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Effects of Ethanol Bark Extract of Mahogany (*Swietenia macrophylla*) on Clinico-Pathological Parameters of Cockerels Challenged with Kudu 113 NDV Strain

[1]Hauwa'u Umar Mungadi, [2]Samiru Ladan Argungu, [1]YunusaAlhaji Adamu and [3]Usman Musa
[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine,
Usmanu Danfodiyo University Sokoto, Sokoto, Nigeria
[2]Zonal Veterinary Clinic Argungu, Kebbi State, Nigeria
[3]Department of Veterinary Pathology, Faculty of Veterinary Medicine,
Usmanu Danfodiyo University Sokoto, Sokoto, Nigeria

ABSTRACT

Background and Objective: Newcastle disease (ND) is an economically important disease and a major threat to the poultry industry. This study was carried out to investigate the effects of ethanol bark extract of mahogany (*Swietenia macrophylla*) in the management of ND in chickens challenged with Kudu 113, a velogenic strain of Newcastle Disease Virus (vNDVirus). **Materials and Methods:** Ethanol bark extraction of *Swietenia macrophylla* was carried out, phytochemical and proximate analysis was done and its safety on chickens was determined using up and procedure. One hundred and fifty, 4 weeks old Cockerels were used for the study. The 5 groups were constituting of A: Negative control given normal feed and water, B: Positive control given only the extract, C: Given normal feed and water and challenged with the virus, D: Given the extract after challenge and E: Given the extract before challenge. **Results:** The extract contained some secondary metabolites and had a wide safety margin. Group E had delayed onset of ND by 24 hrs compared to C and D. Morbidity rate was (100.0%) in group C, followed by group D (90.0%) and group E (73.3%) while the mortality rate was (93.3%) in group C, followed by D (86.7%) and lowest in E (66.7%) with 95% confidence interval using Chi-square and $p<0.05$ considered significant. **Conclusion:** Oral administration of ethanol bark extract of *S. macrophylla* in drinking water at 5500 mg/kg resulted in low morbidity and mortality rates, mild to moderate clinical signs and mild to moderate post-mortem lesions in chickens challenged with Newcastle disease virus Kudu 113 strain.

KEYWORDS

Clinico-pathological, Cockerels, Kudu 113 strain, Newcastle disease, *Swietenia macrophylla*

INTRODUCTION

Ethnoveterinary medicine is the oldest method of curing diseases and infections[1]. Many phytochemicals of several chemical classes have *in vitro* inhibitory effects on all types of microorganis[2]. Aloe species, *Azadirachta indica* (neem) and *Commiphora swynnertonii* (Burtt) are plants often used in management of diseases of animals[3]. Meliaceae plants are attracting considerable interest because of their significant

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biological activities. Secondary metabolites like anthraquinones, alkaloids, flavonoids, tannins, phenolic compounds, terpenoids and glycosides found in the plants hold various pharmacological properties and have antimicrobial activities[4]. *Swietenia macrophylla* plants have a long history of use in ethnoveterinary medicine and can be a rich source of substances for the treatment of infectious diseases such as ND. Also the *S. macrophylla* belonging to Meliaceae family (mahogany tree) is available and affordable[5]. Newcastle disease (ND) is a severe viral disease that affects birds worldwide. The etiology is Newcastle disease virus of the genus *Avulovirus* and family of Paramyxoviridae[6]. The disease presents in three forms: Lentogenic or mild, mesogenic or moderate and velogenic or very virulent form. The lentogenic strains cause few ND outbreaks while the velogenic strains are more virulent and cause high mortality rate[7].

The clinical signs of ND mostly of respiratory, but depression, nervous manifestations or diarrhea may be the prominent clinical form and virus is present in all parts of the carcass of an infected bird and highly contagious[8]. The clinical signs vary widely and are dependent on factors such as: The strain of the virus, species of bird affected, the age of the host (young birds are most susceptible), concurrent infection with other organisms, environmental stress and immune status[9]. Mortality is variable but can be as high as 100%[10]. It is regarded throughout the world as one of the two most important diseases of poultry and other birds, the other being highly pathogenic Avian influenza. Clinical signs and postmortem (PM) lesions include diarrhea, expiratory dyspnea, cloacal haemorrhage, focal glandular, gastric bleeding or ulcers, intestinal mucosal bleeding and necrosis of the pancreas or the spleen. Mean death time (MDT) measured in hours to death, intracerebral pathogenicity index (ICPI) based on an average score of clinical signs over time (mininum 0.0-maximum 2.0) and intravenous pathogenicity indices (IVPI) are used as pathogenecity index for ND[9]. Laboratory testing in embryos or chickens using these standard pathogenicity parameters can be done; all involve the use of numeric criteria[10]. The velogenic strains are characterised by gross lesions which are often significant and include periorbital swelling, swelling of the head, interstitial oedema or oedema of peritracheal tissue of the neck, petechial and ecchymotic hemorrhages of the mucosa of proventriculus. Other lesions include edema, hemorrhages, necrosis or ulceration of respiratory/digestive lymphoid tissues, including cecal tonsil and Peyers's patches. Edema, hemorrhages or degeneration of ovaries are also seen although less evident in the older birds, haemorrhages of the thymus and bursa of fabricius may occur, spleen may appear enlarged and friable and dark red or mottled, some cases may present pulmonary oedema and pancreatic necrosis[11]. The Kudu 113, an NDV isolated from ducks in Nigeria, has been classified as a velogenic strain and is believed to be one of the VNDVs causing ND in chickens in Nigeria[12].

This study was carried out to investigate the effects of ethanol bark extract of mahogany (*Swietenia macrophylla*) in management of ND in chickens challenged with velogenic strain of Newcastle Disease Virus (vNDVirus) through assessment of clinico-pathologic parameters (disease onset, clinical signs and gross postmortem lesions) of the infected chickens.

MATERIALS AND METHODS

Study area: The study was carried out at poultry pen of the Faculty of Veterinary Medicine City Campus, Usmanu Danfodiyo University, Sokoto, Nigeria from August, 2023 to January, 2024.

Ethical clearance: Considering animal rights, ethical clearance was sought and approval was obtained from Institutional Animal Care and Use Committee Usmanu Danfodiyo University Sokoto with reference number UDUS/IACUC/2024/R05.

Study design: Ethanol extraction of the bark of mahogany (*Swietenia macrophylla*) was carried out to experiment with its effect on the management of Newcastle disease in chickens. Phytochemical constituents as well as LD_{50} of the extract were determined. Twelve chickens were used to determine of safety of the extract. One hundred and fifty day-old commercial Cockerels were obtained and used for the study. Antibody titre was evaluated before the experimental infection. Disease onset, clinical signs and postmortem lesions were observed.

Table 1: Design for experimental infection of Cockerels with vNDV and treated with *S. macrophylla* ethanol bark extract

Group	Number of birds	Treatment	Aim
A	30	None	Negative control
B	30	Extract only	Positive control
C	30	Challenge only	Positive control
D	30	Challenge+extract	Therapeutic treatment
E	30	Extract+challenge	Prophylactic treatment

Plant material: The 2 kg of bark of mahogany (*S. macrophylla*) were obtained in the premises of Usmanu Danfodiyo University Sokoto, Nigeria. The plant was authenticated at Department of Botany, Faculty of Biological Science, Usmanu Danfodiyo University Sokoto, Nigeria with reference number UDUH/ANS/0123.

Preparation and extraction of *S. macrophylla* bark using ethanol: The ethanol extraction of *S. macrophylla* bark was done according to Falah *et al.*[13] at the Toxicology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria.

Phytochemical determination of *S. macrophylla* bark extract: The phytochemical test was carried out on the extract using standard procedures to find out its chemical constituents. Qualitative analysis of the crude extract was carried out as described by Edeoga *et al.*[14].

Safety of the extract: The determination of acute oral toxicity (Median Lethal dose) of ethanol bark extract of *S. macrophylla* in chickens in this study was carried out according to Abubakar *et al.*[15]. Twelve separate Cockerels were used for the experiment.

Experimental birds: One hundred and fifty day old commercial Cockerels procured from CHI® hatchery, Ibadan, Nigeria, they were housed in a cage at the poultry unit of Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria. The birds were fed with commercial chick feed *ad libitum* and provided with clean drinking water. The birds were reared in a battery cage from day old to 4 weeks and later divided into 5 groups each containing thirty birds (Group A, B, C, D and E). Group C, D and E were transferred to different cages in a separate room away from the battery cage at 4 weeks before being challenged with Kudu 113 NDV. The remaining control groups (A and B) not challenged with the virus were left in the cage.

Biosecurity measures: Strict biosecurity measures were taken to prevent the occurrence and spread of disease.

Newcastle Disease Challenge Virus (NDV Kudu 113): Newcastle disease virus Kudu 113 strain was isolated from ducks in Nigeria. The viral inoculum was obtained from Regional Laboratory for Animal Influenza and Other Transboundary Animal Diseases, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. An ampoule of viral content was reconstituted with 10 mL of phosphate-buffered saline (PBS) (1:10). Guidelines from the laboratory were followed. The LD_{50} titer of the virus was 108.5 per mL with a storage temperature of 20±4°C.

Groupings and experimental infection: The day old Cockerels were 30 in number for each group, they were grouped A to E as follows (summary in Table 1).

Group A: Served as negative control group fed with chick mash and clean water *ad libitum* for 5 weeks.

Group B: Served as a positive control group fed with chick mash and clean water *ad libitum*. From 4 weeks they were given the plant extract in drinking water at the dose rate of 3000 mg/kg of bird/day for a period of 7 days.

Group C: Served as a positive control group containing day old Cockerels reared for a period of 4 weeks. They were given chick mash and clean water *ad libitum*. At 4 weeks, they were challenged with the diluted velogenic NDV (1:10), 0.1 mL intraocular and clinical signs of ND were looked out for.

Group D: Served as a treatment group reared for a period of 4 weeks and given chick mash and clean water *ad libitum*. They were then challenged with the diluted velogenic NDV virus (1:10), 0.1 mL intraocular followed by the extract at the dose rate of 3000 mg/kg of bird/day for 5 days when signs of ND started manifesting.

Group E: Served as a prophylactic group given chick mash and clean water *ad libitum*. At 3 weeks and 5 days they were given the plant extract at the dose rate of 3000 mg/kg of bird/day for prophylaxis for 2 days and then challenged with the diluted virus (1:10) 0.1 mL intraocular at 4 weeks.

Determination of antibody titer against Newcastle disease virus through haemagglutination assay: The antibody titer of the challenged Cockerels against Newcastle disease virus was determined according to procedure described by Killian[16]. Protective antibody titre value is 1:32 for Newcastle disease[17].

Clinico-pathological determination: The clinico-pathological determination (disease onset, clinical signs and gross pathological lesions of ND) was carried out according to Oyebanji *et al*.[18].

Statistical analysis: The data collected on disease onset, haemmaglutination test, clinical signs, morbidity and mortality rates, as well as post-mortem findings were recorded, tabulated and analyzed using descriptive statistics and presented in forms of percentages, tables and pictures showing the severity of lesions where necessary. The SPSS 21.0 version was used and values where $p<0.05$ were considered to be statistically significant using Chi-square.

RESULTS

Phytochemical determination of ethanol bark extract of *Swietenia macrophylla*: The result of phytochemical analysis of the ethanol bark extract of *Swietenia macrophylla* revealed the presence of secondary metabolites (alkaloid, tannins, saponins, flavonoids, anthraquinone, glycoside, cardiac glycoside, terpenes, steroids and phenols).

LD_{50} of *Swietenia macrophylla*: The extract was found to be safe for consumption by the birds at highest dose given (5500 mg/kg b.wt./day).

Hemagglutination assay in 4 weeks old Cockerels before experimental infection with vNDV: The result of haemagglutination assay carried out at 4 weeks of age in group C, D and E revealed that each group had antibody titre of 1:20. That indicated group C, D and E had unprotective antibody titres against Newcastle disease before the experiment.

Onset and manifestation of clinical signs of Newcastle disease following experimental infection of 4 weeks old Cockerels with vNDV: The onset of ND in this study was noticed at day 3 post-challenge in group C and D while it was noticed 4 days post-challenge in group E (prophylaxis group) (Table 2). Table 3 presented the percentages of observed clinical signs of ND in each group while Fig. 1 showed percentage morbidity. Morbidity rate was (100%) in group C, followed by group D (90.0%) and group E (73.3%) while the mortality rate was (93.3%) in group C, followed by D (86.7%) and lowest in E (66.7%). Figure 2 shows percentage mortality in group C, D and E on day 4, 6, 8, 10 and 12. There was significant statistical difference between group C and D with group E, in overall morbidity rate and morbidity and mortality rates in day six ($p<0.5$).

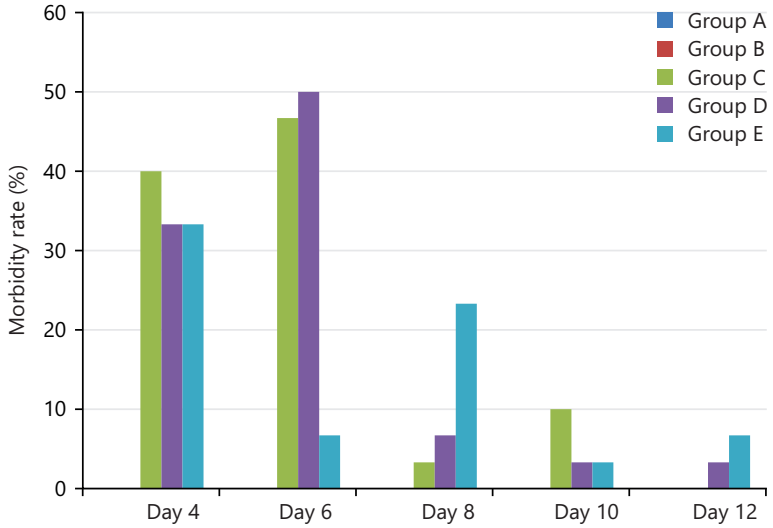

Fig. 1: Percentage morbidity for 30 Cockerels in each group experimentally infected with vNDV at 4 weeks of age on day 4, 6, 8, 10 and 12 post-challenge

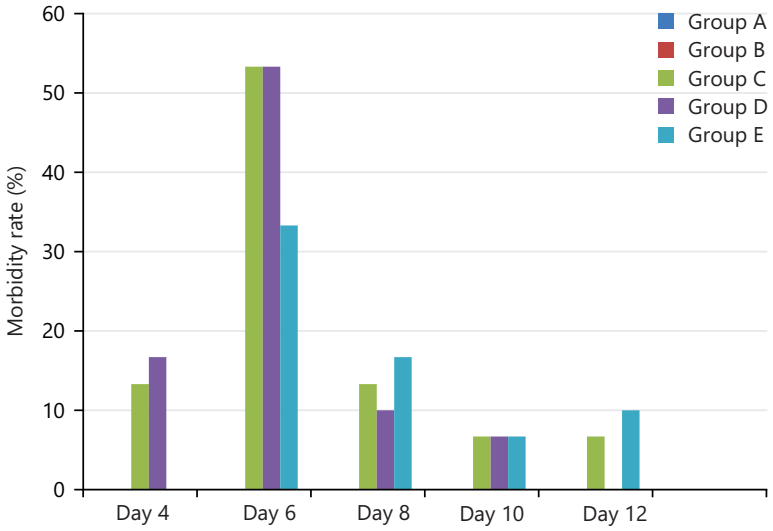

Fig. 2: Percentage mortality for 30 Cockerels in each group experimentally infected with vNDV at 4 weeks of age on day 4, 6, 8, 10 and 12 post-challenge

Table 2: Manifestation of clinical signs of Newcastle disease in Cockerels experimentally infected with vNDV at 4 weeks of age

Group	Day 1	Day 2	Day 3	Day 4	Day 5
A	-	-	-	-	-
B	-	-	-	-	-
C	-	-	+	+	+
D	-	-	+	+	+
E	-	-	-	+	+

+: Presence of clinical signs and -: Absence of clinical signs

Gross lesions in Cockerels experimentally infected with vNDV at 4 weeks of age: The postmortem gross lesions of the Cockerels in group A and B were 0% while for group C, D and E following challenge with vNDV Kudu 113 showed various degrees of pathology on day 4, 6, 8, 10 and 12. The postmortem lesions were scored into three categories based on the degree of manifestation: (1 = Mild, 2 = Moderate and 3 = Severe). Severe petechial hemorrhages of proventriculus (score 3) were observed in Cockerels of group C, moderate (score 2) in group D and mild (score 1) in group E as shown in Fig. 3 and 4. The carcasses were dehydrated; there were also greenish intestinal contents in all the challenged groups.

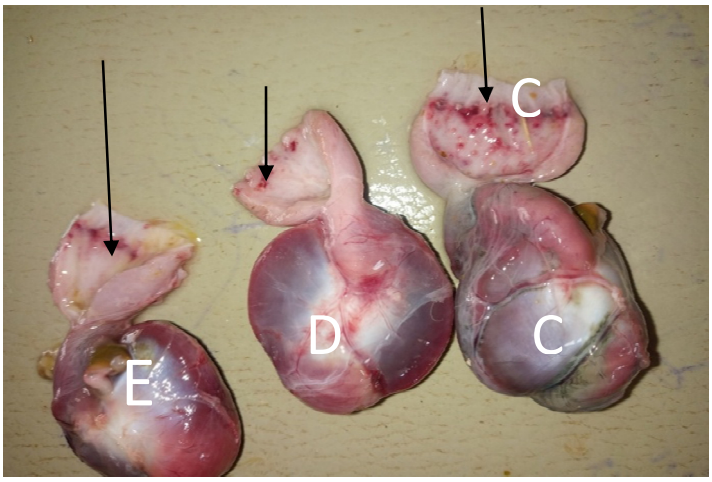

Fig. 3: Petechial hemorrhages on proventriculus of Cockerels (arrow) in group C, D and E on day 4 post-challenge with vNDV

Group C which did not receive any treatment after experimental infection with NDV showed severe hemorrhage while groups D and E showed moderate hemorrhages after receiving *S. macrophylla* bark ethanol extract as therapeutic and prophylactic treatments for ND, respectively

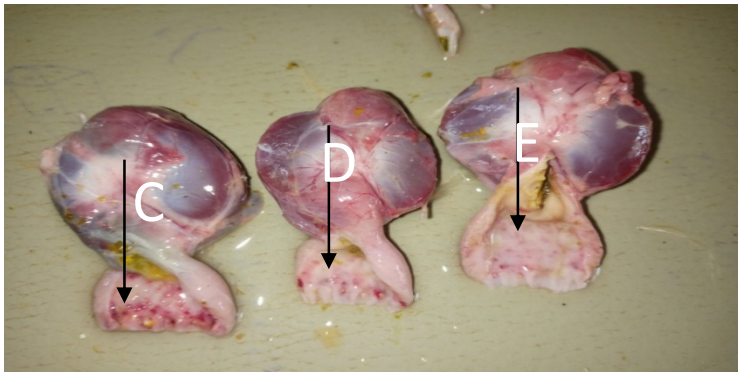

Fig. 4: Petechial hemorrhages on proventriculus of Cockerels (arrow) in group C, D and E on day 6 post-challenge with vNDV

Group C which did not receive any treatment after experimental infection with NDV showed severe hemorrhage, group D showed moderate hemorrhage and group E showed mild hemorrhage after receiving *S. macrophylla* bark ethanol extract as therapeutic and prophylactic treatments for ND, respectively

Table 3: Observed clinical signs following experimental infection of 4 weeks old Cockerels with vNDV

Clinical signs	Group C (%)	Group D (%)	Group E (%)
Depression	93.3	86.7	53.3
Ruffled feathers	93.3	90.0	73.3
Sneezing	20.0	20.0	0.0
Tracheal rales	23.3	16.7	10.0
diarrhoea	83.3	70.0	50.0
Prostration	86.7	76.7	70.0
Paresis of wings	0.0	0.0	6.7
Torticollis	0.0	0.0	6.7
Death	93.3	86.7	66.7

No statistically significant association was observed using Chi-square in all the clinical signs ($p > 0.05$) except for depression, sneezing and diarrhea ($p < 0.05$)

DISCUSSION

The manifestation of signs of ND in this study was observed in Cockerels in group C (challenge-only group), D (treatment group) and E (prophylaxis group) challenged with NDV Kudu 113 velogenic strain of NDV. This is because all the Cockerels used in this experiment had antibody titres low for protection

against NDV which supports the result of Aldous and Alexander[19] on assessment of haemagglutination inhibition titres before vaccination against Newcastle disease. The clinical signs of ND manifested in these groups are the same as reported by other researchers on ND in different parts of the world like[20-22]. This experiment showed that the challenged groups and to which ethanol bark extract of *S. macrophylla* was administered (group D and E) had a milder effect of ND compared to group C which was only infected with the virus. Group C manifested severe clinical signs of a velogenic form of ND. The course of ND pattern post-challenge in this research followed the usual course of vNDV from 2 to 7 days post-infection. This was agreed with the findings of Oyebanji *et al.*[18] who carried out a clinicopathological evaluation of Newcastle disease virus vaccination using gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents in chickens challenged with NDV. Nooruzzaman *et al.*[23] also reported similar post-mortem findings in chickens experimentally infected with vNDV in Bangladesh. The delay in the manifestation of clinical signs by one day in prophylaxis group compared with group C and D (infected only and treatment groups, respectively) shows ethanol bark extract of *S. macrophylla* can delay the onset of ND. This may be attributed to antiviral effect possessed by the extract. The delay in the manifestation of mild signs of ND in the prophylactic group E and treatment group D compared to more severe lesions in challenge challenge-only group C could be attributed to antiviral and anti-inflammatory composition of the extract. Jassim and Naji[24] and Wu *et al.*[25] reported that *S. macrophylla* contains notable metabolites that comprise alkaloids, coumarins, terpenes, flavonoids, anthraquinones and naphthoquinones which possess aniviral activities by interfering with viral replication. An assay of superoxide anion generated by neutrophils in response to fMet-Phe showed anti-inflammatory effects on linomoid compounds: Swietemahonin E, 6-O-acetyi-swietemahonin G[26]. Maiti *et al.*[27] reported that methanol seed extract of *S. macrophylla* has shown antibacterial effect on some strains of *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* which could cause secondary infection in the course of ND. Therefore, the ethanol extract used in this study might have prevented or controlled the activity of bacteria that would have possibly caused secondary infection as commonly seen in ND which leads to manifestation of different clinical signs.

The glycosides contained in the plant are predicted to have anti-diarrheal effects because they have the potential to prevent autacoids and prostaglandin[28]. The tannin compounds present in the extract have been shown to reduce the rate of defecation and improve the consistency of feces as reported by Maiti *et al.*[27] in their *in vivo* study which revealed that seeds of *S. macrophylla* may be a source of antidiarrhoeal drug in the future. In addition, the absence or milder neurologic signs observed in the experimentally infected groups may be attributed to the fact that the extract has been found to provide significant protection to primary cerebellar cultures against oxidative stress from the work of Pamplona *et al.*[29]. The authors also reported that the bark of *S. macrophylla* has been associated with potent antioxidant activity due to the presence of catechin and epicatechin.

In a similar work, Salisu *et al.*[30] reported antiviral effect of aqueous extract of *Momordica balsamina* and *Cucumis metuliferus* fruits on Newcaslte disease in pullets. Their findings were in agreement with the current findings due to the fact that both plants have antiviral effects especially on NDV. The results revealed curability of *M. balsamina* for ND at 2.5 g/100 L of water with recorded lower clinical signs and higher antibody titer, while the same fruit had the best results for prophylaxis at 5.0 g/100 L of water with least clinical signs and highest antibody titers.

Total percentages of morbidity in group C, D and E were 100.0, 90.6 and 73.3%, respectively and total percentages of mortality in group C, D and E were 93.3, 86.7 and 66.7%, respectively. Survival rates in group C, D and E, were 6.7, 13.3 and 33.3%, respectively. There was a significant statistical difference between group C and D with group E, in overall morbidity rate and morbidity and mortality rates in day 6 ($p<0.5$). At day 6 post infection with NDV, it is assumed that high level of vireamia has reached and presence or absence of body protection will determine the morbidity and mortality. Therefore it is

assumed that the *S. macrophylla* extract lowered the morbidity and mortality rates in the Cockerels infected with vNDV. Ampitan *et al*.[31] reported methanol extract of *Momordica balsamina* at 2.5 g/100 L of water to have curative abilities on Avian paramyxovirus-1 infection in broilers. Also, the research of Eze *et al*.[32] revealed survival of 0, 32, 88 and 100% in chickens in groups IV, I, II and III, respectively that were given daily oral treatment of methanol extract of *Moringa oleifera* at 200 mg/kg b.wt., from day 42 until day 56 of age. All the groups were challenged with Kudu 113 velogenic strain of ND virus on day 56 of age Groups II and III at 42 days of age were vaccinated with the La Sota strain of ND vaccine. Group I was not vaccinated, while IV was left as untreated/unvaccinated control. The results of this research were similar to what was obtained in this study. Similarly, the study by Faeji *et al*.[33] revealed n-hexane leaf extract from *Phyllanthus amarus* to possess significant antiviral activities against ND virus in broilers. Prophylactic administration of the extract at 500 mg/L was found to be more effective in minimizing the rates of mortality of birds infected with NDV compared to the administration for therapeutic purposes.

On the contrary, Okoroafor *et al*.[34], after their studies on the effect of methanol stem bark extract of *Azadirachta indica* on morbidity and mortality of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113), had 100% mortality of the infected birds. This indicated that oral administration of methanol stems bark extract of *Azadirachta indica* does not protect birds from ND as in *S. macrophylla* extract.

CONCLUSION AND RECOMMENDATIONS

Administration of ethanol bark extract of *Swietenia macrophylla* in drinking water resulted in lowering morbidity and mortality rates and severity of postmortem lesions of Newcastle disease in chickens. This suggests that the extract has an antiviral effect. Total elimination of the disease is dependent on good management practices and biosecurity of poultry houses. Higher concentration of the extract than what was given in this study within a safe range (up to 5500 mg/kg of bird/day) or an increase in the duration of administration of the extract from what was given in this study, especially for prophylaxis is recommended to assess better treatment and prophylaxis result. Similar studies are recommended to be conducted on other parts of the plant such as the leaves and roots to come up with the best management option.

SIGNIFICANCE STATEMENT

This study evaluated the efficacy of ethanol bark extract of *Swietenia macrophylla* for the treatment of Newcastle disease. The extract was found to contain secondary metabolites and was safe for chickens at a concentration of 5500 mg/kg orally. One hundred and fifty grower Cockerels were used for the study. The 5 groups of 30 chickens each were formed A and B served as negative and positive controls, respectively while C was infected with Kudu 113 velogenic ND virus without any treatment, D was given the extract after infection for therapeutic treatment and E was given the extract for prophylactic treatment. Group C showed severe disease followed by group D and lastly group E. This study indicates that the extract has the potential for treatment of ND.

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