *et al.,* 2022). Recent development in molecular biology and genomic sequencing has greatly opened doors for understanding the biology and replication of the Newcastle disease virus (NDV) (Fellahi and Boudouma, 2021).

The sources of NDV include respiratory secretions/discharges and feces of infected birds and all parts of carcasses, it can survive for several weeks in the environment, most especially in cool weather. Virus is shed during clinical stages and for a limited period during convalescence, wild birds and waterfowl may act as reservoir hosts for lentogenic pathogen of ND; subsequently, these viruses could become virulent following mutation upon establishment in domestic poultry. Infected birds can shed the virus during incubation period and for short time during recovery (Mahamud *et al.,* 2022). Phytotherapy, or herbalism, is defined as the use of plants or herbs to prevent or treat diseases in human and animals. This practice is on the increase among medical practitioners as well as veterinarians and livestock farmers. There have been several reports which have shown the positive outcome of the use of herbal extracts as antiviral agents used in animals as prophylaxis and remedy. The use of herbs may reduce the incidence of drug resistance and may modulate the immune system in preventing viral-related diseases (Yasmin *et al*, (2020). *Sweitenia macrophylla* plants (mahogany) have a long history of use in ethno veterinary medicine and can be a rich source of substances for the treatment of infectious disease such as ND. Also, the *S. macrophylla* tree, like many herbs, is cheap, available, and affordable, therefore can serve as a cheaper alternative in treatment of viral diseases (Duraipandiyan *et al.,* 2006). This study was carried out to determine the effect of ethanol bark extract of Mahogany (*S.macrophylla*) on Newcastle disease virus shedding in chickens experimentally infected with NDV Kudu 113 strain.

MATERIALS AND METHODS

Study area

The study was conducted at poultry pen of the Faculty of Veterinary Medicine Usmanu Danfodiyo University, Sokoto city campus.

Study design

Ethanol extraction of bark of Mahogany (*Sweitenia macrophylla*) was carried out to determine its antiviral effect and subsequent viral shedding in cockerels experimentally infected with Kudu 113 vNDV before and after administration of the extract. Phytochemical constituents as well as safetyof the extract were determined.

One hundred and fifty (150) day old commercial cockerels were obtained and divided into five groups namely; A, B, C, D and E. Group A served as negative control where the birds were given normal feed and water, group B was positive control given only the extract, group C were only challenged with Kudu 113 velogenic strain of Newcastle Disease Virus (vNDVirus), group D were given the ethanol extract of Mahogany bark after challenge with the virus, and group E were given the extract before challenge (Table 1). Antibody titre was evaluated at four weeks before the experimental infection. Shedding of the virus was determined by real time polymerase chain reaction qRT-PCR. The birds were raised for 8 weeks (from November to December 2023).

Ethical clearance

Ethical approval was sought from Institutional Animal Care and Use Committee Usmanu Danfodiyo University Sokoto

with reference number UDUS/IACUC/2024/R05.

Sourcing, Preparation and Extraction of *S. macrophylla* Bark Using Ethanol

Mahogany (*S. macrophylla*) tree was identified in the premises of Usmanu Danfodiyo University Sokoto, Nigeria and the bark were obtained. It was authenticated at Botany Department of Faculty of Biological Science Usmanu Danfodiyo University Sokoto, Nigeria with reference number UDUH/ANS/0123. Extraction of *S. macrophylla* bark was done using ethanol according to Abubakar *et al.* (2018) at the Toxicology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto.

Phytochemical Analysis of Ethanol Bark Extractof *S. macrophylla*

Determination of phytochemical constituents of the extract and qualitative analysis of the crude extract were carried out using standard procedure described by Edeoga *et al.,* (2005).

Determination of Safetyof *S. macrophylla* Bark Extract

Determination of acute oral toxicity of the extract in chickens used for this research was carried out according to OECD (2008).

The Challenge virus (NDV Kudu 113)

Newcastle disease virus Kudu 113 strain inoculum was obtained from Regional Laboratory for Animal influenza and other Transboundary Animal Diseases, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. An ampule of viral content was reconstituted with 10ml of phosphate buffered saline (1:10). Guidelines from the Regional Laboratory for Avian influenza and other transboundary animal diseases were followed. LD_{50} titer of the virus was $10^{8.5}$ per ml with storage temperature of 20^0C±4°C.

Birds sourcing, grouping and experimental infection

Day old commercial cockerels (150 in number) were procured from a hatchery in Ibadan, Nigeria and housed in poultry house of Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto. The birds were fed with commercial chick feed *ad libitum*and provided with clean drinking water. The birds were raised for four (4) weeks and later divided into five groups each containing thirty (30) birds (Groups A, B, C, D and E). Biosecurity measures were taken to prevent any incident of infectious diseases in the poultry house. **Group A:** Negative control group fed with chick mash and clean water *ad libitum* for five weeks.

Group B: Positive control group fed with chick mash and clean water *adlibitum*. At four weeks they were given the plant extract for a period of seven days.

Groups C and D were day old cockerels reared for a period of four weeks, given chick mash and clean water *ad libitum*.

Group C (Positive control group): At four weeks, they were challenged with the diluted velogenic NDV (1:10), 0.1ml intranasal and manifestation of clinical signs of ND was monitored.

Group D (Treatment group): Were given the extract for five days when some signs of ND were observed.

Group E (Prophylactic group): At three weeks five days they were given the plant extract for prophylaxis for two days and then challenged with the diluted virus (1:10) 0.1ml intranasal at four weeks.

Determination of antibody titer against Newcastle disease virus through Haemagglutination Assay

The antibody titer against Newcastle disease virus was determined through Haemagglutination assay.

Viral shedding

Cloacal swabs were taken using sterile swab sticks at days 1, 3, 5, and 7 post challenge from the cockerels and stored in plain sample bottles containing 3mls of phosphate buffered saline at 4^0C to analyze viral shedding. Viral shedding determination was carried out by qRT-PCR analysis for quantification at day 8 post challenge.

RNA extraction and real-time reverse transcription-polymerase chain reaction (RT-PCR) to amplify f gene of Newcastle disease Virus

Total viral RNA extraction was carried out using QIAamp mini viral RNA extraction kit following the manufacturer's instructions. Micro-tube containing the extracted RNA was caped and stored at -20^0 C until required. RT-PCR was performed using previously published primers (Farkas *et al*., 2009) targeted to the F gene (Forward and reverse primers: FT_NDV_VF1 GAY TCY ATC CGY AGG ATA CAA GRG TC 4832–4857 99 and FT_NDV_VR2 AAC CCC AAG AGC TAC ACY RCC 4930–4910). The specificity of primers/probe was tested by using a GenBank BLAST search to exclude the possibility of false-positive results with heterologous sequences. All components of the TransStart® Green qPCR SuperMix on ice were thawed. A master mix was prepared for the reactions by adding for a 20 µl reaction: TransStart® Green qPCR SuperMix, 10 µl, forward primer (10 µM): 0.4

µl, reverse primer (10 µM), 0.4 µl, NDV RNA template, 2 µl (concentration dependent on optimization), nuclease-free water to reach 20 µl total volume. The master mix was added to PCR tubes.

Polymerase chain reaction cycling

The following were performed in Qiagen Rotor-Gene qPCR machine with the following thermal cycling program: Initial denaturation and Enzyme Activation; hold 1 at 55°C for 10 minutes and hold 2 at 95°C for 1 minute, with step1 95 °C for 10 seconds, step2 (annealing) 55°C for 30 seconds and melting at 60°C for 90 seconds on 1st step and 5 seconds on next step and elongation at 72°C for 1minute until the reaction was stopped. PCR cycling was repeated for 45 cycles. The Rotor-Gene Q machine was set up to collect fluorescence data at each cycle in the primer elongation phase according to the manufacturer's instructions.

Statistical analysis

The data collected on viral shedding were recorded and tabulated and analyzed using descriptive statistics and presented in forms of tables and graphs. Analysis of variance (ANOVA) was also used to compare the results.

RESULTS

Phytochemical Determination of Ethanolic Bark Extract of *Sweitenia macrophylla*

Result of phytochemical analysis of ethanolic bark extract of *Sweitenia macrophylla* revealed the presence of secondary metabolites with glycosides and steroids having moderate and high reactions respectively. There were5.4% saponins, 1.66% flavonoids, 0.44% alkaloid, 0.6% anthraquinone, 0.56% tannin and 0.87% phenol.

Safety of the ethanol bark extract of *Sweitenia macropylla*

The extract was found to be safe for consumption by the birds at highest dose given (5500mg/kg body weight) using twelve cockerels for the experiment.

Hemagglutination assay for the experiment groups before infection

The result of haemagglutination assay carried out at four weeks of in all the groups to check antibody titer of NDV revealed that all the groups had unprotective titers.

Viral shedding at different days in groups C, D and E infected with velogenic Newcastle Disease virus in copies per ml

The results of NDV shedding as summarized in (Table 1), showed that the viral shedding in copies/ml was highest in group C (Positive control, challenge only group) at day 3 (1,209,058,509), followed by day 5 (126,974,145) then day 7 (57,586) and lowest at day 1 (19,474). Group D (Challenge plus extract group) was second in viral shedding which was highest in day 3 (109, 457,234), followed by day 1 (16,295), then day 5 (1,400) and lowest in day 7 (584). The group E (extracts plus challenge group) was the least in viral shedding compared to groups C and D. In group E, the viral shedding was highest at day 1(1,363), followed by day 3 (584), day 5 (221) and loweat in day7 (77) shown in (Table 1). Statistically using repeated measure ANOVA within the groups (C, D and E), the difference in results from all the groups were statistically significant in viral shedding with $P<0.05$.

Table 1: Viral shedding at different days in groups C, D and E infected with velogenic Newcastle virus.

Group	Day 1	Day 3 DNA Copies/mL	Day 5	Day 7
C	19,474	1,209,058,509	126,974,145	57,586
D	16,295	109, 457,234	1,400	584
E	1,363	584	221	77

Raw Data for A. Green qPCR Cycling

Figure 1 shows the raw data of A. Green PCR cycling involving the florescence against each cycle.

Quantification data for A. Green qPCR Cycling

Figure 2 shows the quantification data of A Green PCR. cycling involving the florescence against each cycle. Figure 3 shows standard curve of the Qpcr.

DISCUSSION

Routine vaccination program against ND is practiced in the study area like in other parts of the country, but still ND outbreaks are frequently reported in the field. Traditional medicinal plants are known to provide rich sources of antiviral agents (Chiang *et al.,* 2003). There were many researches through challenge studies in relation to the use of tree extracts in management of Newcastle disease by determining antiviral, prophylaxis and immunological effects of the extracts on velogenic Newcastle disease virus. This study investigated the effects of ethanol bark extract of Mahogany (*Sweitenia macrophylla)* in shedding of velogenic Newcastle disease virus (kudu 113) at preventive and treatment levels in cockerels experimentally infected with the virus. Cloacal swab samples of the infected cockerels belonging to 3 different

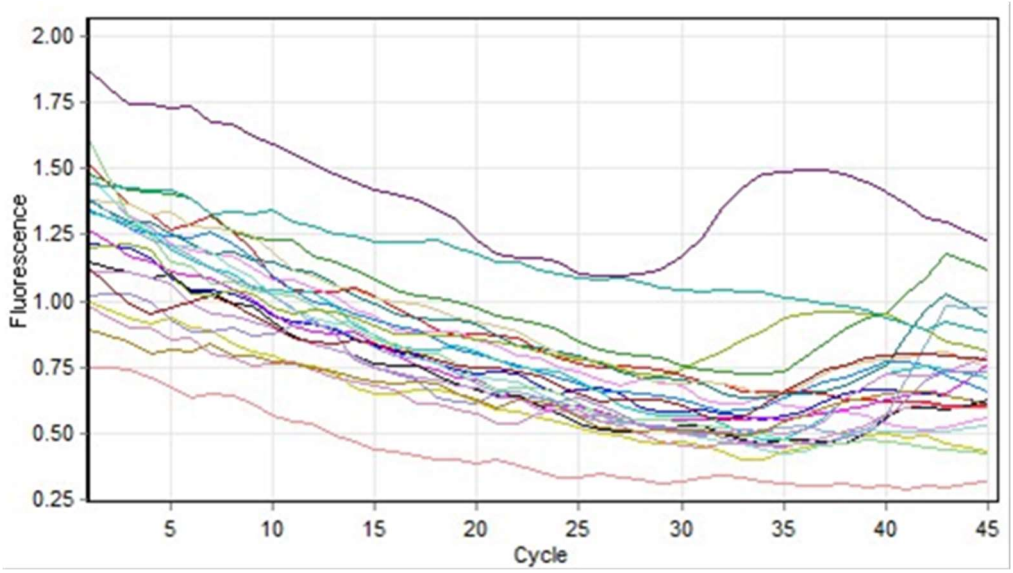

Figure 1: Raw Data For A Green qPCR Cycling

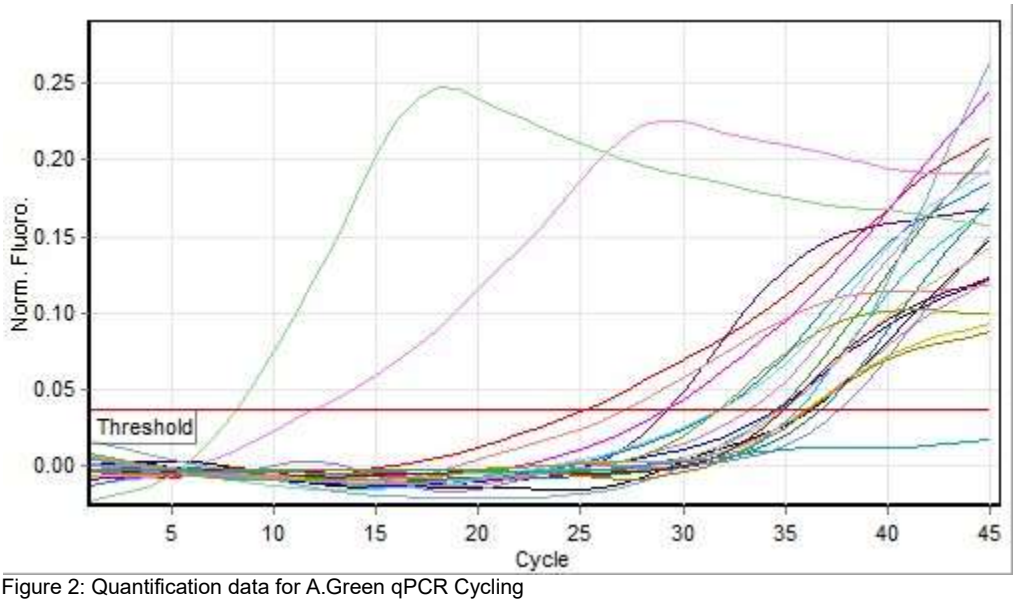

Figure 2: Quantification data for A.Green qPCR Cycling

groups viz; group C with 30 cockerels infected with the virus and not given the plant extract, group D with 30 cockerels given the extract after infection and group E with 30 cockerels given the extract before infection. The cloacal swab samples were used in the study to assess viral load. NDV shedding from cloacal and oropharyngeal routes were compared in study conducted by Sajo *et al.* (2023), the results showed a higher prevalence (42.2%) of NDV shedding from cloacal route while the oropharyngeal route was (26.6%). This shows that cloacal route can be a preferred route for checking ND virus shedding. *Sweitenia macrophylla* plant contains metabolites such as Alkaloid, Coumarins, Terpenes, Flavonoids, Anthraquinones and naphthoquinones which possess antiviral activities by interfering with viral replication (Jassim and Naiji, 2003).Frage *et al*. (2022) stated that alkaloids at concentrations lower than 10µM showed effective anti-viral activities with no significant toxicity. Viol (2013) reported that the small phytochemicals such as alkaloids, flavonoids, phenols, sugar bearing molecules and terpenes contained in *S. macrophylla* were found to contain anti-viral agents among other components. The tree has glycosides and carbohydrates which are considered possessing a helpful immune mechanism by

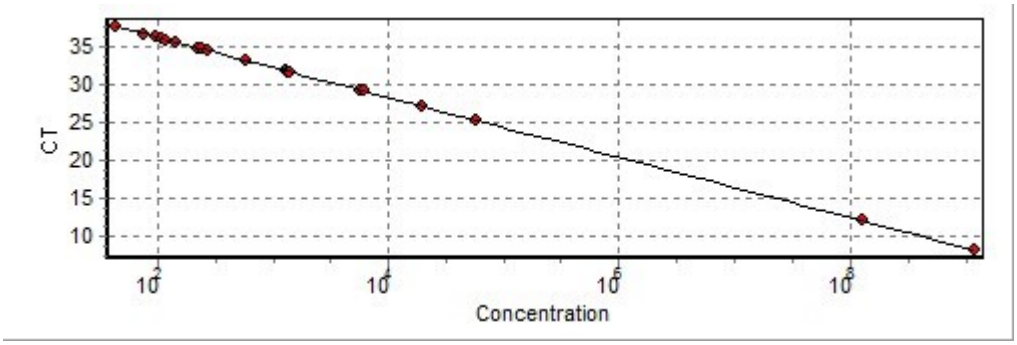

Figure 3: Standard curve of qPCR showing cycle threshold against Log Copies/mL

enhancing body and dietary additives (Yadav*et al.,* 2014). In the present study, *S. macrophylla* was found to contain 5.4% saponins, 1.66% flavonoids, 0.44% alkaloid, 0.6% anthraquinone, 0.56% tannin and 0.87% phenol. Steroids and glycosides were positive with high and moderate reactions respectively. Research sources indicate that saponin compounds have physiological effects such as anti-viral, blood purifying, anti-inflammatory, anti-diabetic, anti-atherosclerotic, immunomodulatory effects and serve protective functions like gastroprotective and hypolipidaemic (Banno *et al.,* 2004). Kumar *et al*. (2013) reported that flavonoids apart from possessing antioxidative, coronary heart disease prevention, hepatoprotective, anti-inflammatory and anti-cancer activities, and some flavonoids exhibit potential antiviral activities. Glycosides contained in *S. macrophylla* extract are predicted to have anti-diarrheal effects due to their ability to prevent the activities of autocoids and prostaglandin (Tiwari *et al.,* 2011). Anthraquinone has been found to exhibit in vitro antiviral activity against poliovirus. Zohaib *et al.* (2015) reported anti-Cytomegalovirus activity of anthraquinones. Zahra *et al.* (2020) reported tannin to possess antiviral, antiparasitic, antioxidant and anti-inflammatory properties which can be valuable in replacing some antibiotics in animal feed. Maghsoud *et al.* (2022) reported tannins to improveimmunity in animals. Sonia *et al.* (2015) reported that phenolic compound from leave of *Sweitenia macrophylla* has in vitro cytoprotective effects and antioxidant capacity. Wu *et al.* (2012) mentioned that *S. macrophylla* demonstrated antiviral effect in vivo on Hepatitis C virus by its activity from its component (3-hydroxy caruilignan C) through suppression of RNA replication by the combination of 3-HCL-C, interferon-Alpha, and RNA virus polymerase inhibitor, protease inhibitor. The suppression was by inducing interferon stimulated response element transcription and interferon dependent antiviral gene expression. Ashraf *et al.,* (2017) reported in-vivo antiviral potential of *Glycyrrhiza glabra* extract against Newcastle disease virus. The findings of Irivboje *et al.,* (2021) on a survey of phytogenic extracts commonly used in the control of Newcastle disease in indigenous chickens raised in Yewa South local government area of Ogun State Nigeria revealed positive outcome. Feji *et al.,* (2019) reported anti-viral activity of *Phyllanthus amarus* leaf extract against NDV in unvaccinated broilers. Hala *et al.,* (2022) reported immunological effect of *Moringea oleifera* leaf extract on vaccinated and unvaccinated Hubbard chickens experimentally infected with NDV. Priya *et al.,* (2022) reported antiviral effect of herbal mixture (garlic, nilavembu, turmeric, coriander and fenugreek) on Newcastle disease virus in ovo, using specific pathogen free eggs. Results of studies conducted by Ampitan *et al.,* (2023) revealed prophylactic potency of methanol extract of *Momordica balsamina* against Avian paramyxovirus-1 infection in Broilers.

The results of NDV shedding in the present study showed the viral load in (copies/ml) in groups C, D and E at days 1, 3, 5 and 7 post challenge with Kudu 113 NDV. In group C (Positive control, challenge only group) the viral load was highest (1,209,058,509) at day 3, followed by day 5 (126,974,145), then day 7 (57,586) and lowest in day 1 (19,474). This could mean at day 1, the virus was at multiplication phase, at day 3, the virus had multiplied and the immune response was at lag phase therefore the load of the virus was high. The decrease in the viral load at days 5 and 7 might be as a result of natural immune response at exponential phase.

Group D (the treatment group) was second in viral shedding, and this could be attributed to administration of the extract after observation of clinical signs of ND in the group (day 3post challenge) for possible treatment. The viral load in this group was found to be highest at day 3 (109, 457,234), followed by day 1 (16,295), day 5 (1,400) and lowest at day 7 (584). The reason the viral load was highest at day 3 is possibly the virus had multiplied and the birds had not fully developed immunity against it, at days

5 and 7 the extract was believed to have had effects on the virus while at day one the virus had not multiplied to a large number and no intervention was made.

Group E (prophylaxis group) was the least in viral shedding compared to groups C and D. Mild clinical signs of ND started manifesting at day 4 post-challenge. In this group, viral shedding was highest at day 1 (1,363), followed by day 3 (584), then day 5 (221) and lowest at day 7 (77). The gradual reduction of viral load seen could be as a result of the effects of the extract given before the experimental infection which minimized multiplication of the virus. Using repeated measure ANOVA within the groups (C, D and E), the viral load was statistically significantwithin the groups with ($P<0.05$).

Conclusion

From the results of this research, it is concluded that ethanol bark extract of Mahogany (*Sweitenia macrophylla*) has the potential of reducing Newcastle disease viral shedding in chickens when administered as prophylactic and treatment agent with least shedding when given for prophylaxis.

Recommendation

It is recommended that ethanol bark extract of Mahogany (*Sweitenia macrophylla*) should be considered as an alternative to symptomatic antibiotic and other drugs therapy usually practiced in the management of Newcastle disease.

Conflict of Interest

There was no conflict of interest in whatever way among the authors of this work.

Acknowledgements

The authors hereby acknowledge the assistance of Prof. P.A. Abdu of Department of Veterinary Medicine Ahmadu Bello University Zaria, the technical support of staff of Faculty of Pharmaceutical Sciences, Centre for Advanced Medical Research and Training (CAMRET), Clinical Pathology Laboratory, Faculty of Veterinary Medicine and Fleming laboratory.

REFERENCES

Aldous, E. W. and Alexander, D. J. (2001). Detection and differentiation of Newcastle disease virus (Avian paramyxovirus type 1). *Avian Virology*. **30**(2):117.

Ampitan, T. A., Garba, M. H., Adekanmbi, D. I., Ampitan, A. A. A., Adelakum K. M. (2023). Prophylatic Potency of Methalonic Extract of *Momordica balsamina* L. Against Avian Paramyxovirus-1 Infection in Broiler Chicken. *Egytian Journal of Production*, **60**(1):25-32.

Ashraf, A., Ashraf, M.M., Rafiqe, A., Aslam, B., Galani, S., Zafar, S., Asad, F., Asghar, R.D., AKram, S., Ahmed, H., Shah, S.M.A., Asif, R. (2017). In vivo antiviral potential of Glycyrrhiza glabra extract against Newcastle disease virus. *Pakistan Journal of Pharmaceutical Sciences*,**30** (2):567-572.

Banno, N., Akihisa, T., Yasukawa, K., Higashihara, H., Ukiya, M., Watanabe, K. (2004). Triterpene acids from the leaves of Perilla frutescenes and their anti-inflammatory and antitumour-promoting effects. *Bioscience Biotechnology and Biochemistry*, 68, 85-90.

Bello, M. B., Yusoff, K., Ideris, A., Bejo, M.H., Peeters, B.P.H., Omar, A. (2018). Diagnostic and Vaccination Approaches for Newcastle Disease Virus in Poultry: The Current and Emerging Perspectives. *International Biomedical Research Journal*, 10.1155/2018/7278459 10.3390/ani11123567.

Cattoli, G., Susta, L., Terregino, C., Brown, C. (2011). A review of Newcastle disease of field recognition and current methods of laboratory detection. *Journal of Veterinary Diagnostic Investigation* **23**(4):637-65

Chiang, L., C., Cheng, H. Y., Chiang, W., and Lin, C.C. (2003). Antiviral activity of eight commonly used medicinal plants in Taiwan. *Journal of Clinical Medicine* 31(6)897-905.

Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S. (2006). Antimicrobial activity of some ethno medicinal plants used by Paliyar tribe from Tamil Nadu, India. *Complementary and Alternative Medicine,* 6 edition 35. Evolution **10**(1):26-35.

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemicals constituent of some Nigeria medical plants. *African Journal of Biotechnology,* **4** (7): 685-688.

Farkas, T., Székely, E., Belák, S., & Kiss, I. (2009). Real-time PCR-based pathotyping of Newcastle disease virus by use of TaqMan minor groove binder probes. *Journal of Clinical Microbiology,* 47(7), 2114–2123. 10.1128/JCM.01652-08 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Feji, C. O., Oladunmoye, M. K., Adebayo, I. A., Adebolu, T. T. (2019). Anti-Viral Effect of *Phyllanthus amarus* Leaf Extract Against Newcastle Disease Virus in Broilers. *Asian Plant Research Journal*, **2**(4). Pp1-9. ISSN 2581-9992.

Fellahi, S. and Boudouma F. (2021). Virus de la maladie de Newcastle chez la volaille: Perspectives actuelles et émergentes [Newcastle disease virus in poultry: Current and emerging perspectives]. *Revue Marocaine des Sciences Agronomiques et Vétérinaires*, 9(3): 453-460. Available athttps://www.agromaroc.com/index.php/Actes_IAVH2/article/view/1021.

Frage, L. A., Bader, S., A. and Aman, U. (2022). Potential Antiviral Action of Alkaloids. *Molecules*; 27(3):903. Doi:10.3390/molecules27030903PMCID:PMC8839337 | PMID:35164173.

Hala, M. N., Tolba, A. A., Elmaaty, G. K., Farag, D. A., Mansour, H. A. E. (2022). Immunological Effect of *Moringa Oleifera* leaf extracts on Vaccinated and Non-Vaccinated Hubbard Chickens Experimentally Infected with Newcastle Virus. *Saudi journal of Biological sciences*, **29**(1):420-426.

Hu, Z., He, X., Deng, J., Hu, J., and Liu, X. (2022). Current situation and future direction of Newcastle disease vaccines. *Veterinary Research*, 53:Pp 99. DOI: https://www.doi.org/10.1186/s13567-022-01118-w

Irivboje., O. A., Olufaya, O. O., Irivboje, Y. I. (2021). A survey on Phytogenic Extract Commonly Used in the Control of Newcastle Disease in Indigeneous Chickens Raised in Yewa South Local Government Area of Nigeria, *Nigerian Journal of Animal Science*, **23**(1):86-94.

Jamil, F., Aslam, L., Laraib, Ali H., Shoukat, K., Rasheed, M.A., Raza, S., and Ibrahim, M. (2022). An in silico study of derivative of Newcastle disease virus epitopes based vaccine against hemagglutunin neuraminidase protein. *Journal of Animal Science*, 101: skac375, DOI: https://www.doi.org/10.1093/jas/skac375.Epub ahead of print. PMID: 36371806; PMCID: PMC9883717

Jassim, S. A. and Naiji, M. A. (2003). Novel antiviral agents: A medicinal plant perspective. *Journal of Applied Microbiology.***95**(1)412-427.

Kumar, S., and Pandey, A. k. (2013). Chemistry and biological activities of flavonoids: An overview. *Journal of World Science*. **16** (10)1155.

Maghsoud, B., Aristide, M., Valiollah, P., Adem, K., Muhammad, J., Huseyin, E., Pasquale, D,. Jose, M. L. (2022). Tannin in Ruminant Review. *Multidisciplinary Digital Publishing Institute Journal*. 27(23): 8273 Doi 10.3390/molecules27238273 PMID: 36500366.

Mahamud, S.N.A., Bello, M.B., Ideris, A. and Omar, A.R. (2022). Efficacy of genotype-matched Newcastle disease virus vaccine formulated in carboxymethyl sago starch acid hydrogel in chickens vaccinated via different routes. *Journal of Veterinary Science*, 23(4): e25. DOI: https://www.doi.org/10.4142/jvs.21242.

Miller, P. J. and Koch, G. (2013). Newcastle disease. In: D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, V. L. Nair (Editors), *Diseases of Poultry,* 13th Edition. Wiley-Blackwell.,Pp. 89-107, 120-130.

Naz, D., Rahman, S., Aslam, M.A., and Muhammad, F. (2022). Newcastle disease virus in poultry with an interface as a human vector. *Veterinary Vaccine*, 1(1): 100003. DOI:https://www.doi.org/10.1016/j.vetvac.2

OECD, O., (2008). Guideline 425: Acute oral toxicity—Up-and- down procedure. *OECD Guidelines for the Testing ofChemicals*, *2*.

Office International Des Epizooties OIE. (2012). Newcastle disease. *Manual of Diagnostic Test and Vaccine for Terrestrial Animals.* Chapter 2.3.14 http// www. OIE.

Orsi, M. A., Doretto, J. R., Camillo, S. C. A., Reischak, D., Ribeiro S., Ramazzoti, A., Mendonca, A. O., Spilki (2010). Prevalence of Newcastle disease virus in Broiler chickens (*Gallus gallus*) in Brazil. *Brazilian Journal of Microbiology.**41**:349-357*

Priya, M. S., Murthy T. R. G. K., Vijayanand T (2022). Antiviral Effect of Herbal Mixture (Garlic nilavembu, turmeric coriander, and fenugreek) Against Newcastle Disease Virus in Ovo. *Journal of Applied Poultry Research*, **31**(1):100229.

Sajo, M. U., Hamisu, T. M., Saidu, A. S., Haruna, N. M., Shettima, Y. M., El-Yuguda , A. D., Abubakar, M. B., Madu, E. D., Waziri, M. M. (2023). Comparative Analysis of Newcastle Disease Virus Shedding from Naturally Infected Breeds of Poultry in Maiduguri, Nigeria. *Sahel Journal of Veterinary Sciences*, 20(3). eISSN: print ISSN: 1117-6210.

Sonia, Pamplona., Paulo Sa., Dielly Lopse., Edmar Costa., Elizabeth Yamada., Consuelo E. Silva., Mara Arruda., Jesus Souza., Milton Da Silva. (2015).In Vitro Cytoprotective Effects and Antioxidant Capacity of Phenolic Compound from Leave of Sweitenia macrophylla.

Tabbu, C. R. (2000). Penyakit Ayam dan Penanggulangannya. Poultry Diseases and Their Treatment. Penerbit Kanisius. *Yogyakarta.* Vol. 1, Pp14-20.

Tiwari, A. K., Katari, R. S., Nanthakumar, T., Dash, B. B., Desai, G. (2004). Differential detection of Newcastle disease virus strain by degenerate primers base RT-PCR. *Journal Comparative Immunology Microbiology Infectious Disease*. Pp **27**:163-169.

Viol, D. I., (2013). Screening of traditional medicinal plants from Zimbabwe for phytochemistry, antioxidant, antimicrobial, antiviral and toxicological activities. http://ir.uz.ac.zw/bitstream/ 10646/1105/1/*Viol-thesis.*

Wu, S. F., Lin, C. k., Chuang, Y. S., Chang, F. R., Tseng, C. K., Wu, Y. C., Lee, J. C. (2012). Anti Hepatitis C virus activity of 3-hydroxy caruilignan C from *Swietenia macrophylla* stems. *Journal of Viral Hepatitis*. **19**:364-370.

Yasmin, A.R., Chia, S.L., Looi, Q.H., Omar, A.R., Noordin, M.M., Ideris, A. (2020). Herbal extracts as antiviral agents. *Feed Additives*. 115–132. doi: 10.1016/B978-0-12-814700-9.00007-8. Epub 2020 Jan 24. PMCID: PMC7150227.

Yassmin, E., Walied A., Ehad Kotb E., Ghada Allam E., Ahmed E., Nagwan E., Samah M. M., Maha S. L., Samad M. M., Maha S. L., Ashraf A., Samah A. A. (2021). Pathogenesis of Velogenic Genotype VII.I.I Newcastle Disease Virus Isolated From Chicken in Egypt Via Different Inoculation Routes: Molecular, Histopathological, and Immunuhistochemistry Study. *National Centre for Biotechnology Information*, **11**(12):3567, doi:

Zahra, M. H., Tlou, G. M., Letlhogonolo, S., Monnye, M. (2020). The Effects of Tannins in Monogastric Animals with Special Reference to Alternative Feed Ingredients. *Multidisciplinary Digital Publishing Institute Journal*. 25(20):4680 Doi 10.3390/molecules25204680 PMID: 33066367.

Zohaib, A., Zainab, A., Yali Z., Zachary, M., Deborah, S. P., Hard I. P., Glen, E. K., Alison K., and Michael A. M. (2015). Anti- Cytomegalovirus Activity of the Anthraquinone Atanyl Blue, *Natioanl Centre for Biotechnology Information*. 114:86-95.