

Antiviral effect of *Anthocleista nobilis* root extract on the liver homogenate indices of poultry fowls infected with Newcastle Disease Virus (NDV)

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Abstract

This study reports the preliminary investigation of the antiviral effect of *Anthocleista nobilis* root extract on the liver homogenate indices of poultry fowls treated for Newcastle Disease (ND). Eighteen (18) weeks-old fowls were used for this study. These were divided into 3 groups, A (infected and with treatment), B (infected and 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 0.5 mg per 100 g of body weight for 28 days. All the fowls were also given tetracycline antibiotic to eliminate bacterial infections. The average body weight and the temperature were monitored. The cytological examination of the fowls in group B showed that there was ulceration in the intestinal lining. There was increase in the average body temperature of the fowls in group B ($41.9 \pm 0.2^\circ\text{C}$). There was also a drop in their average body weight from $510 \pm 0.2\text{g}$ to $508 \pm 0.2\text{g}$ between days 1 to 6. The liver homogenate values obtained before challenging the fowls with NDV indicated that all the fowls were healthy and no physiological abnormalities were traced in them. The values obtained at day 7 of treatment with the extract indicated a drastic drop in the mean total protein and protein fractions of the fowls in group B (8.20g/l, 4.30g/l, and 3.90g/l), followed by those of group A (7.15g/l, 2.95g/l, and 4.20g/l) and C tended toward normal ranges (51.48g/l, 22.83g/l, and 28.65g/l). At day 28, the result showed that fowls in group A tended toward normal mean values of total protein (29.35g/l), albumin (11.70g/l), and globulin (17.65g/l), followed by group C total protein (52.80g/l), albumin (24.10g/l), and globulin (28.70g/l). The study showed physiological evidence that the treatment of ND with the extract is equally effective at day 28. After day 28, there were no survivors among the fowls in group B. No ulceration was observed in group A fowls. The study also showed a drastic drop in liver homogenate indices of group B fowls and those of group A. Fowls in group C were with normal ranges at day 7. This suggests that the extract of *A. nobilis* was able to either prevent the multiplication of NDV or ameliorate the toxic effect leading to ulceration. These positives changes observed in group A is thus physiological evidence that the treatment of ND with the ethanolic root extract of *A. nobilis* is effective. With these physiological evidences, it can be concluded that the extract was effective to impact immunity in fowls infected with NDV. Thus, there is need to create awareness among farmers and poultry dealers to encourage them to use the ethanolic root extract of *A. nobilis* for the treatment of ND. The extract was able to correct the physiological alteration (liver homogenate indices) associated with Newcastle disease.

Keywords: *Anthocleista nobilis*, Antiviral Effect, Liver Homogenate Indices, Fowls, Ulceration

Introduction

Poultry production has been improved significantly in the last three decades and plays a vital role in the economy of most countries of the world. The provision of quality protein in the shortest period of time in the form of meat and eggs is the major contributing role of poultry birds in human nutrition (Sajjad et al, 2011). Paramyxovirus 1 (PMV-1) or Newcastle Disease (ND) is considered among the most important disease of

poultry and outbreaks with mortality up to 100% are common (Alders and Spreadbrow, 2001; Saidu and Abdu, 2008). ND infection takes place through virus inhalation or ingestion and it spreads from one bird to another depends on the availability of the virus in its virulent infectious form (Whiteman and Bickford, 1983) and its short incubation period of 5-6 days (Chansiripornchai and Sasipreeyajan, 2006). The disease usually affects the respiratory, gastrointestinal and nervous systems with common signs of listlessness,

increased respiratory rate, yellowish to greenish diarrhea and weakness followed by prostration and death (Chansiripornchai and Sasipreeyajan, 2006). It is a highly contagious viral disease affecting poultry of all ages. Affected species include chickens, turkeys, pigeons and ducks. The condition is rarely diagnosed in ducks but is a possible cause of production drop/fertility problems (McMullin, 2004).

In most parts of the developed world, antiviral chemotherapy has come of age. Until a few years ago, the general consensus was that no antiviral chemotherapy could be envisaged that would not be complicated by toxicity for the host cell. This dogmatic view has proved incorrect with the advent of selective anti-herpes agent. All the antiviral drugs now known were discovered by random search in the laboratory, chance equally played an important role in the discovery of active antiviral molecules. Although the drugs now available are little used in clinical medicine, they serve to demonstrate that chemotherapy of viral infection is not (as some have thought) impracticable. Unfortunately, they give few clues to ways of discovering new & more effective agents. 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).

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 natured, reduced diseases associated risks, lowering blood cholesterol level and diversified functions in improving performance, growth rate, feed conversion rate and weight gain in birds (Lewis et al., 2003; Sajjad et al., 2011). Any plant that have one or more of its organs containing substances that can be used for chemotherapeutic purpose or which are precursors for the synthesis of useful drugs is regarded as a medicinal plant (Lewington, 1990). Medicinal plants constitute a very important part biological heritage of the earth. Traditional sciences place a high value on this inheritance which they express through their ultimate relationship with nature (Banso et al., 2003).

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 sap is considered in the treatment of ear ache and ophthalmia. All parts of the plants are found to contain alkaloids (e.g. leaf 0.05%). The root is the most active pharmacologically and is the most used as a purgative, a poison antidote, an emmenagogue, abortifacient to treat leprosy, edemas and elephantiasis of the scrotum. A root decoction is taken in Serra Leone for constipation and gonorrhea. 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).

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 physiological evidence (liver homogenate indices) that can be obtained during the treatment of poultry fowls against Newcastle disease virus. The liver homogenate indices were also examined before, during and after the administration of the *Anthocleista nobilis* root extracts and to determine and identify the physiological changes in the target organs (liver, kidney and intestine).

Materials and Methods

Eighteen poultry fowls of 4 weeks old were gotten and allowed to grow for another 4 weeks more, this was to enable the birds to develop their own Immunity, since the birds (fowls) were vaccinated immediately after hatch. The fowls were randomly distributed into 3 different groups namely A, B, and C and each group was kept in different strata. Groups A, B and C contained 6 fowls each, group C serve as the control. 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 ample. It was kept in the refrigerator at 4°C to avoid deterioration of the viral pathogen which was a velogenic strain. 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*. At week 7 and 28 of the experiment, blood samples were collected randomly from two (2) poultry fowls per treatment for the determination of the haematological indices. Samples were collected from the neck vein of the fowls by venipuncture using disposable needle (21-gauge needle) and syringes. The fowls were fasted overnight (12hrs) and normally bled in the morning (7.00–8.00am) to avoid excessive bleeding. The collection site was cleaned with alcohol

and xylene to dilate the veins. Sterile cotton was used to cover the collected in sample bottles containing no dipotassium salt of ethylene diamine–tetra acetic acid (EDTA–K2+).

The fowls in groups A and B were injected intravenously with 1ml of the NDV (0.11mg/ml concentration each). Treatment commenced immediately after challenging the fowls with NDV. 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 at interval of 6 hours. In order to eliminate bacterial and other infections, all the fowls were given tetracycline antibiotics by diluting it in their drinking water. The weight and temperature and signs and symptoms were observed twice a day (morning and evening) while the weight was monitored at the interval of 5 days. Two fowls from each group were slaughtered and dissected after 7 days to observe the damages that the Newcastle virus had inflicted on some of the major organs. Such organs include; the kidney, the intestine, and the liver. At week 7 of the experiment, blood samples were collected randomly from three (4) fowls per treatment for the determination of the liver homogenate parameters (total protein and protein fractions such as albumin and globulin) using standard methods as described by Cheesbrough (2006). The fowls were fasted overnight (12hrs) and normally bled in the morning (7.00–8.00am) to avoid excessive bleeding. The collection site was cleaned with alcohol and xylene to dilate the veins. Sterile cotton was used to cover the collected in sample bottles containing no dipotassium salt of ethylene diamine–tetra acetic acid (EDTA–K2+).

The blood samples collected without coagulant were used to determine the liver homogenate components such as albumin, total protein and globulin using the methods described by other workers (Spencer and Price, 1997; Ajagbonna et al., 1999; Uko et al., 2000; Mohammed et al., 2008, 2011). The liver homogenate analysis of blood samples were carried out at the Department of Science Laboratory Technology, The Polytechnic, Bida, Niger State, Nigeria, using the routinely available clinical methods (Bush, 1975). The blood samples collected without coagulant were used to determine the liver homogenate components such as albumin, total protein, and globulin using the methods described by elsewhere (Spencer and Price, 1997; Ajagbonna et al., 1999; Uko et al., 2000; Mohammed et al., 2008, 2011). The liver homogenates include total protein and protein fractions (albumin and globulin). Total protein was determined by using biuret method (Reinhold, 1953). Globulin was calculated by subtracting albumin from total protein (AL-Eissa and Alkahtani, 2011).

Results

The study was conducted to investigate the effect of administration of ethanolic root extract of *Anthocleista nobilis* on liver homogenate of poultry fowls. The results of the changes in the body weight of the test fowls are shown in tables 1. As evident from the table 1, no weight record was made for the birds in group B at the period because none of the fowls could survive the disease as about 66.7% died while 33.3% of the fowls were used for cytological examination. For the fowls in group A, the percentage survival was 83.3%. So, the average change in body weight of the fowls showed a drop in body weight of the tested fowls in group B as a result of the infection, while those of group A and C increased gradually.

Table 1: Average change (mean±SE) in body weight of test fowls (N=6)

(Days)	Group A	Group B	Group C
01	540 ± 0.2	510 ± 0.2	562 ± 0.2
06	543 ± 0.1	508 ± 0.2	568 ± 0.2
11	548 ± 0.1	-	575 ± 0.2
16	555 ± 0.2	-	580 ± 0.1
21	561 ± 0.2	-	587 ± 0.2
26	568 ± 0.1	-	591 ± 0.1

SD = Standard deviation; n = Number of Fowls per group; A = Fowls that were infected and with treatment; B = Fowls that were infected without treatment; C = Fowls that were not infected but were given treatment (control)

The average change in body temperature of the fowls from the first day of exposure to the last day of treatment for group A and C was initially high and later tender toward normal. Temperature of the birds in group B was higher in groups A and C (Table 2).

The results of the effect of the viral pathogens on the tested fowls are shown in table 3. The cytological examination of the test fowls in group B showed that there was ulceration of the intestinal lining of the fowls. However, ulceration of the intestinal lining was not observed in those of groups A and C. No visible damage was inflicted on the kidneys and livers of the fowls in all the groups.

Table 4 shows values of total protein, albumin and globulin obtained before challenging the fowls with the viral pathogens. It showed that all the fowls were healthy and no physiological abnormalities were traced in them. The total protein values across the groups are within normal ranges (48.50l/l–54.20 g/l).

Table 5 shows the antiviral effect of *Anthocleista nobilis* root extract on total protein, albumin and globulin obtained 7 days after challenging the fowls with New castle virus (NDV). These parameters showed a drastic decrease in group A and B.

Table 2: Average Change (mean±SE) in body temperature of the test fowls per day

Period (days)	Temperature (⁰ C)		
	A ± SE (n = 6)	B ± SE (n = 6)	C ± SE (n = 6)
1	40.7±0.2	40.7±0.2	40.5±0.2
2	40.3±0.2	41.1±0.2	40.3±0.2
3	40.9±0.2	42.3±0.2	40.3±0.2
4	40.6±0.2	41.8±0.2	40.4±0.2
5	40.9±0.2	42.3±0.2	40.2±0.2
6	40.6±0.2	41.8±0.2	40.3±0.2
7	40.6±0.2	42.5±0.2	40.0±0.2
8	40.4±0.2	42.6±0.2	40.2±0.2
9	40.6±0.2	42.3±0.2	40.2±0.2
10	40.6±0.2	-	40.3±0.2
11	40.5±0.2	-	40.3±0.2
12	40.3±0.2	-	40.4±0.2
13	40.4±0.2	-	40.2±0.2
14	40.4±0.2	-	40.4±0.2
15	40.3±0.2	-	40.3±0.2
16	40.4±0.2	-	40.0±0.2
17	40.3±0.2	-	40.5±0.2
18	40.2±0.2	-	40.4±0.2
19	40.4±0.2	-	40.3±0.2
20	40.3±0.2	-	40.4±0.2
21	40.2±0.2	-	40.3±0.2
22	40.3±0.2	-	40.2±0.2
23	40.4±0.2	-	40.3±0.2
24	40.5±0.2	-	40.2±0.2
25	40.3±0.2	-	40.4±0.2
26	40.4±0.2	-	40.3±0.2
27	40.2±0.2	-	40.4±0.2
28	40.3±0.2	-	40.3±0.2
Average Mean	40.4 ±0.2	41.9±0.2	40.3±0.2

Keys: SD= Standard Deviation; n = Numbers of Fowls per group with treatment; A = Fowls that were infected and treated; B = Fowls that were infected without treatment; C = Fowls that were not infected but given treatment (control)

Table 3: Effect of the viral pathogens on the tested fowls (cytological examination)

Group (Fowls)	Ulceration
A	-
B	+
C	-

Keys: - = No. Ulceration; + = Presence of Ulceration; A = Fowls that were infected and with treatment; B = Fowls that were infected without treatment; C = Fowls that were not infected but were given treatment (control)

Table 6 shows the effect of *Anthocleista nobilis* root extract on total protein, albumin and globulin of fowls challenged with New castle virus (NDV) following 28 days administration of the extract (i.e.

after 28 days of treatment of New Castle Disease). It showed that the liver homogenate values obtained 28 days after the administration of the extract among those in group A tended toward normal range.

Discussion

The beneficial health effects of extracts from many types of plant that are used as seasoning agents in food and beverages have been claimed for centuries (Friedman et al., 2002). Many hundreds of plants worldwide are also used in traditional medicine as treatments for bacterial infections (Martin and Ernst, 2003; Okoli and Iroegbu, 2004). Scientists from divergent fields are investigating plants with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory on all types of microorganisms *in vitro* (Cowan, 1999).

Our study showed that the average change in body weight of the fowls in group B dropped drastically. This could be attributed to the infection of fowls in group B (group that was inoculated with ND virus but not given the plant extract). This is in agreement with what was reported by Chen et al. (2011), who found that chickens not receiving bamboo extract (BFRE) and exposed to 10 °C showed a significant decrease in the body weight gain. There is an evidence to suggest that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects (Kamel, 2001). The finding of average body weight reported in this study is in agreement with what was reported by other authors in closely related studies (Cabuk *et al.*, 2003; Lee *et al.*, 2004; Al-Kassie 2009; Al-Kassie and Jameel, 2009). The effect of ground thyme and cinnamon on the performance of broilers was studied by Al-Kassie and Jameel (2009), who found their effect on the live weight gain and the improvement of the health of poultry, in addition to other performance traits, feed conversion ratio and feed intake. In another study by Al-Kassie (2009), extract oil derived from thyme and cinnamon in broiler diets improved body weight gain, feed intake and feed conversion ratio, which may be due to active materials in these plants which are considered as digestion stimulating factors, in addition to their antimicrobial activity against bacteria found in the intestine (Cabuk *et al.*, 2003).

The finding of average body weight reported in this study deviates from what was reported by Mtambo et al. (1999), who in a similar study that mean body weights of birds in group B declined markedly compared to the other groups. Mtambo et al. (1999) also reported that there was no prophylactic or therapeutic value of the plant extract against ND and

Table 4: Mean \pm SE of total protein, albumin and globulin in liver homogenate before challenging the fowls with Newcastle virus (NDV)

Group	Total Protein (g/l)	Mean Total Protein (g/l)	Albumin (g/l)	Mean Albumin (g/l)	Globulin (g/l)	Mean Globulin (g/l)
A ₁	54.20	52.65 \pm 0.2	23.00	23.05 \pm 0.2	31.20	29.60 \pm 0.2
A ₂	51.10		23.10		28.00	
B ¹	53.00	50.75 \pm 0.2	25.00	23.60 \pm 0.2	28.00	27.15 \pm 0.2
B ²	48.50		22.20		26.30	
C ¹	49.70	50.95 \pm 0.2	24.40	23.70 \pm 0.2	25.30	27.25 \pm 0.2
C ²	52.20		23.00		29.20	

Keys: A = Fowls that were infected and with treatment; B = Fowls that were infected without treatment; C = Fowls that were not infected but were given treatment (control)

Table 5: Effect of *Anthocleista nobilis* root extract on total protein, albumin and globulin (Mean \pm SE) after 7 days administration of the Newcastle virus (NDV) and root extract

Group	Total Protein (g/l)	Mean Total Protein (g/l)	Albumin (g/l)	Mean Albumin (g/l)	Globulin (g/l)	Mean Globulin (g/l)
A ₁	06.30	07.15 \pm 0.2	02.30	02.95 \pm 0.2	04.00	04.20 \pm 0.2
A ₂	08.00		03.60		04.40	
B ¹	06.00	08.20 \pm 0.2	02.70	04.30 \pm 0.2	03.30	03.90 \pm 0.2
B ²	10.40		05.90		04.50	
C ¹	51.85	51.48 \pm 0.2	23.05	22.83 \pm 0.2	29.80	28.65 \pm 0.2
C ²	50.10		22.60		27.50	

Keys: A = Fowls that were infected and with treatment; B = Fowls that were infected without treatment; C = Fowls that were not infected but were given treatment (control)

Table 6: Effect of *Anthocleista nobilis* root extract on total protein, albumin and globulin (mean \pm SE) of fowls after 28 days of administration of Newcastle virus (NDV)

Group	Total Protein(g/l)	Mean Total Protein(g/l)	Albumin (g/l)	Mean Albumin (g/l)	Globulin (g/l)	Mean Globulin (g/l)
A ₁	30.30	29.35 \pm 0.2	12.00	11.70 \pm 0.1	18.30	17.65 \pm 0.1
A ₂	28.40		11.40		17.00	
B ¹	-	-	-	-	-	-
B ²	-	-	-	-	-	-
C ¹	54.10	52.80 \pm 0.2	24.00	24.10 \pm 0.2	30.10	28.70 \pm 0.2
C ²	51.50		24.20		27.30	

Keys: A = Fowls that were infected and with treatment; B = Fowls that were infected without treatment; C = Fowls that were not infected but were given treatment (control)

that plant extract showed a negative effect on body weights in birds with ND. In our study, the average change in body weight of the fowls in group A (group that was inoculated with ND virus and treated with the plant extract) and group C (controls) increased gradually. This is in agreement with what was reported by Chen et al. (2011), who found that chickens not receiving bamboo extract (BFRE) had their body weight gains maintained within the normal range when the ambient temperature was 10°C. However, Chen et al. (2011) also showed that the plant extract used in their study was an effective additive in preventing chickens from losing body weight during cold stress.

The result obtained showed changes in the body temperature of the poultry fowls used in this study. This agrees with the fact that they suffered from Newcastle

disease (Beard, 1998). The occurrence of ulceration has been reported by Jordan (1990) as an indicator of Newcastle disease. Such ulceration was not observed in the treated group A suggests 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. Plant extracts always showed immuno-modulatory (Fakeye, 2008), anti-inflammatory (Castaldo and Capasso, 2002), antimicrobial and growth promoting effects (Kumari et al., 2007). It has been reported that plant extracts often contain flavonoids, bioactive polysaccharides, and other that have been found to be beneficial in promoting chicken growth and resistance to disease (Chen et al., 2011).

According to Akinmutimi (2004), biochemical blood components are influenced by the quantity and

quality of feed. Biochemical 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). Hence, serum chemistry is becoming increasingly important diagnostic tools. Blood parameters are used as an aid tool for the diagnosis of infectious and several parasitic diseases (AL-Eissa and Alkahtani, 2011). The liver homogenate values obtained before challenging the fowls with NDV indicated that all the fowls were healthy and no physiological abnormalities were traced in them. The liver homogenate values obtained after 7 days of treatment with the extract indicated a drastic drop in the mean total protein and protein fractions of the fowls in group B followed by those of group A and C tended toward normal ranges. These positives changes observed in group A shows that there are physiological evidence that the treatment of ND with the ethanolic root extract of *Anthocleista nobilis* is effective.

In the same vein, the liver homogenate values obtained 28 days after the administration of the extract showed that poultry fowls in group A tended toward normal mean values of total protein (29.35g/l), albumin (11.70g/l), and globulin (17.65g/l), followed by group C with mean values of total protein (52.80g/l), albumin (24.10g/l), and globulin (28.70g/l). This similar trend as observed after 7 days after administration of the extract also show physiological evidence that the treatment of ND with the ethanolic root extract of *Anthocleista nobilis* is equally effective after 28 days. However, the no survivor observed among the fowls in group B is an indication that these fowls could not survive the ND to that period of time. The normal range of values observed for treatments A and C suggest adequate protein for normal metabolic and physiological activities (Mohammed et al., 2011).

In conclusion, the fowls in groups A and B injected intravenously with the same dose of NDV. The confirmatory symptoms observed in group B was initially mild in group A and later became unnoticeable after the treatment with the root extract of *Anthocleisia nobilis*. With these physiological evidences, it can be concluded that the root extract of *Anthocleisia nobilis* was effective to impact immunity in fowls suffering from ND. Therefore, there is need to create awareness among farmers and poultry dealers for the ethanolic root extract of *Anthocleisia nobilis* to be used for the treatment of Newcastle disease.

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