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Research Article
Effects of Hydromethanol Extract of Stem Bark of *Anacardium occidentale* on Gross and Histopathological changes Associated with Experimental Velogenic Newcastle Disease Virus Infection in Chickens

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Abstract
Objective: The aim of this study was to evaluate the effect of hydromethanol stem bark extract of *Anacardium occidentale* on the clinical and pathologic manifestations of velogenic Newcastle disease virus infection in chickens. **Materials and Methods:** Two hundred chickens were randomly assigned to 8 groups (Groups 1-8) comprising 25 birds in each group. At 6 weeks of age chickens in groups 1, 2 and 3 were each drenched with 1000, 500 and 250 mg/kg body weight of hydromethanol stem bark extract of *Anacardium occidentale*, respectively (prophylactic groups) for 7 days. All the chickens in groups 1, 2, 3, 4, 5, 6, 7 were later inoculated with velogenic Newcastle disease virus intramuscularly. On observation of clinical signs on day 2 post infection, chickens in groups 4, 5 and 6 were each drenched with 1000, 500, 250 mg/kg body weight of hydromethanol stem bark extract of *Anacardium occidentale*, respectively (therapeutic groups) for 7 days. Group 7 was infected and not treated while group 8 was uninfected and untreated. The birds were weighed before and after treatment. **Results:** There was significant ($p<0.05$) reduction in weight and variation in the gross, histopathological changes and mortality patterns. **Conclusion:** It can be concluded that hydromethanol bark extract of *Anacardium occidentale*, can reduce the lesion and mortality in chickens infected with velogenic Newcastle disease virus infection.

Key words: *Anacardium occidentale*, chickens, poultry disease, poultry industry, stem bark extract, velogenic Newcastle disease virus

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry industry is globally being threatened by a highly devastating poultry disease of birds known as Newcastle disease. Outbreaks of Newcastle disease (ND) caused by virulent Newcastle disease virus (vNDV) have been identified as limiting factor to the growth of the poultry industry. Velogenic ND (vND) is endemic in many parts of the world including countries in Asia, the Middle East, Africa and Central and South America[1].The disease was first reported in Newcastle Upon Tyne, in England in the year 1926 and later in Nigeria in 1957[2-4]. The disease is caused by ND virus (NDV) that belongs to the genus *Avian orthoavulavirus* 1 (AOAV-1), subfamily *avulavirinae* and family *Paramyxoviridae*[5,6]. The *Paramyxoviruses* isolated from avian species have been classified into eleven subtypes designated APMV-1 to APMV-11 by serological testing and phylogenetic analysis[7]. NDV strains have been classified into 18 genotypes (Class II, Genotypes I-XVIII) based on phylogenetic analysis with the partial hyper variable nucleotide sequences of the F gene[8]. The disease is present globally causing severe losses in the poultry industry[9]. It is associated with severe respiratory, gastrointestinal and neurological lesions in chicken leading to high morbidity and mortality and affects over 250 species of birds of all ages[10]. Newcastle disease has caused devastation of the Nigerian poultry industry which has resulted in many farmers abandoning poultry business[11]. Many countries where poultry are raised commercially rely on vaccination to keep the disease under control. However, outbreaks have been reported in vaccinated populations[12]. Furthermore, it has been reported that vaccination can protect against the clinical signs of vNDV infection but not against the shedding of the vNDV in the faeces, saliva and egg in chickens and turkeys[12-17], atrophy of the lymphoid organs[15,18,19] and drop in egg production[15,20]. In many countries of Africa, Middle East and Asia where the local chickens are on free range and constitute reservoirs of infection to commercial poultry biosecurity is very poorly practiced. So, effective means of prevention and control of vNDV infection in poultry is still yet to be discovered.

The medicinal use of cashew tree (*Anacardium occidentale)* (AO) has been reported globally. *Anacardium occidentale* belongs to the family *Anacardiaceae* and popularly known as cashew tree, originated from Brazil. The *Anacardiaceae* family consists of several plants with immense pharmacological activity. Of all the plants in *Anacardiaceae* family AO has been reported to have immense pharmacological and therapeutic activities[21]. Stem bark extract of AO possesses phyto-constituents such as saponins, tannins and flavonoids, which have been reported to exert antioxidant activities[22-24]. *Anacardium occidentale* tree has been known as multipurpose tree whose leaf, stem and bark extracts are used extensively for treatment of different diseases[22,25,26]. The effect of stem extract of AO on haematology and serum biochemistry has been observed to be relatively safe in chicken[27]. Antibacterial activity of stem bark extract of AO has been established by *in vitro* studies using bacterial sensitivity tests in petri dishes. There is dearth of information on the effect of hydromethanol stem bark extract of AO in diseases of chickens. The aim of this study was to investigate the effect of hydromethanol stem bark extract of AO on pathology of vNDV infection in chickens.

MATERIALS AND METHODS

Collection and identification of the AO stem bark: The fresh stem bark of cashew tree was sourced from Nguru cashew Plantation in Nsukka Local Government Area, Enugu State of Southeast Nigeria. The specimens were identified by a taxonomist in Herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka (UNN). The samples were kept in the Departmental herbarium section (UNN|H.AO|2018.1).

Preparation and extraction of AO stem bark: The pieces of stem barks were chopped into small bits and dried at room temperature on top of laboratory bench and were ground into coarse powder using the hammer mill. The pulverized fresh stem bark were extracted using cold maceration by soaking 1000 g in 2 litres of 70% hydro-methanol for 48 hrs with intermittent shaking. The extract was filtered using No. 1 Whatman filter paper and the filtrate was evaporated to dryness in a rotary evaporator (Buchi, Switzerland). The dried extract was stored in a refrigerator at 4°C. The solubility of the extract was carried out using distilled water.

The brine shrimp lethality and acute toxicity test of stem bark: The results of brine shrimp lethality and acute toxicity test of stem bark have been described in an earlier publication[28].

Experimental animals and design: Two hundred one-day-old cockerels were purchased from reputable local commercial hatchery. They were kept in isolation at the departmental poultry disease experimental unit. Brooding was by deep litter. Feed and water were supplied *ad libitum.* At six weeks of age, the birds were randomly assigned to eight groups (1-8) of

25 chickens each. Groups 1, 2 and 3 were drenched daily with 1000, 500, 250 mg/kg body weight (BW) of the extract respectively, for 7 days. These constituted the prophylactic groups. All the birds in groups 1, 2, 3, 4, 5, 6 and 7 were inoculated with 0.1 mL of the NDV inoculum intramuscularly (IM). The therapeutic groups (groups 4, 5 and 6) were drenched with 1000, 500, 250 mg/kg BW of the extract following the observation of the clinical signs on day 2 PI. Birds in group 7 were challenged but not treated (positive control), while birds in group 8 were neither infected nor treated (negative control). The principles governing the humane use and conduct of experiments with animals were strictly observed during this study and the experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty Veterinary Medicine, UNN (Approval Reference Number: FVM-UNN-IACUC-2019-0448).

Preparation of Newcastle disease virus inoculum: The velogenic viscerotropic NDV (vvNDV), duck/Nigeria/Plateau/Kuru/113/1992 obtained from the National Veterinary Research Institute, Vom, Nigeria, was used. It was isolated from an apparently healthy duck and characterized by Echeonwu *et al.*[29] and Igwe *et al.*[30]. The strain belongs to NDV class II, genotype XVII[31]. The inoculum had a median embryo infective dose (EID_{50}) of $10^{6.46}$ per mL.

Clinical signs and body weights: The chickens were observed twice daily for clinical signs and mortality. Ten chickens were randomly selected and weighed in each group on days 0, 7 post prophylactic treatment (PPT) and 3, 10 post inoculation (PI).

Pathology examination

Gross pathology: Post-mortem examination was carried out on dead carcass on days 3 and 6 PI. The distribution and persistence of the lesions were also observed and recorded.

Histopathological study: This was also carried out in lymphoid organs (spleen, bursa of Fabricius and thymus) on days 3 and 6 PI. The organs were fixed in 10% neutral buffered formalin and processed by paraffin embedding technique using the method of OIE (Animal Health)[32]. Sections were cut at 5 micron thickness with automatic section cutting machine (Leica, Germany) and stained with haematoxylin and eosin (H and E) using the method of Suvarna *et al.*[33]. The slides were studied under the light microscope and the pictures were taken using 2.0 Photometric mutican.

Virus isolation

Virus Isolation and Identification: Samples of the bursa, thymus, spleen and brain were collected from 3 dead chickens in each group on days 3 and 6 PI. Virus isolation was done in embryonated chicken eggs using the method of OIE[32]. The tissues were processed as a pool according to standard protocol.

Serology: Two milliliter of blood was collected from 10 chickens randomly selected in each group on days 0 PI. The serum samples were assayed for HI antibody using the HA/HI method of OIE (Animal Health)[32]. The geometrical mean titre (GMT) was calculated using the Tube Method by Villegas and Purchase[34]

Statistical analysis: One-way analysis of variance (ANOVA) was used to analyze the results using statistical packages version 15.0 computer software. Variant means were separated Post-hoc using the Least Significant Difference (LSD) methods. The level of significance was accepted at $p \leq 0.05$. Summary of the data from all groups were presented as Mean ± SEM in tables.

RESULTS

Clinical signs of Newcastle disease: Effects of hydro-methanolic stem bark extract of *Anacardium occidentale* (AO) were determined in experimental infection of vNDV in chickens by observing the clinical signs and post mortem changes for 13 days PI.

The prophylactic groups: The clinical signs appeared first in chickens of groups 1 and 7 (groups that received highest dose and non treated group respectively), on day 2 PI. The clinical signs observed were drop in feed and water consumption and depression. On day 3 PI all the birds in positive control group (group 7) were (100%) depressed. In this group, birds were showing signs of dyspnoea, coughing with frothing and croaking sound and serous ocular discharges, anorexia and greenish diarrhoea, while birds in groups 1, 2 and 3 (prophylactic groups) were passing green to yellowish diarrhoea which lasted for few days. Mortalities were recorded on day 3 PI in all the groups except group 8 (Negative control group). The birds in all the infected groups showed torticollis, incoordination, coughing with frothing and croaking sound. The birds were also shaking and jerking their heads upward and downward on day 4 PI in all the groups but the clinical signs appeared more severe in groups 1 and 7 when compared with birds in groups 2 and 3. In groups 2 and

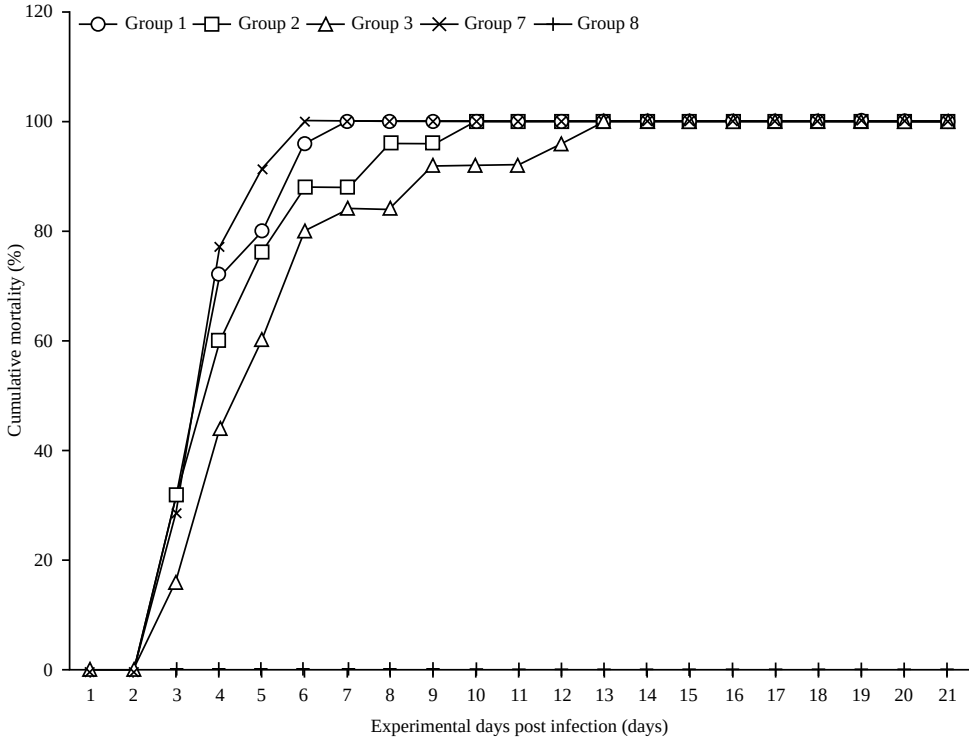

Fig. 1: Cumulative mortality pattern in groups* of cockerels treated prophylactically with varied doses of *Anacardium occidentale* stem bark extract and infected with velogenic Newcastle disease virus

*Group 1: Treated with prophylactic 1000 mg/kg extract and infected, Group 2: Treated with prophylactic 500 mg/kg extract and infected, Group 3: Treated with prophylactic 250 mg/kg extract and infected, Group 7: Infected Untreated Control and Group 8: Uninfected Untreated Control

3 few birds showed torticolis and wing paralysis. The mortality was 100% in all the infected groups but the pattern of mortality varied. Group 7 (positive control group) had 100% mortality on day 6 PI while groups 1, 2 and 3 had 100% mortality on days 7, 10 and 13 PI, respectively (Fig. 1). In group 8 (Negative control group) no mortality was observed throughout the experimental period. The persistence and distribution of the lesions in prophylactic are presented in Table 1 and 2. These lesions were more severe in birds in groups 1 and 2 when compare to birds in group 3 that received the lower dose of the extract.

Therapeutic groups: Clinical signs were observed in one bird on day 2 PI in group 4 and treatment with the AO stem bark extract started the same day. On day 3 PI clinical signs were seen in birds in all the groups except in birds in control group 8. The clinical signs observed were similar to those of the prophylactic groups. Mortality was first recorded on day 3 PI in all the groups. Percentage mortalities recorded in all the groups are shown in Fig. 2. In the infected therapeutic groups, 100% mortality occurred in group 7 (infected and not treated) on day 6 PI, while the group that received lowest dose of the extract had 100% mortality on day 15 PI (Fig. 2).

Changes in body weights: On days 0 and 7 post administration (PA), there was no significant difference ($p>0.05$) in mean BW of all the birds in all the groups (Table 3) while on day 3 PI significant difference ($p<0.05$) in BW was observed in group 3 when compared with group 7. The mean BW of all the birds did not differ significantly ($p>0.05$) on days 0 and 3 PI in birds that were infected and given therapeutic doses of the extract (Table 4).

Gross lesions: The lesions in the infected groups were congestion of the skeletal muscles, haemorrhages of the proventricular glands forming band between the crop and the proventriculus, intestinal ulcers, swollen and haemorrhagic caecal tonsils. In the prophylactic section, the atrophy of the bursa, spleen and thymus was more severe in positive control group (group 7) when compare to other infected and treated groups (Fig. 3-6). The atrophy observed in group 3 that received the lowest dose was very mild when compared to other treated groups. The distribution and persistence of the lesions, in chickens of group 1 and 2 was mild when compared with group 3 which received the lowest dose of the extract (Table 1 and 2). There was no proventricular haemorrhage in the group 3 chickens throughout the experiment.

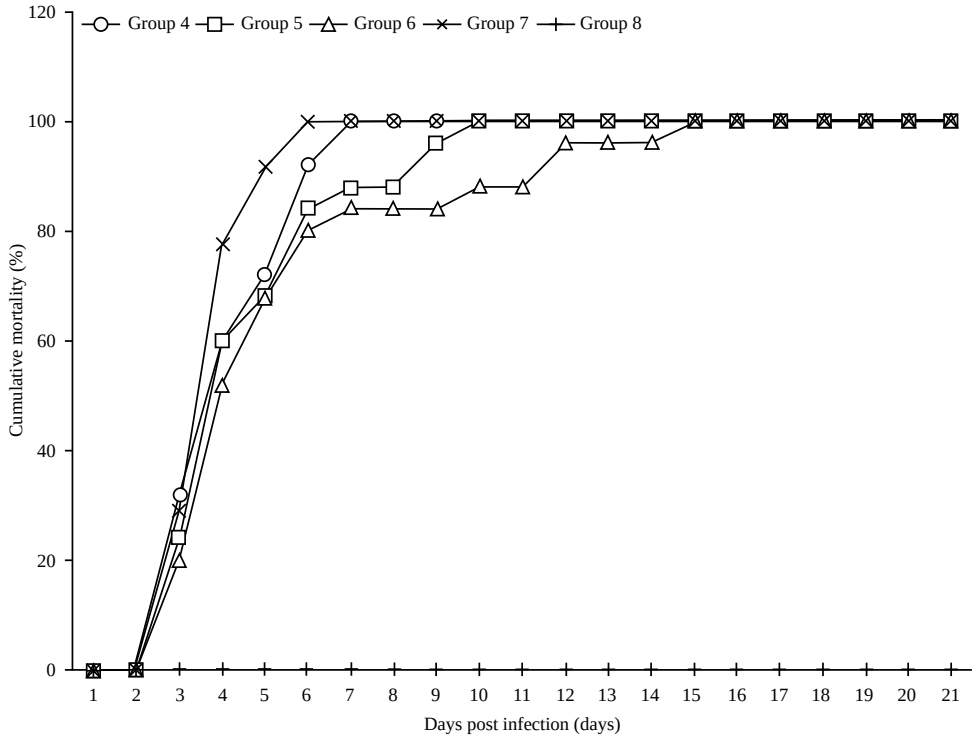

Fig. 2: Cumulative mortality pattern in groups* of cockerels infected with Newcastle disease virus and treated with varied therapeutic doses of stem bark extract of *Anacardium occidentale*

*Groups: 4: Infected and treated with therapeutic 1000 mg/kg extract, 5: Infected and treated with therapeutic 500 mg/kg extract, 6: Infected and treated with therapeutic 250 mg/kg extract, 7: Infected Untreated Control and 8: Uninfected untreated control

Table 1: Distribution and persistence of the gross lesions in prophylactic groups on day 3 PI

		Groups				
Organs lesions	Day 3 PI	1	2	3	7	8
Muscles	Congestion of thighs and breast	3/3	3/3	1/3	3/3	0/3
Proventriculus	Hemorrhage	2/3	1/3	0/3	3/3	0/3
Bursa	Atrophy/enlargement	3/3	0/3	3/3	0/3	1/3
		0/3	3/3	0/3	0/3	0/3
Spleen	Atrophy/enlargement	1/3	0/3	1/3	0/3	1/3
		0/3	1/3	0/3	0/3	0/3
Thymus	Atrophy	3/3	2/3	1/3	3/3	0/3
Caecal tonsil	Caecal tonsil hemorrhage	2/3	2/3	1/3	3/3	0/3
Kidney	Congestion and enlargement	1/3	1/3	0/3	1/3	0/3
Liver	Parboiled	2/3	0/3	0/3	1/3	0/3
Intestine	Button-like ulcers	3/3	1/3	1/3	2/3	0/3

The gross lesions observed in the therapeutic section (Group 4-6) followed the same pattern as seen in prophylactic section (Table 5 and 6). The atrophy of the lymphoid organs (bursa, spleen and thymus) was also more severe in group 7 when compared with infected and treated groups.

Histopathology: On day 3 PI, microscopic lesions observed in bursa in birds of group 7 were ballooning degeneration, necrosis, depletion of lymphocytes and hyperplasia of follicular epithelium (Fig. 7a). In group 3 that received the lowest dose of the extract in prophylactic groups, the bursa showed mild depletion of lymphocytes (Fig. 7b). The spleen and thymus also showed necrosis and depletion of lymphocytes. Fibrin deposition was observed in the thymus which was more severe in group 7 (Fig. 8a) when compare to group 3 that received lower dose of the extract in prophylactic group (Fig. 8b).

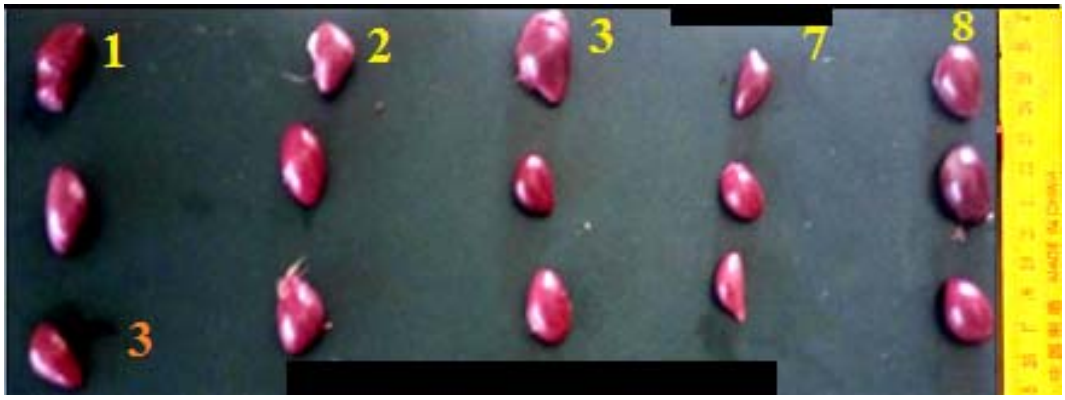

Fig. 3: Spleen in prophylactic groups showing atrophy in group 7 on day 3 PI

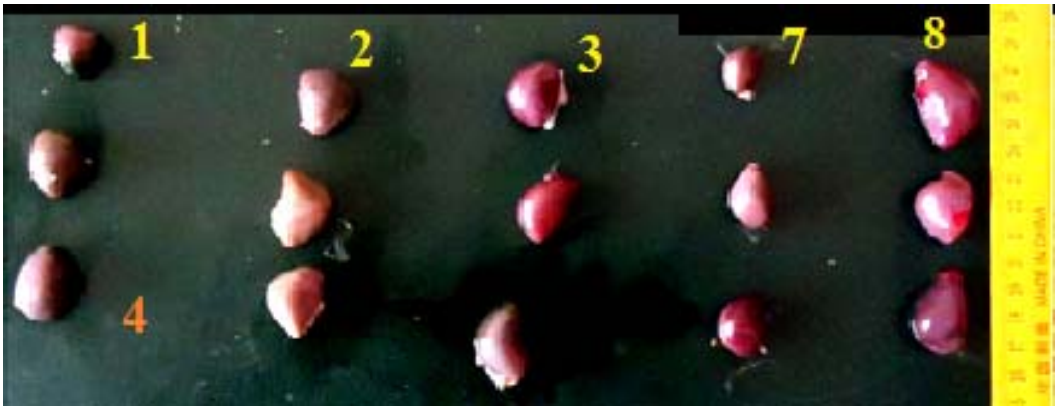

Fig. 4: Spleen showing severe atrophy in group 7 but mild in other groups when compared with group 8 on day 6 PI

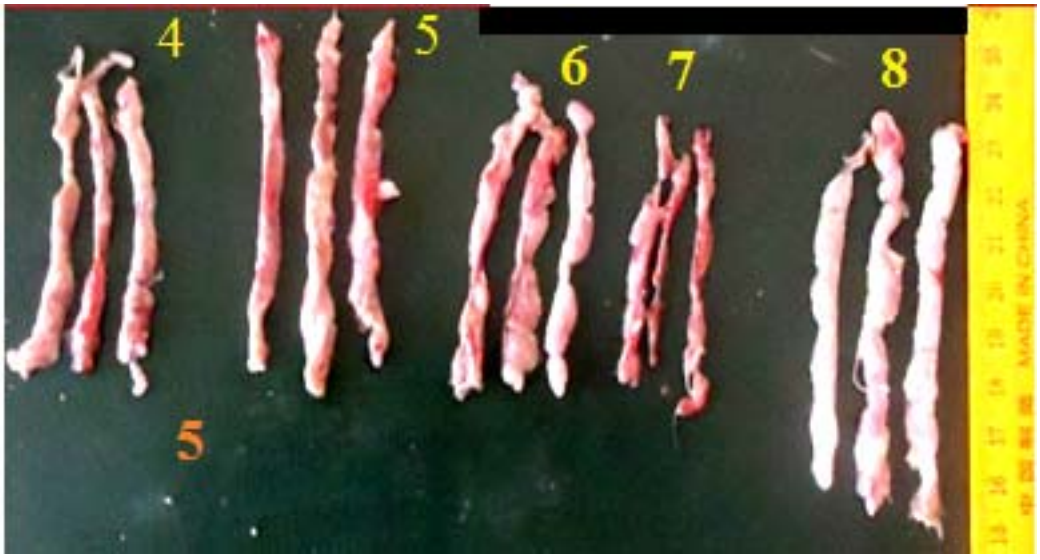

Fig. 5: Thymus showing severe atrophy in group 7 on day 4 PI

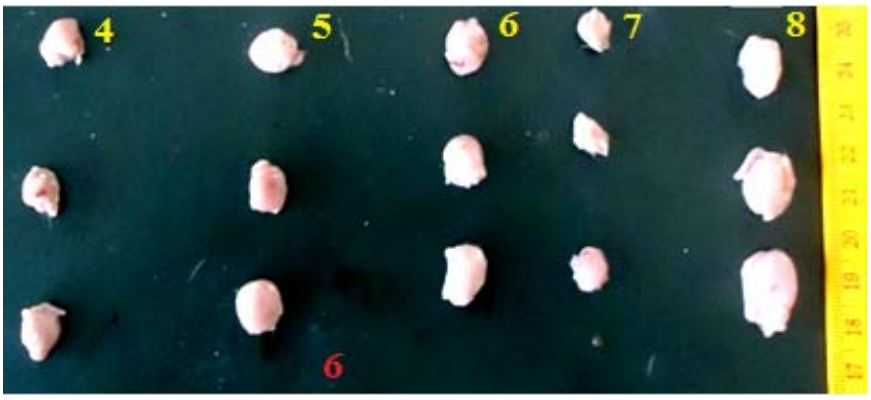

Fig. 6: Bursa of Fabricius showing mild atrophy in groups 4, 5 and 6 but severe atrophy in group 7 on day 7 PI

Table 2: Distribution and persistence of the gross lesions in prophylactic groups on day 6 PI

	Day 6 PI	Groups				
Organs	Lesions	1	2	3	7	8
Muscles	Congestion of thighs and breast	1/3	0/3	0/3	2/3	0/3
Proventriculus	Hemorrhage	2/3	1/3	0/3	2/3	0/3
Bursa	Atrophy/enlargement	1/3	0/3	1/3	0/3	1/3
		0/3	3/3	0/3	0/3	0/3
Spleen	Atrophy/enlargement	1/3	0/3	1/3	0/3	1/3
		0/3	1/3	0/3	0/3	0/3
Thymus	Atrophy	3/3	3/3	2/3	3/3	0/3
Caecal tonsil	Caecal tonsil hemorrhage	2/3	2/3	1/3	1/3	0/3
Kidney	Congestion and enlargement	1/3	1/3	0/3	1/3	0/3
Liver	Parboiled	2/3	0/3	0/3	1/3	0/3
Intestine	Button-like ulcers	2/3	1/3	1/3	2/3	0/3

Table 3: The mean body weights (g) of birds treated prophylactically with varied doses of *Anacardium occidentale* stem bark extract and infected with velogenic Newcastle disease virus

	Means of body weights (g) during the experimental period, with standard error in brackets		
Experimental groups	Day 0	Day 7	Day 10 (Day 3 PI)
Group 1			
(1000 mg/kg b.wt., SBE-PR+Infected)	321.90 (9.09)	397.60 (22.40)	464.70^{ab} (19.86)
Group 2			
(500 mg/kg b.wt., SBE-PR+Infected)	319.10 (7.67)	384.20 (32.09)	448.10^{a} (25.36)
Group 3			
(250 mg/kg b.wt., SBE-PR+Infected)	325.20 (9.99)	387.00 (18.67)	511.50^{b} (16.85)
Group 7			
(Infected untreated control)	336.90 (10.62)	380.90 (13.04)	439.90^{a} (17.89)
Group 8			
(Uninfected control)	329.50 (8.04)	403.70 (16.18)	471.70^{ab} (16.74)

^{ab}Different alphabetical superscripts in a column indicate significant differences between the means of the groups, p<0.05 and SBE-PR: Stem bark extract-prophylactic

Virus isolation: In all the infected groups, vNDV was isolated from all the organs evaluated in both prophylactic and therapeutic sections (Table 7 and 8).

Serology: The HI antibody titres were significantly (p<0.5) low on day 0 PI in both prophylactic and therapeutic groups (Table 9 and 10). Samples could not be assayed at later dates because by day 6 PI over 80% of the birds had died in all the infected groups.

DISCUSSION

The incubation period (IP) of the virus in prophylactic and therapeutic groups was 2 days PI. This supported the results of Igwe *et al.*[20], Omeke *et al.*[35] and Onyema *et al.*[36] who reported that in chickens infected with vNDV the IP was 2-3 days. Birds in groups 1 and 7 showed clinical signs of ND on day 2 PI, while birds in group 3 (those that received the lowest dose of the extract) showed clinical signs on day 3 PI. The disparity

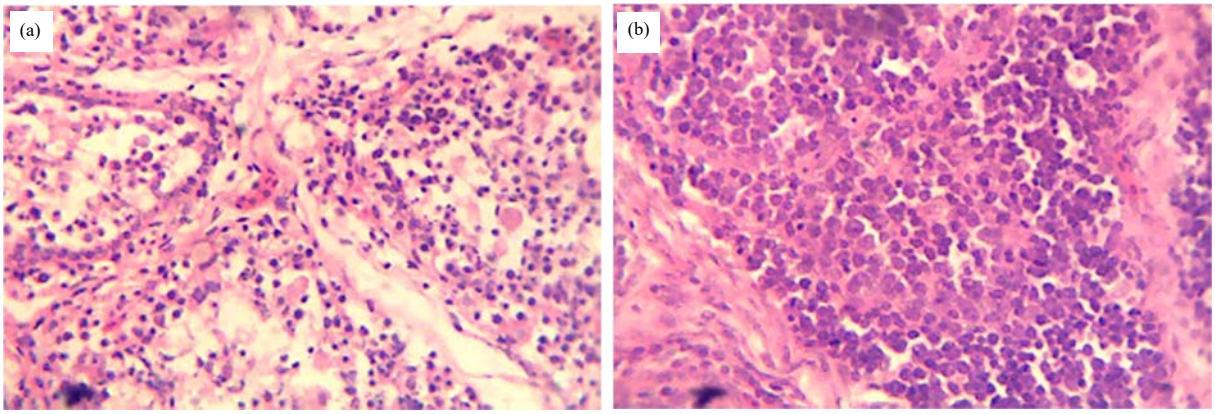

Fig 7(a,b): (a) Bursa of group 7 chicken showing severe necrosis, depletion of lymphocytes and hyperplasia (H) of bursal epithelium on day 3 PI. H&E ×200 and (b) Bursa of group 3 chicken showing mild depletion of lymphocytes on day 3 PI. H&Ex200

Table 4: The mean body weights (g) of birds infected with velogenic Newcastle disease virus and treated with varied therapeutic doses of *Anacardium occidentale* stem bark extracts

	Means of body weights (g) during the experimental period, with standard error in brackets	
Experimental groups	Day 0	Day 10 (Day 3 PI)
Group 4		
(Infected+1000 mg/kg b.wt., SBE-TH)	455.80 (11.44)	434.90 (11.78)
Group 5		
(Infected+500 mg/kg b.wt., SBE-TH)	463.90 (18.83)	450.20 (17.06)
Group 6		
(Infected+250 mg/kg b.wt., SBE-TH)	475.00 (12.49)	444.40 (18.03)
Group 7		
(Infected untreated control)	439.40 (12.76)	439.90 (17.89)
Group 8		
(Uninfected control)	449.70 (16.62)	471.70 (16.74)

No significant differences between the means of the groups, ps>0.05. Stem bark extract-therapeutic

Table 5: Distribution and persistence of the gross lesions in therapeutic groups on day 4 PI

	Day 4 PI	Groups				
Organs	Lesions	4	5	6	7	8
Muscles	Congestion of thighs and breast	2/3	1/3	0/3	3/3	0/3
proventriculus	Hemorrhage	2/3	1/3	0/3	3/3	0/3
Bursa	Atrophy/enlargement	3/3	0/3	0/3	0/3	1/3
		0/3	3/3	0/3	0/3	0/3
Spleen	Atrophy/enlargement	1/3	0/3	0/3	0/3	1/3
		0/3	3/3	0/3	0/3	0/3
Thymus	Atrophy	3/3	1/3	1/3	3/3	0/3
Caecal tonsil	Caecal tonsil hemorrhage	2/3	2/3	1/3	1/3	0/3
Kidney	Congestion and enlargement	1/3	1/3	0/3	1/3	0/3
Liver	Parboiled	2/3	0/3	0/3	1/3	0/3
Intestine	Button-like ulcers	2/3	1/3	1/3	2/3	0/3

showed that the low dose had effect in extending the IP of vNDV. In this experiment also, the disease showed marked effect on the body weight of the birds in all the infected groups. This decrease in mean BW in NDV infection has been observed by many researchers[19,30,36,37]. The observation of no significant difference in mean BW of the prophylactic groups on day 7 PA showed that stem bark extract may not have adverse effect on feed and water intake of the birds. The reduction in mean BW is a common occurrence in septicaemic and viraemic diseases due to reduction in feed and water intake[37,38]. The result of this experiment showed that the stem bark extract at 250 mg/kg BW reduced the loss of body weight due to vNDV infection. The clinical signs observed in all the infected (prophylactic and therapeutic) groups were similar to those described for vNDV by Igwe *et al.*[20], Omeke *et al.*[35] and Onyema *et al.*[36]. These researchers observed depression,

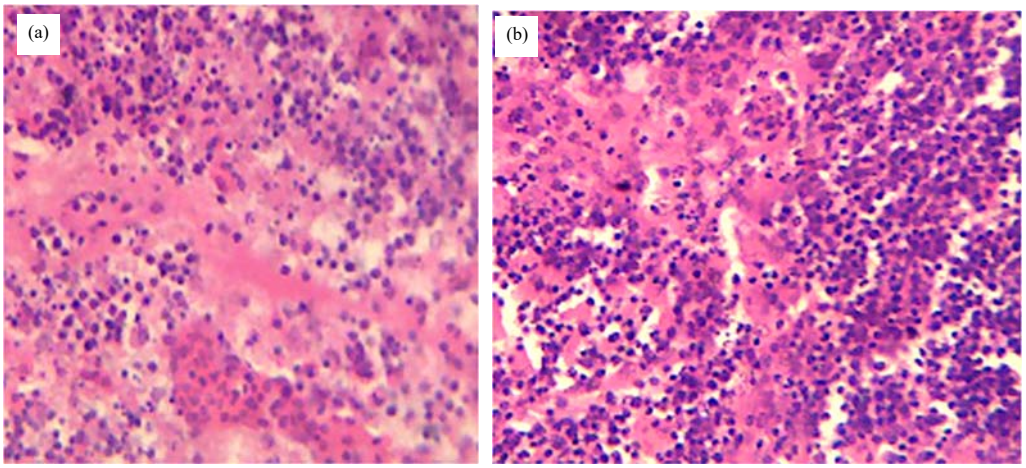

Fig. 8(a,b): (a) Thymus of group 7 chicken showing severe necrosis, depletion of lymphocytes and fibrin deposition on day 3 PI. H and E×200 and (b) Thymus of group 3 chicken showing moderate necrosis, depletion of lymphocytes and mild fibrin deposition day 3 PI. H and E×200

Table 6: Distribution and persistence of the gross lesions in therapeutic groups on day 6 PI

	Day 6 PI	Groups				
Organs	Lesions	4	5	6	7	8
Muscles	Congestion of thighs and breast	3/3	1/3	1/3	3/3	0/3
Proventriculus	Hemorrhage	3/3	2/3	2/3	3/3	0/3
Bursa	Atrophy/enlargement	2/3	1/3	2/3	3/3	0/3
Spleen	Atrophy/enlargement	1/3	0/3	0/3	2/3	0/3
Thymus	Atrophy	1/3	0/3	0/3	2/3	0/3
Caecal tonsil	Caecal tonsil hemorrhage	2/3	1/3	1/3	2/3	0/3
Kidney	Congestion and enlargement	2/3	2/3	0/3	2/3	0/3
Liver	Parboiled	1/3	2/3	1/3	2/3	0/3
Intestine	Button-like ulcers	2/3	3/3	0/3	3/3	0/3

Table 7: Viral isolation in prophylactic groups

Days PI	Groups	Bursa	Spleen	Thymus	Brain
3	1	$3^A/5^B$ $(60)^C$	4/5 (80)	4/5 (80)	1/5 (20)
	2	2/5 (40)	2/5 (40)	3/5 (60)	2/5 (40)
	3	3/5 (60)	2/5 (40)	1/5 (20)	0/5 (0)
	7	3/5 (60)	5/5 (100)	3/5 (60)	0/5 (0)
	8	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)
6	1	4/5 (80)	3/5 (60)	4/5 (80)	2/5 (40)
	2	5/5 (100)	4/5 (80)	5/5 (100)	2/5 (40)
	3	3/5 (60)	2/5 (40)	1/5 (20)	1/5 (20)
	7	3/5 (60)	5/5 (100)	3/5 (60)	2/5 (40)
	8	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)

A: Number positive for the virus, B: Total number organs assayed and C: The percentage of organs positive

Table 8: Virus isolation in therapeutic groups

Days PI	Groups	Bursa	Spleen	Thymus	Brain
3	4	$2^A/5^B$ $(40)^C$	3/5 (60)	2/5 (40)	2/5 (40)
	5	1/5 (20)	3/5 (60)	3/5 (60)	1/5 (20)
	6	3/5 (60)	3/5 (60)	4/5 (80)	1/5 (20)
	7	4/5 (80)	4/5 (80)	2/5 (60)	1/5 (20)
	8	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)
6	4	4/5 (80)	3/5 (60)	4/5 (80)	2/5 (40)
	5	3/5 (60)	4/5 (80)	3/5 (60)	2/5 (40)
	6	2/5 (40)	2/5 (40)	1/5 (20)	1/5 (20)
	7	3/5 (60)	5/5 (100)	3/5 (60)	2/5 (40)
	8	0/5 (0)	0/5 (0)	0/5 (0)	0/5 (0)

A: Number positive for the virus, B: Total number organs assayed and C: The percentage of organs positive

Table 9: Haemaglutination inhibition titre in prophylactic groups

	Day 0 PI				
S/No.	Group 1	Group 2	Group 3	Group 7	Group 8
1	0	0	0	2	2
2	0	2	0	0	0
3	0	0	2	0	0
4	0	0	0	0	0
5	0	0	2	0	0
6	0	0	0	0	0
7	2	0	0	0	0
8	0	2	0	0	0
9	0	0	2	0	0
10	0	0	0	2	0
GMT	1.1	1.3	1.5	1.4	1.1

Table 10: Haemaglutination inhibition titre in therapeutic groups

	Day 0 PI				
S/No.	Group 4	Group 5	Group 6	Group 7	Group 8
1	2	0	0	2	2
2	0	2	2	0	0
3	2	2	2	0	0
4	0	0	0	0	2
5	0	0	4	2	2
6	2	0	0	2	0
7	2	2	0	0	0
8	0	2	2	0	0
9	2	2	2	0	0
10	2	0	0	2	2
GMT	2.3	2.0	2.3	1.7	1.7

partial or incomplete in appetite, huddling together and greenish diarrhoea. The greenish diarrhoea observed in this study is an indicative of gastrointestinal lesions. Fever seen in septicaemic and viraemic diseases in chickens lead to hemolysis and excess bile production which turns the faeces greenish in colour. Nervous signs that occurred in this study which include ataxia, paralysis, circling and torticolis have all been reported in previous studies[35,36]. The high mortality recorded in this experiment has been reported by some researchers Wakamatsu *et al.*[39] and Onyema *et al.*[36] who studied different strains of vNDV in 4 and 6 weeks old birds and reported 90 and 100% mortality, respectively. Oladele *et al.*[40] observed 52% mortality in non immunized chickens infected with NDV KUDU 113 strain, while Onyema *et al.*[36] reported 100% mortality in 6 weeks old broilers infected with NDV KUDU 113 strain of vNDV. Variation in mortality seen in vNDV may be due to many factors such as strain of the virus, species of the birds, immune status of the host, environmental condition etc. In this study, the pattern of mortality observed was different from what has been reported by other researchers. The birds that received the highest dose (group 1) of the stem bark extract were dying following the same pattern of the birds that did not receive any treatment (positive control group 7), while birds that received the lowest dose in prophylactic groups were dying gradually. All the birds in groups 1 and 7 died on day 7 and 6 PI, respectively, while birds in groups 2 and 3 died on day 10 and 13 PI, respectively. This suggested that low dose of stem bark extract has protective effect against vNDV while high dose may be toxic to birds. This supported the observation of Omeke *et al.*[28] who reported that stem bark extract may be toxic when given at high dose of 3000 mL/kg BW. Omeke *et al.*[27] also reported that high dose of stem extract of AO can be toxic in haematology and serum biochemistry of chickens. In therapeutic groups also the last mortality in groups 4 and 7 were on days 7 and 6, respectively, while groups 5 and 6 that received lower doses were on days 10 and 15, respectively. The total mortality observed in this study did not differ in all the groups but the rate at which the birds were dying differed markedly. The pattern of mortality recorded was an indication that the stem bark extract at low dose has protective effect against NDV. This also agrees with the finding of Okonkwo *et al.*[41] who reported that aqueous leaf extract of AO had protective effect on the liver at low dose, while high dose could exert toxic effect on the liver. The gross lesions such as congestion of the breast and thigh muscles, hemorrhagic button-like ulcers of the ceacal tonsil, atrophy of the lymphoid organs recorded in this study have been

observed earlier[20,35-37,42]. Severe ulceration of the overlying intestinal epithelium observed in vNDV infection may be due to active viral replication in these parts of the body[43]. In this study, there was clear difference in severity of the haemorrhage and congestion observed in different organs of dead birds in different groups. In dead birds in group 3 and 6 (groups that received lowest doses in prophylactic and therapeutic respectively) the congestion of the breast and the thigh muscles and proventricular haemorrhage were very mild when compared with the carcasses of birds in other infected groups. The frequency and severity of lesions in each organ were probably related to tissue tropism of the virus. The observation of mild lesions in birds that received the lower doses was an indication that the extract at low dose reduced the replication of the virus in these organs. Haemorrhagic intestinal ulcers have been described as characteristic lesions or prominent pathological feature of vNDV[44]. Lesions in the GIT are found only in chickens with vNDV infection and are suspected to be responsible for the very high mortality found in chickens when compared with other avian species[17]. The atrophy, lymphocytic necrosis and depletion of lymphocytes observed in lymphoid organs (Bursa of Fabricus, Spleen and Thymus) have been reported in vNDV infection in domestic poultry[20,39,45]. The results of this experiment showed that the stem bark extract administration reduced the vNDV atrophy of the bursa, spleen and thymus in both prophylactic and therapeutic groups when compared with the untreated groups. This may reduce or prevent immunosuppression associated with vNDV infection[19]. The acute necrotic changes seen in the spleen around the sheathered arterioles suggested an active trapping of the antigen by reticular cells[46].

CONCLUSION

It can be concluded that stem bark extract at low dose has protective effect against vNDV infection which are beneficial to poultry farmers. Haemagglutination inhibition antibodies (either absent or present at very low titres) at day 0 PI did not affect the vNDV infection. The organs bursa, spleen and thymus can be reliably used in virus isolation in the field cases of vNDV infection.

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