

Effect of *Anthocleista nobilis* root extract on the haematological indices of poultry chicken challenged with Newcastle disease virus (NDV)

Ayodele PO^a, Okonko IO*^b, Evans E^a, Okerentugba PO^b, Nwanze JC^c, Onoh CC^c,

^aDepartment of Science Laboratory Technology, The Federal Polytechnic, P.M.B. 55, Bida, Niger State, Nigeria;

^bDepartment of Microbiology, University of Port Harcourt, P.M.B 5323 Uniport Post Office, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria;

^cDepartment of Pharmacology and Therapeutics, Igbinedion University, Okada, Edo State, Nigeria
Tel: +234-80-35380891; Email: iheanyi.okonko@uniport.edu.ng, mac2finney@yahoo.com

ABSTRACT: This study reports the effect of *Anthocleista nobilis* root extract on the haematological indices of poultry chicken challenged with Newcastle disease virus (NDV). Eighteen (18)-weeks-old chickens were used for this study. They were divided into 3 groups, A (chickens infected + treatment), B (chickens infected without treatment) and C (control). Groups A and B were challenged with Newcastle disease virus (NDV). Group A and C were given ethanolic root extract of *A. nobilis* orally at intervals of 6 h at 0.5mg per 100g of body weight for 28 days. All the chickens were given tetracycline antibiotic to eliminate bacterial infections. The average body weight and the temperature were monitored. The cytological examination of the chickens in group B showed that there was ulceration in the intestinal lining. All values for blood parameters were within the normal range for chickens before they were challenged with NDV. From our findings most of hematological indices of poultry chickens tend towards normal after treatment with the root extract of *A. nobilis*. This showed the ability of this plant extract to impact immunity in chickens suffering from Newcastle disease (ND). The packed cell volume (PCV), hemoglobin (Hb) and full blood count (TWBCC) were significantly different ($P < 0.05$) between the Group A and Group B. The hematological indices of poultry chickens in group A were significantly ($P < 0.05$) influenced by the treatments except for mean corpuscular haemoglobin concentration estimation (MCHC). It showed negligible differences ($P > 0.05$) among the three groups, A (33.3% and 33.8% respectively), B (32.4%) and C (32.1%). They fell within the normal range of 30.0%-36.0%. This showed that the NDV did not affect that aspect. The study indicated a drop in the hematological indices of the infected and untreated chickens (Group B) while those of group A and C fell within normal range. The study showed that ethanolic root extract of *Anthocleista nobilis* was able to correct the haematological and physiological alteration associated with Newcastle disease among infected poultry chickens. The ability of group A poultry chickens to tend towards normal after treatment are physiological evidences of the antiviral effect of the root extract of *A. nobilis* on hematological indices of the poultry chickens studies. The study also showed that *Anthocleista nobilis* root extract was able to prevent further NDV infection of the poultry chickens. Thus, further studies on phyto-chemical and toxicological properties of *Anthocleista nobilis* as well as its antiviral property are advocated.

[Ayodele PO, Okonko IO, Okerentugba PO, Nwanze JC, Onoh CC. **Effect of *Anthocleista nobilis* root extract on the haematological indices of poultry chicken challenged with Newcastle disease virus (NDV).** *Nat Sci* 2013;11(3):82-xx]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 13

Keywords: *A. nobilis*, Antiviral effect, New Castle disease, NDV, hematological indices, chickens, ulceration, Nigeria

1. Introduction

Rural poultry are the dominant form of poultry kept in the developing world. They are a natural resource whose potential is not fully exploited for the welfare of rural populations (Awan et al., 1994). Some research has been carried out during the past few years and it now appears that rural poultry are receiving increasing support for research and development from many government and international funding agencies throughout the world (Awan et al., 1994). Diseases seriously affect family poultry and constitute one of its major threats (Ndahi et al., 2011). The most devastating disease of rural poultry is New Castle disease (NCD). Newcastle disease is a major constraint to poultry

production in Africa, in both commercial and village rearing systems (Nwanta et al., 2008). Moreki et al. (2010) ascribed losses in family poultry to diseases, diseases and parasites, predation, a combination of diseases, parasites and predation. In order of prevalence, the common diseases of poultry are coccidiosis, infectious coryza, fowl pox, infectious bursal disease (IBD) and Newcastle disease (Moreki et al., 2011; Moreki, 2012). NCD is the most widespread infectious disease in Africa (Moreki, 2012). Similarly, Moreki (2010) identified NCD to be a major constraint in family poultry, causing up to 100% mortality in unprotected flocks.

Newcastle Disease is a viral disease of birds caused by a filterable virus Newcastle Disease Virus (NDV) which belongs to the family Paramyxoviridae (Okwor and Eze, 2010). ND is considered among the most important disease of poultry and outbreaks with mortality up to 100% are common (Saidu and Abdu, 2008). Paramyxovirus 1 (PMV-1) or Newcastle Disease (ND) is a highly contagious zoonotic viral disease affecting poultry of all ages (McMullin, 2004)

Although the first outbreaks recognized as Newcastle disease occurred in Indonesia in 1926, it has been suggested that a large outbreak in Scotland in 1896 was due to Newcastle Disease Virus (NDV) (Sadiq et al., 2011). The first documented outbreak of Newcastle disease in Nigeria occurred between December, 1952 and February 1953 in and around Ibadan (Okwor and Eze, 2010). The disease has since this time remained a notable problem in the country (Oladele *et al.*, 2002) and has become endemic in Nigeria in both, local and commercial poultry with annual epidemics recorded in highly susceptible flocks with pockets of outbreaks occurring in between the annual epidemic periods (Saidu and Abdu, 2008; Okwor and Eze, 2010).

Outbreaks of Newcastle disease in Nigeria were reported to be more likely in farms that kept exotic birds together with local chickens and other poultry species like ducks and turkeys (Abdu *et al.*, 2005a). The outbreaks of Newcastle disease were more common in layers than in broilers (Abdu *et al.*, 2005a). Newcastle Disease (ND) is the most important disease and it causes very high mortality (Sonaiya, 2009). The disease was also reported to be more common during the dry harmattan (November-March) (Abdu *et al.*, 2005b; Sonaiya, 2009; Sadiq et al., 2011). ND in Nigeria has age and species differences (Abdu *et al.*, 2005b). In rural Nigeria, it is common to find a combination of different poultry species and breeds being kept in the same compound, including chickens, turkeys, Muscovy ducks and pigeons. At present, it is customary to find ostriches, peacocks, geese and mallard ducks in the same compound in cities and in some poultry farms (Adene and Oguntade, 2006; Friend, 2006; Sadiq et al., 2011).

New Castle Disease (NCD) is the most important disease that affects these chickens resulting in very high morbidity, mortality and case fatality rates. Based on this study which indicates that this disease usually starts at the onset of the cold dry season that is around November, NCD vaccine (Lasota) could be administered just before the onset of this period and during the period in order to curtail this disease. Also, fowl pox vaccination could be carried out to prevent further occurrence of the disease. Anthelmintics could be given before the onset of raining season and during raining season to guard against syngamus trachea

infection which is said to occur from the month of June to October.

All strains of Newcastle disease virus (NDV) occur in rural poultry, but velogenic strains are reported to be more common. Serological surveys in conjunction with isolation studies have shown that velogenic NDV strains are endemic in rural poultry populations even in isolated villages and possibly in isolated flocks (Awan et al., 1994). Although NDV is endemic in village poultry, the clinical disease usually follows an epidemic pattern. ND outbreaks often occur once or twice a year at regular intervals affirming the endemicity of the virus, however, 'mini' outbreaks in individual flocks and sporadic cases in individual birds may occur. Epidemics usually occur at times of climatic stress, leading to seasonal occurrence (Awan et al., 1994). The spread of NDV within and between village poultry populations is relatively slow due to a low contact rate (Awan et al., 1994). The major mode of transmission appears to be by the faecal-oral route. The respiratory route may also play a role in flocks where close bird-to-bird associations exist. Other poultry species, wild and feral birds, wild animals, communal water reservoirs and domestic animals may play a role in transmission; however, their role has not been properly investigated (Awan et al., 1994).

There is currently a large and ever-expanding global population base that prefers the use of natural products in treating and preventing medical problems. This has influenced many pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. At present, plant and herb resources are unlimited, have provided mankind remedies for many infectious diseases and continue to play a major role in primary health care as therapeutic remedies in developing countries. The search for biological active extracts based traditionally used plants is still relevant due to induction of resistance of pathogens to chemical drugs and the prevalence of the fatal different infections. Medicinal plants are used in pharmaceuticals, nutraceuticals, cosmetics, and food supplements and even as traditional source of medicines because of their antitumor, antiarthritic and antithrombotic functions (Sajjad et al, 2011). The consumption and demand for medicinal plants have been adopted in many countries because of low cost, easy availability, affordability for a common farmer, good antimicrobial nature, reduced diseases associated risks, lowering blood cholesterol level and diversified functions in improving performance, growth rate, feed conversion rate and weight gain in birds (Sajjad et al, 2011).

The use of ethnoveterinary medicine in the management of animal healthcare is as old as the domestication of various livestock species (Ndahi et al., 2011). Compared to Western modern medicine, EVM is

widely utilized by the family poultry rearers across the country (Ndahi et al., 2011). *Anthocleista nobilis* which is commonly called the candelabrum or cabbage tree in English language, Duwa Kuchi in Nupe language, Kwari in Hausa language and Apa Ora in Yoruba language belongs to the family Loganiaceae. The plant is used with boiled root of *Combretum smeathmanni* (combretaceae) pepper and ash taken as a drink for chest pain, the liquid resulting from boiling dry falling leaves is drunk in Sierra Leone to treat Jaundice, the leaf sap is reputed to be haemostatic. The bark is used in Nigeria for its antipyretic, tonic purgative properties. In Congo, its pulped bark is applied as antiseptic and Cistercian on sores, swollen buboes and abscesses and to treat yaws (Lewington, 1990). The root is the most active pharmacologically and is the most used as a purgative and dietary, a poison antidote, an emmenagogue, abortifacient to treat leprosy, edemas and elephantiasis of the scrotum. A root decoction is taken in Sierra Leone for constipation and gonorrhoea. In Congo the root decoction is given to women as a purgative to cleanse the abdomen and to ensure that the urinogenital parts return to its proper place (Burkill, 1995).

Furthermore, scientists and researchers are trying to combat against fatal diseases in poultry through the use of medicinal plants, containing the most active ingredients to promote growth, weight gain, and immunostimulant (Sajjad et al, 2011). *Aloe spp.* and *N. tabacum* were also used against internal parasites while wood ashes, especially those from *Peltophorum africanum* and *Combretum imberbe* were used against external parasites (Ndahi et al., 2011). Because of its unpleasant and strong smell, *Thamnosma rhodesica* leaves were placed in chicken shelters in order to repel external parasites (Ndahi et al., 2011).

In Nigeria, Musa et al. (2008) noted that these remedies that are used by rural farmers may or may not have direct effect on NCD virus but could affect protozoan and helminths parasites of rural poultry by reducing the parasites burden, and boosting the immunity of birds against infection. Deeba (2009) in Pakistan reported the use of *Nicotiana tabacum* in treatment of NCD. Mwale et al. (2005) reported that *A. vera* and *Aloe spicata* were the predominantly used plant species for chicken health management in Zimbabwe. In agreement with Moreki et al. (2010), Mwale et al. (2005) mentioned that *A. vera* acts like a broadspectrum antibiotic remedy. Moreng (2008) reported that feeding chickens a concoction of Moringa (*Moringa oleifera*) tree leaves is an effective deworming practice. The study of Ogbe and Affiku (2011) in Nigeria showed that *M. oleifera* leaves contained appreciable amounts of carbohydrate, protein and minerals, which are nutritional requirements for poultry. The authors also mentioned that *M. oleifera*

could be useful as feed supplement and as medicine in poultry to improve health and growth performance (Moreki, 2012).

Masimba et al. (2011) pointed out that ethnoveterinary knowledge was mostly in the custody of older men and women who passed it orally to younger generations by word of mouth. According to Sri Balaji and Vikrama Chakravarthi (2010), ethnoveterinary practices concern to animal health care is as old as the domestication of various livestock species. They comprise beliefs, knowledge, practices and skills pertaining to health care and management of livestock (Ndahi et al., 2011).

In Kenya, Lagu and Kanyanja(2010) reported that traditional healers play limited roles in treating local chickens as many farmers collect, concoct and administer the local herbs themselves. Many of the plants used to prepare indigenous medicine contain valuable active ingredients (Ndahi et al., 2011). Previous study by Moreki et al. (2010) showed that 86.7% of family poultry rearers used EVM, whereas the remainder used modern medicines (vaccines and drugs). The most common forms of EVM preparations are powders, poultice, ointment, decoction, infusion, cold ware extract, tincture and fumigation (Toyang et al., 2007; Sri Balaji and Vikrama Chakravarthi, 2010; Ndahi et al., 2011).

In contrast to vaccines, therapeutic remedies are village traditions. Rural poultry producers use many local natural products to treat or prevent diseases in their livestock. Their chickens also receive treatment. Workers with village chickens have become interested in ethnoveterinary medicine and lists of traditional remedies are becoming available. This study was carried out to ascertain in greater details the relationship between the active ingredient in the ethanol root extracts of *Anthocleista nobilis* and the haematological evidence that can be obtained during the treatment of poultry chickens against Newcastle disease virus. The haematological indices were examined before, during and after the administration of the *A. nobilis* root extracts.

2. Material And Methods / Experimental Details / Methodology

2.1. Source of Newcastle disease virus (NDV)

The Newcastle disease virus used in this study was obtained from National Veterinary Research Institute (NVRI) Vom, Jos. The Newcastle disease virus was in a freeze dried form, prepared and stored in amples. It was kept in the refrigerator at 4°C to avoid deterioration of the viral pathogen which was a velogenic strain.

2.2. Collection and identification of plant

The plant material was collected from a neighboring village called Kusogi very close to the

Federal Polytechnic, Bada, Niger State. The plant was identified based on the criteria stipulated by International Committee for Botanical Nomenclatures (ICBN), as *Anthocleista nobilis*.

2.3. Collection blood samples

At week 7 and 28 of the experiment, blood samples were collected randomly from two (2) poultry chickens per treatment for the determination of the haematological indices. Samples were collected from the wing (brachial) vein of the chickens by venipuncture in commercial vacutainer® EDTA K²⁺ which served as an anticoagulant for haematology. The poultry chickens were fasted overnight (12hrs) and normally bled in the morning (7.00–8.00am) to avoid excessive bleeding.

2.4. Determination of the effectiveness of the *Anthocleista nobilis* root extract on haematological indices of poultry chickens challenged with Newcastle disease virus

Eighteen poultry chickens of 4 weeks old were gotten and were allowed to grow for another 4 weeks more, this was to enable the birds to develop their own Immunity, since the birds (chickens) were vaccinated immediately after hatch. The chickens were randomly distributed into 3 different groups namely A, B, and C and each group was kept in different apartments. Groups A, B and C contained 6 chickens each, group C serve as the control. The chickens in groups A and B were injected intraocularly route with 0.2ml (10^{5.0-6.0} LD₅₀) of the viral pathogen concentration under a biocontainment condition. Treatment commenced immediately after challenging these poultry chickens with the viral pathogens. Group A was treated while group B was left without treatment. Only groups A and C were given the plant extract at a dose of 0.5mg orally per 100g body weight the treatment was done at interval of 6 hours. In order to eliminate bacterial and other infections, all the chickens were given an oxytetracycline treatment was administered at 4 weeks of age via the birds' water supply at a dose of 25 mg/lb for 5 days. The blood samples of the chickens were also collected to determine hematological indices which include using standard methods as described by other workers (Spencer and Price, 1997; Ajagbonna et al., 1999; Uko et al., 2000; Cheesbrough, 2006; Mohammed et al., 2008, 2011; AL-Eissa and Alkahtani, 2011). The hematological indices include the pack cell volume (PCV), %; hemoglobin (Hb), g/l; full blood count (TWBCC) cells/mm⁻³; and mean corpuscular haemoglobin concentration (MCHC), %.

2.5. Data Analysis

Data were analyzed using the general linear model procedure, ANOVA and independent t-test to compare the level of significant difference between the

treated groups and the controls. Indicator of statistical significance is $P \leq 0.05$.

3. Results Analysis

The study was conducted to investigate the effect of *Anthocleista nobilis* root extract on the haematological indices of poultry chickens challenged with Newcastle disease virus (NDV). The results of the haematological indices of the test chickens challenged with New Castle Disease are shown in Tables 1, 2 and 3. The haematological indices obtained before challenging the chickens with NDV indicated that all the chickens were healthy and no physiological abnormalities were traced in them. The packed cell volume; PCV (%), haemoglobin; Hb (g/l), mean corpuscular haemoglobin concentration (MCHC) (g/l) and total white blood count concentration; TWBCC (g/l) of all the groups tends towards normal. It ranged from 0.35% – 0.43%, 120.0g/l – 140.0g/l, 302.3g/l – 357.1g/l and $4.0 \times 10^3 \text{mm}^{-3}$ – $4.6 \times 10^3 \text{mm}^{-3}$ respectively (Table 1). In the same vein, the mean PCV (%), Hb (g/l), MCHC (%) and TWBCC (g/l) ranged from 37.0–42.0%, 127.5–130.0g/l, 32.6–34.5% and 4.15×10^3 – $4.4 \times 10^3 \text{mm}^{-3}$ respectively (Table 1) and these values are normal.

Means PCV level is also presented in Table 1. Group C receiving no NDV and ethanolic extract showed higher PCV (42.0%) level. This was closely followed by Group B (39.0%) receiving only NDV and no ethanolic extract of *A. nobilis*. While Group A (37.0%) receiving both NDV and ethanolic extract of *A. nobilis*. Means Hemoglobin estimation (Hb) level is presented in Table 1. Group C receiving no NDV and ethanolic extract and Group B receiving only NDV and no ethanolic extract of *A. nobilis* showed higher Hb level (13.00 g/l) as compared to Group A receiving both NDV and ethanolic extract of *A. nobilis* having Hb level of 127.5 g/l. There was no significant difference between the Hb level of the treatments and the control (130.0g/l vs. 135.0g/l, $P > 0.05$). Means corpuscular haemoglobin concentration estimation (MCHC) level is also presented in Table 1. Group A receiving both NDV and ethanolic extract of *A. nobilis* showed higher ($P > 0.05$) MCHC level (34.5%). This was followed by Group C receiving no NDV and ethanolic extract with MCHC level of 33.7% and Group B receiving only NDV and no ethanolic extract of *A. nobilis* showed lower ($P > 0.05$) MCHC level (32.6%) as compared to other groups. There was no significant difference between the MCHC level of the treatments and the control (33.7g/l vs. 32.6g/l, $P > 0.05$). Means total white blood count concentration (TWBCC) level is also presented in Table 1. Group B receiving only NDV and no ethanolic extract of *A. nobilis* showed higher MCHC level ($4.4 \times 10^3 \text{Cellsmm}^{-3}$). This was followed by Group A receiving both NDV and ethanolic extract of

A. nobilis and Group C receiving no NDV and ethanolic extract with mean TWBCC level of 4.2×10^3 Cells mm^{-3} as compared to Group B. However, these differences were not statistically different (4.4×10^3 Cells mm^{-3} vs.

4.2×10^3 Cells mm^{-3} , $P > 0.05$) in the haematological indices obtained before challenging the poultry chicken with NDV.

Table 1: Haematological indices obtained before challenging the poultry chickens with NDV

Group	PCV (%)	Mean PCV (%)	Hb (g/l)	Mean Hb (g/l)	MCHC (%)	Mean MCHC (%)	MCHC	TWBCC (Cells mm^{-3})	Mean TWBCC (Cells mm^{-3})
A ₁	35.0		125.0		35.7			4.0×10^3	
A ₂	39.0	37.0 ^b	130.0	127.5 ^b	33.3	34.5 ^b		4.3×10^3	4.2×10^{3b}
B ¹	37.0		120.0		32.4			4.6×10^3	
B ²	40.0	39.0 ^b	140.0	130.0 ^b	35.0	33.7 ^b		4.2×10^3	4.4×10^{3b}
C ¹	43.0		130.0		30.2			4.4×10^3	
C ²	40.0	42.0 ^b	140.0	135.0 ^b	35.0	32.6 ^b		4.0×10^3	4.2×10^{3b}

Keys: PCV – Packed Cell Volume %, Hb – Hemoglobin g/l, MCHC – mean corpuscular haemoglobin concentration g/l, TWBCC – Full blood count (cells mm^{-3}); A = Chickens that were challenged and treated; B = Chickens that were challenged and without treatment; C = Chickens that were not challenged and were given treatment (control); ^b = Not Significant ($P > 0.05$)

The hematological indices obtained following the challenging with NDV and no administration of the *Anthocleista nobilis* root extract (after 7 days of treatment) showed decrease in the PCV (%) and Hb (g/l) levels in the blood of chickens in group B. While those of groups A and C maintained the normal ranges. The mean TWBCC cell mm^{-3} values of groups A and B increased ranging from 4.2×10^3 – $7.6 \times 10^3 \text{mm}^{-3}$ and 4.4×10^3 – $9.4 \times 10^3 \text{mm}^{-3}$ respectively. The MCHC (%) of all the groups showed negligible differences (Table 2). Means PCV level after challenging with NDV and after 7 days of administration or no administration of ethanolic root extract of *A. nobilis* is presented in Table 2. The mean PCV level of Group C receiving no NDV and *Anthocleista nobilis* root extract remained the same after 7 days (42.0%). A slightly decrease in mean PCV level of the chickens in Group A from 37.0% (Table 1) to 32.0% (Table 2) was observed. However, there was drastic decrease ($P < 0.05$) in mean PCV level from 39.0% (Table 1) to 15.0% (Table 2) among chickens in group B which received only NDV and no treatment

with ethanolic extract of *A. nobilis*. There was significant differences in mean PCV level between treatments and control (32.0% vs. 15.0%; 32.0% vs. 42.0%; 42.0% vs. 15.0%, $P < 0.05$).

Means Hemoglobin estimation (Hb) level is also presented in Table 2. Group C receiving no NDV and no treatment with the extract showed no increase or decrease ($P > 0.05$) in Hb level (135.0g/l) as shown in Tables 1, 2, and 3. Group A receiving both NDV and ethanolic extract of *A. nobilis* showed a decrease in Hb level from 127.5g/l (Table 1) to 105.0g/l (Table 2). However, these differences were statistically different (105.0g/l vs. 50.5g/l; 135.0g/l vs. 105.0g/l; 50.0g/l vs. 135g/l, $P < 0.05$). When compared to Group B chickens which received only NDV and no treatment with the *Anthocleista nobilis* root extract, there was a drastic decrease ($P < 0.05$) in Hb level from 130g/l (Table 1) to 50.5g/l (Table 2). Means corpuscular haemoglobin concentration estimation (MCHC) level of all the groups showed negligible differences ($P > 0.05$).

Table 2: Hematological indices results obtained 7 days after the administration of Newcastle disease virus and *A. nobilis* root extract

Group	PCV (%)	Mean PCV (%)	Hb (g/l)	Mean Hb (g/l)	MCHC (%)	Mean MCHC (%)	MCHC	TWBCC (Cells mm^{-3})	Mean TWBCC (Cells mm^{-3})
A ₁	31.0		100.0		32.3			7.2×10^3	
A ₂	32.0	32.0 ^a	110.0	105.0 ^a	34.4	33.3 ^b		8.0×10^3	7.6×10^{3a}
B ¹	16.0		54.0		31.3			8.8×10^3	
B ²	14.0	15.0 ^a	47.0	50.5 ^a	33.6	32.4 ^b		1.0×10^4	9.4×10^{3a}
C ¹	43.0		130.0		31.7			4.2×10^3	
C ²	41.0	42.0 ^a	140.0	135.0 ^a	32.6	32.2 ^b		4.0×10^3	4.1×10^{3a}

Keys: PCV–Packed Cell Volume %, Hb–Haemoglobin g/l, MCHC–mean corpuscular haemoglobin concentration %, TWBCC–Full blood count (cells mm^{-3}); A = Chickens that were challenged and treated; B = Chickens that were challenged and without treatment; C = Chickens that were not challenged and were given treatment (control); ^a = Significant ($P < 0.05$); ^b = Not Significant ($P > 0.05$)

Mean total white blood count concentration (TWBCC) level is also presented in Table 2. Group B receiving only NDV and no treatment with the extract

showed higher ($P < 0.05$) TWBCC level (9.4×10^3 Cells mm^{-3}). This was followed by Group A receiving both NDV and treatment with the extract (7.6×10^3

Cells mm^{-3}). The mean TWBCC level of Group C chickens receiving no NDV and no treatment showed negligible difference from day 1 (4.1×10^3 Cells mm^{-3}). However, these differences in mean total white blood count concentration (TWBCC) level were statistically different (7.6×10^3 Cells mm^{-3} vs. 9.4×10^3 Cells mm^{-3} ; 7.6×10^3 Cells mm^{-3} vs. 4.1×10^3 Cells mm^{-3} ; 9.4×10^3 Cells mm^{-3} vs. 4.1×10^3 Cells mm^{-3} ; $P < 0.05$). Generally, there was significant different ($P < 0.05$) in some of the haematological indices between Group A (with treatment) and Group B (without treatment) as well as Group B and Group C (controls).

The hematological indices results obtained 28 days after challenging with NDV and the administration or no administration of *Anthocleista nobilis* root extract showed that the mean PCV values ranged from 0.0% to 42.0%. The mean Hb value ranged from 0.0 g/l -140.0 g/l. mean MCHC estimation ranged from 0.0% to 35.0% while the mean TWBCC level ranged from 0.0 g/l to 5.3×10^3 g/l. The poultry chickens in group A tended toward normal as those of group C, none of the chickens in group B survived the challenging with Newcastle disease virus to that period of time since they were not treated with *Anthocleista nobilis* root extract (Table 3). However, mean PCV level after 28 days of administration of ethanolic root extract of *A. nobilis* is also presented in Table 3. The mean PCV level of Group C receiving no NDV and ethanolic extract remained the same after 28 days (42.0%). Mean PCV level of the chickens in Group A also remained the same (37.0%). There was significant differences in mean PCV level between treatments (Group A and Group B) and control (37.0% vs. 0.0%; 42.0% vs. 0.0%, $P < 0.05$) but no significant differences exist between mean PCV level of Group A and controls (37.0% vs. 42.0%, $P > 0.05$).

Mean hemoglobin estimation (Hb) level is also presented in Table 3. Group C receiving no NDV and no treatment with the *Anthocleista nobilis* root extract showed an increase ($P < 0.05$) in Hb level from 130.0g/l (Table 1) to 135.0g/l (Table 3). Group A receiving both NDV and ethanolic extract of *A. nobilis* showed an increase ($P < 0.05$) in Hb level from 127.0g/l (Table 1) to 135.0g/l (Table 3). However, these differences were statistically different (128.0g/l vs. 0.0g/l; 135.0g/l vs. 0.0g/l, $P < 0.05$) but no significant difference exists between mean PCV level of Group A and controls (128.0g/l vs. 135.0g/l, $P > 0.05$).

MCHC level of all the groups showed negligible differences except for those in group B which had no survivors (Table 3). Means TWBCC level is also presented in Table 3. Group A receiving only NDV and no treatment with the *Anthocleista nobilis* root extract showed higher ($P < 0.05$) TWBCC level (5.3×10^3 Cells mm^{-3}) after 28 days. The mean TWBCC level of group C chickens receiving no NDV and no treatment showed negligible difference between before and after treatments (4.1×10^3 Cells mm^{-3}) as shown in Table 3. However, these differences in mean total white blood count concentration (TWBCC) level were statistically different (5.3×10^3 Cells mm^{-3} vs. 0.0×10^3 Cells mm^{-3} ; 0.0×10^3 Cells mm^{-3} vs. 4.1×10^3 Cells mm^{-3} , $P < 0.05$) but there was no significant difference in mean TWBCC level Group A and controls (5.3×10^3 Cells mm^{-3} vs. 4.1×10^3 Cells mm^{-3} , $P > 0.05$).

Generally, there was significant different ($P < 0.05$) in some of the haematological indices after 28 days between Group A (with treatment) and Group B (without treatment) as well as Group B and Group C (controls) as shown in Table 3.

Table 3: Haematological indices obtained 28 days following the administration of Newcastle disease virus and *A. nobilis* root extract

Group	PCV (%)	Mean PCV (%)	Hb (g/l)	Mean Hb (g/l)	MCHC (%)	Mean MCHC (%)	TWBCC (Cells mm^{-3})	Mean TWBCC (Cells mm^{-3})
A ₁	37.0		126.0		33.0		5.4×10^3	
A ₂	38.0	37.0 ^a	130.0	128.0 ^a	34.6	33.8 ^a	5.2×10^3	5.3×10^{3a}
B ¹	0.0		0.0		0.0		0.0	
B ²	0.0	0.0 ^a	0.0	0.0 ^a	0.0	0.0 ^a	0.0	0.0 ^a
C ¹	41.0		130.6		31.2		4.2×10^3	
C ²	41.0	42.0 ^a	140.0	135.0 ^a	35.0	32.1 ^a	4.0×10^3	4.1×10^{3a}

Keys: PCV – Packed Cell Volume %, Hb - Haemoglobin g/l, MCHC – mean corpuscular haemoglobin concentration %, TWBCC – Full blood count (cells mm^{-3}); A = Chickens that were challenged and treated; B = Chickens that were challenged and without treatment; C = Chickens that were not challenged and were given treatment (control); 0.0 = No survivor; ^a = Significant ($P < 0.05$)

4. Discussion

Newcastle disease is a viral and often fatal disease that has been reported to affect a wide range of avian hosts, irrespective of age and sex. It is reported to be a major constraint to the development, survival and

productivity of village poultry (Nwanta et al., 2008a). ND is regarded as the most economically important disease that devastates village poultry in Nigeria as it causes death of millions of birds (particularly young birds) and economic losses through the slaughter of sick

birds (Nwanta et al., 2006a,b). Mortality rate as high as 80% has been recorded in chickens (Nwankiti et al., 2010). This study was carried out to evaluate the antiviral effect of *A. nobilis* root extracts on the haematological indices of poultry chickens infected with Newcastle disease virus (NDV) following its administration. Haematological blood components are influenced by the quantity and quality of feed (Akinmutimi, 2004). Haematological components of blood are sensitive to elements of toxicity in feed, especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998; Mohammed et al., 2011). The phytochemical diversity of tropical forest plants has been the focus of much interest and research. The secondary phytochemicals in the vegetation protect the plants against herbivores. The chemicals are often biologically active and may have antibiotic properties. For these reasons, many chemicals used in drug manufacture are or were originally derived from tropical forest plants, and it is likely that more will be discovered since much of the flora has not yet been researched. The people who inhabit these forests make extensive use of the phytochemicals in their traditional medicine, a factor which is gaining importance in forest management and rural development (Thomas et al., 1989).

Interest in the use of NDV as an anticancer agent has arisen from the ability of NDV to selectively kill human tumor cells with limited toxicity to normal cells (McMullin, 2004). No treatment for NDV exists, but the use of prophylactic vaccines and sanitary measures reduces the likelihood of outbreaks (McMullin, 2004). The cytological examination of the test chickens in group B showed that there was ulceration of the intestinal lining of the chickens. However, ulceration of the intestinal lining was not observed in those of groups A and C, and no visible damage was inflicted on the kidneys and livers of the chickens in all the groups. The occurrence of ulceration has been reported by Jordan *et al.* (1990) is an indicator of Newcastle disease that ulceration was not observed in the treated group A suggested that the root extract of *Anthocleista nobilis* is able to either prevent the multiplication of the virus or ameliorate the toxic effect leading to ulceration. Newcastle disease (ND) is reported as the most important viral disease of poultry in the world including developing countries. It has a devastating effect on commercial as well as village poultry industries (Nwanta et al., 2006). The resource derivable from the chickens cannot be fully utilized unless the disease is controlled particularly in the village poultry flocks that are believed to keep the virus in circulation and act as reservoirs and carriers to themselves and the more susceptible exotic breeds in commercial farms (Nwanta et al., 2006).

The hematological indices of the examination carried out showed a drop in the mean packed cell volume of the chickens in group B (infected but not treated) from 39.0% at day 1 to 15.0% at day 7 and 0.0% at day 28. Those of group A (challenged with NDV and treated) though tended toward normal dropped from 39.0% before administration of the NDV and extract at day 1 to 31.0%, 7 days after treatment with the extract. This finding was similar with that of Sajjad *et al.* (2011), who reported significant decrease in PCV level of broilers chicks administered with medicinal plants infusion of aloe vera gel, barbery, garlic and ginger. Also, from those of group A (infected and treated) though tended toward normal dropped from increased 31.0% at day 7 after treatment to 37.0% at day 28 after treatment with the extract. This finding agrees with Esonu *et al.* (2006), who reported significant increase in PCV level, in layers fed herbal plant neem. In differential count, an abnormally higher monocytes level is synonymous with bacterial infection (Akinmutimi, 2004). Dairo (2005) and Mohammed *et al.* (2005, 2011) reported that the inclusion levels of camel blood-rumen content mixture did not affect PCV, RBC, and MCHC values of growing rabbits.

From the hematological indices of the examination carried out, it showed a drop in the mean haemoglobin levels from 130.0g/l at day 1 before the administration of NDV to 0.50g/l at day 7 and 0.0g/l at day 28 after the administration of the NDV to the chickens in group B. In the same vein, it also showed a drop ($P < 0.05$) in the mean Hb levels from 127.5g/l at day 1 before the administration of NDV and the *A. nobilis* extract to the chickens in group A to 105.0g/l after 7 days of treatment. The findings of this study were similar with the findings of Sajjad *et al.* (2011), who reported that water based infusion of Aloe vera gel, Barbery, Garlic and Ginger had significant effect on hemoglobin concentration. However, haemoglobin levels of chickens in group A also increased slightly from 127.0g/l at day 1 before the administration of NDV and the extract and from 105.0g/l at day 7 following its administration to 138.0g/l at day 28 following the administration of both NDV and the extract. Also, Group C (serving as control) with no NDV and ethanolic extract of *A. nobilis* showed higher Hb level (135.0g/l) as compared to Group A receiving both NDV and ethanolic extract of *A. nobilis* with a lower Hb level (128.0g/l). The findings of this study were similar with the findings of Esonu *et al.* (2006), who observed significant increase in Hb level while feeding herbal plant (neem) to the laying hen. Results of our findings is also in contrast with the findings of Gautam *et al.* (2004), who noticed that no significant effect on Hb level was observed, in animals fed *Withania somnifera*. However, it is also in agreement with the result of Sham *et al.* (2003), who reported

significant effect on hemoglobin and red cell count, while feeding *Withania somnifera* to animals. Mohammed et al. (2011) reported that the values for HB differed significantly ($P < 0.05$) among the treatment groups (growing rabbits) fed with camel blood-rumen content mixture.

This study showed that 7 and 28 days following administration of NDV and the ethanolic root extract of *A. nobilis*, mean corpuscular haemoglobin concentration estimation (MCHC) level showed negligible differences ($P > 0.05$) among the three groups, A (33.3% and 33.8% respectively), B (32.4%) and C (32.1%). Since, they fall within the normal range of 30.0%-36.0%. It showed perhaps that the disease does not affect that aspect. Means total white blood count concentration (TWBCC) level as presented Table 3b and 3c showed that the presence of Newcastle disease in those of group A and B gave rise to increase in the full blood count (TWBCC) cellmm⁻³. While those of group A and group C (controls) fell within normal ranges of 4.0×10^3 - 5.5×10^3 cellsmm⁻³, those of group B had no survivor 28 days after the administration of the NDV and the extract. Finding of this study is in disagreement with the findings of Gautam et al. (2004), who noticed that no significant effect on lymphocyte and WBC counts was observed, while feeding *Withania somnifera* to animals. Our result can also be comparable with the findings of Sham et al. (2003) and who reported significant increase in white cell counts while feeding *Withania somnifera* to the mice and that of Sajjad et al. (2011), who reported that water based infusion of Aloe vera gel, Barbary, Garlic and Ginger had significant effect on lymphocyte and WBC counts of rabbits. Mohammed et al. (2011) reported that the values for WBC differed significantly ($P < 0.05$) among the treatment groups (growing rabbits) fed with camel blood-rumen content mixture.

It has been reported that the outbreaks of ND are present on a yearly basis and depend on the season and factors such as age, breed, type of bird and management system of poultry play vital roles in the prevalence of ND in Nigeria (Nwanta et al., 2008b; Okwor and Eze, 2010). The physiological, nutritional and pathological conditions of animals are usually assessed, using haematological and biochemical analyses of their blood (Cetin et al., 2009, 2010; Al-Eissa et al., 2011). From our findings that most of hematological indices of poultry chickens tend towards normal after treatment with the root extract of *A. nobilis* showed the effect of this plant extract on poultry chickens challenged Newcastle disease virus (NDV). This is an encouraging development in that the use of this extract will be of immense help where vaccines are not readily available. Previous studies had reported vaccination as the only safeguard against endemic ND (Usman 2002; Nwanta et al., 2006). Nwanta et al.

(2008b) in their study reported that single vaccination had a significant effect on reducing incidence of Newcastle disease compared to birds that were not vaccinated nor had multiple ND vaccinations. However, the frequent power outages coupled with poor information on the part of the farmers on vaccine procurement and handling makes vaccine failure a common phenomenon in Nigeria (Okwor et al., 2009).

The limitations of this present study were firstly, we did not screen for phyto-chemical properties of *Anthocleista nobilis* and secondly, we were unable to conduct a toxicity study to determine its LD₅₀ and test its antiviral property as a preliminary step. However, following our initial reports on the liver enzymes and other biochemical parameters, lends credence in establishing its safety in the intended host.

5. Conclusion

Most family poultry are threatened by disease outbreak, especially NCD (Moreki, 2012). Family poultry is usually owned and managed by resource-poor farmers who are unable to buy expensive vaccines for their flocks (Moreki, 2012). These vaccines require cold chain that is lacking in the village environment. As a result, EVM is crucial in preserving the health of family poultry because it is cheap, readily available and cost effective (Moreki, 2012). The EVM preparation and administration varies from place to place and also differs depending on the diseases treated (Moreki, 2012). Medicinal plants should be conserved, cultivated and harvested strategically to preserve them for future use (Moreki, 2012). Further studies are required in order to document ethnoveterinary practices used for health management of family poultry (Moreki, 2012).

Conclusively, the mean packed cell volume (PCV), mean haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC) and full blood count (TWBCC), were significantly different ($P < 0.05$) among the treated (group A) and the untreated group (group B). Group A were significantly ($P < 0.05$) influenced by the treatments after 7 days and 28 days following the administration of the *A. nobilis* ethanolic root extract. However, since the chickens in groups A and B were injected intravenously with the same dose of New Castle Virus (NDV) and the confirmatory symptoms observed in group B which was initially mild in group A and later was not noticeable, coupled with the ability of group A poultry chickens to tend towards normal after treatment are physiological evidences of the antiviral effect of the root extract of *Anthocleisia nobilis* on hematological indices of the poultry chickens studies. It also showed that the extract was able to impact immunity in chickens suffering from Newcastle disease. There is therefore, the need for the ethanolic extract of *Anthocleisia nobilis* to be publicized so as to

create awareness to farmers for the treatment of Newcastle disease.

Acknowledgements

The authors acknowledge the assistance of the members of staff of Department of Science Laboratory Technology, School of Applied Arts and Science, The Federal Polytechnic, Bida, Niger State, Nigeria for instrumentation and The National Veterinary Research Institute (NVRI) Vom, Jos, Nigeria for the provision of the viral isolates and materials during the course of this study. We also recognize the contribution and supports of Dr. A. Banso and Miss VI Michael in the Department of Science Laboratory Technology, School of Applied Arts and Science, The Federal Polytechnic, Bida, Niger State, Nigeria for their contributions to this study.

References

- [1] Abdu, P.A., Sa'idu, L., Bawa, E.K., Umoh, J.U. (2005a). Factors that Contribute to Newcastle Disease, Infectious Bursal Disease and Chicken Pox Outbreaks in Chickens. Proceedings of 42nd NVMA Congress (Maiduguri 2005), El-kanemi Hall University of Maiduguri, Borno State, pp: 66-68.
- [2] Abdu, P.A., Bawa, E.K., Umoh, J.U., Sa'idu, L. (2005b). Factors that Contribute to Newcastle Disease, Infectious Bursal Disease and Chicken Pox Outbreaks in Vaccinated Chickens. Proceedings of 42nd NVMA Congress (Maiduguri 2005), El-kanemi Hall University of Maiduguri, Borno State, pp: 68-70.
- [3] Adene, D.F., Oguntade, A.E. (2006). The Structure and Importance of the Commercial and Village Based Poultry Industry in Nigeria. Food and Agricultural organization (FAO) Poultry Production Systems; ECTAD/AGAP, pp: 1-102.
- [4] Akinmutimi, A.H. (2004). Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resource for broiler chickens. A Ph.D Thesis, Michael Okpara University of Agriculture, Umudike, Nigeria.
- [5] AL-Eissa, M.S., Alkahtani, S. (2011). Seasonal influence on some blood and biochemical parameters of Jerboa (*Jaculus jaculus*) in Saudi Arabia. Research Opinions in Animal & Veterinary Sciences (ROAVS) 1(1), 51-54
- [6] Alexander, D.J. (2003). Newcastle disease and other avian Paramyxoviridae infections. In: Disease of Poultry, Ed. Saif, Y.M; Barnes, H.J; Glisson, J.R. Fadly, A.M; McDougald, L.R. and Swayne, D.E. 11th Edn., Iowa State University Press, Ames, IA, pp: 63-87.
- [7] Awan, M.A., Otte, M.J., James, A.D. (1994). The epidemiology of Newcastle disease in rural poultry: A review. Avian Pathology, 23 (3): 405 – 423
- [8] Beard, C.W. (1998). Vetogenic Newcastle Disease. In: Foreign Animal Disease: The Gray Book. Richmond VA, US Animal Health Association, USA
- [9] Bell, J.G., Spradbrow, P.B. (ed). (1992). Newcastle disease in village chickens in North, West and Central Africa.. Proceedings, International Workshop held in Kuala Lumpur, Malaysia Canberra Centre for International Agricultural Research (ACIAR) pp. 142-143. Centre for International Agricultural Research (ACIAR)
- [10] Burkill, H.M. (1995). *The Useful Parts of West Tropical Africa* (2nd ed.) Royal Botanic Gardens KVVU, pp145-150.
- [11] Cetin, N., Bekyurek, T., Cetin, E. (2009). Effects of sex, pregnancy and season on some haematological and biochemical blood parameters in angora rabbits. Scandinavian J. Lab. Animal Sci., 36, 155-162.
- [12] Cetin, E., Kanbur, M., Silici, S., Eraslan, G. (2010). Propetamphos-induced changes in haematological and biochemical parameters of female rats: Protective role of propolis. *Food and Chemical Toxicology*, 48: 1806-1810.
- [13] Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Cambridge University Press, p. 434.
- [14] Dairo, F.A.S. (2005). Assessment of rumen content on the haematological parameters of growing rabbits: Proc. of 10th Annual Conference of Animal Science Association of Nigeria (ASAN), Sept. 12-15. University of Ado Ekiti, Nigeria. Pp. 301–302.
- [15] Esonu, B.O., Opara, M.N., Okoli, I.C., Obikaonu, H.O., Udedibie, C., Iheshiolor, O.O.M. (2006). Physiological Response of Laying Birds to Neem (*Azadirachta Indica*) Leaf Meal-Based Diets: Body Weight Organ Characteristics and Haematology. *Journal of OJHAS*, 5: 972-997.
- [16] Friend, M. (2006). Evolving changes in diseases of waterbirds. In Waterbirds around the world. G.C. Boere, C.A. Galbraith and D.A. Stroud (Eds). The Stationery Office, Edinburgh, UK, pp: 412-417.
- [17] Gautam, M., Diwanay, S.S., Gairolac, S., Shinde, Y.S., Jadhav, S.S., Patwardhan, B.K. (2004). Immune response modulation to DPT vaccine by aqueous extract of *Withania somnifera* in experimental system. *Journal of Ethnopharmacology* 4: 841-849.
- [18] Jordan, F.T.W. (1990). Poultry Disease (3rd edn). Cambridge University Press, Great Britain: p121 – 136.

- [19] Lewington, A. (1990). *Plants for People*. The Natural History Museum London: pp 136 – 144.
- [20] Lewis, M.R., Rose, S.P., Mac Kenzie, A.M., Tucker, L.A. (2003). Effect of dietary inclusion of plant extract on the growth performance of male broiler chicken. *Journal of British Poultry Science*, 20: 78-82.
- [21] McMullin, P. (2004). *A Pocket Guide to Poultry Health and Disease*. 5M Enterprises Ltd., Poultry Health Services, Sheffield, UK.
- [22] Mohammed, G., Igwelbuke, J.U., Kwari, I.D. (2005). Performance of growing rabbits fed graded levels of goat rumen content. *Global Journal of Pure and Applied Sciences*, 11 (1):39–43.
- [23] Mohammed, G., Igwebuikie, J.U., Ubosi, C.O., Alade, N.K. (2008). Comparative study of the nutrient composition, Amino acid profile and microbial assay of fresh and dried cattle, camel, sheep and goat rumen content. Proceeding of the 13th Annual Conference of Animal Science Association of Nigeria. (ASAN), ABU, Zaria, Sept. 15th -19th Pp: 518–520.
- [24] Mohammed, G., Igwebuikie, J.U., Alade, N.K., Adamu, S.B., Raji, A.O. (2011). Haematological and serum biochemical indices of growing rabbits fed camel blood-rumen content mixture. *Research Opinions in Animal & Veterinary Sciences*, 1(1): 44-47
- [25] Nwanta, J.A., Umoh, J.U., Abdu, P.A., Ajogi, I., Alli-Balogun, J.K. (2006a). Management of losses and Newcastle disease in rural poultry in Kaduna State, Nigeria. *Nigerian Journal of Animal Production* 2006, 3(2): 274–285.
- [26] Nwanta, J.A., Umoh, J.U., Abdu, P.A., Ajogi, I., Adeiza, A.A. (2006b). Field trial of Malaysian thermostable Newcastle disease vaccine in village chickens in Kaduna State, Nigeria. *Livestock Research for Rural Development*. Volume 18(5), Article #60. Retrieved April 2, 2011, from <http://www.lrrd.org/lrrd18/5/nwan18060.htm>
- [27] Nwanta, J.A., Abdu, P.A., Ezema, W.S. (2008a). Epidemiology, challenges and prospects for control of Newcastle disease in village poultry in Nigeria. *World's Poultry Science Journal*, 64: 119-127
- [28] Nwanta, J.A., Egege, S.C., Alli-Balogun, J.K., Ezema, W.S. (2008b). Evaluation of prevalence and seasonality of Newcastle disease in chicken in Kaduna, Nigeria. *World's Poultry Science Journal* 64 : 416-423
- [29] Okwor, E.C., Eze, D.C. (2010). The Annual Prevalence of Newcastle Disease in Commercial Chickens Reared in South Eastern Savannah Zone of Nigeria. *Research Journal of Poultry Sciences*, 3(2): 23-26
- [30] Okwor, E.C., Eze, D.C., Uzuegbu, M.O. (2009). Effect of storage conditions on the potency of Newcastle disease vaccine la sota. *Int. J. Poult. Sci.*, 8: 999-1002.
- [31] Oladele, B.S., Abdu, P.A., Nok, A.J., Esiebo, K.A.N, Useh, N.M. (2002). Effect of some inhibitors on neuramidase of Newcastle disease virus Kudu 113 strain. *Veterinarsiarlir*, 72: 185-194.
- [32] Oyawoye, E.O., Ogunkunle, M. (1998). Physiological and biochemical effects of raw jack beans on broiler. *Proceeding of Nigerian Society of Animal Production*, 23:141–142.
- [33] Sadiq, M.A., Nwanta, J.A., Okolocha, E.C., Tijjani, A.N. (2011). Retrospective Study of Newcastle Disease (ND) Cases in Avian Species in Maiduguri, Borno State, North Eastern Nigeria. *International Journal of Poultry Science* 10 (1): 76-81, 2011
- [34] Saidu, L., Abdu, P.A. (2008). Outbreak of viscerotropic velogenic form of Newcastle disease in vaccinated six weeks old pullets. *Sokoto J. Vet. Sci.*, 7: 37-40.
- [35] Sajid ur, R., Durrani, F.R., Chand, N., Khan, R.U., ur Rehman, F. (2011). Comparative efficacy of different schedules of administration of medicinal plants infusion on hematology and serum biochemistry of broiler chicks. *Research Opinions in Animal & Veterinary Sciences (ROAVS)*; 1(1), 8-14
- [36] Sham, D., Chitreb, D., Patwardhan, B. (2003). Immunoprotection by botanical drugs in cancer chemotherapy. *Journal of Ethnopharmacology*, 90: 49-55.
- [37] Thomas, D.W., Thomas, J.M., Bromley, W.A., Mbenkum, F.T. (1989). Korup Ethnobotany Survey. Final Report to the World Wide Fund For Nature. Panda House, Weyside Park, Godalming, Surrey, U.K.
- [38] Thomson, M., Al-Qattan, K.K., Al-Sawan, S.S., Alnaqeeb, M.A., Khan, I., Ali, M. (2003). The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. *Journal of Prostaglandins Leukotriens and Essential Fatty Acids*, 67: 475-478.
- [39] Usman, M. (2002). Effects of vaccination of chickens against Newcastle disease with thermostable V₄ and Lasota vaccines using different grains and their brans as vehicle. M.Sc Thesis, Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria, Nigeria.