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EVALUATING THE EFFECT OF *MOMORDICA BALSAMINA LINN*, SELENIUM AND LAMIVUDINE FOR THE TREATMENT OF NEWCASTLE DISEASE IN PULLETS

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ABSTRACT
Newcastle disease virus (NDV) is an avian paramyxovirus that causes significant economic losses to the poultry industry worldwide. Aim of the study was to evaluate the effect of *M. balsamina,* selenium and lamivudine for the treatment of ND. While the objectives of the study were to: Determine the Midian Lethal dose (MLD) of *M. balsamina,* selenium and lamivudine for the treatment of ND. A total of three hundred day-old dominant black pullets obtained from a commercial hatchery in Ibadan, Nigeria were randomly assigned into six groups. Group I was pretreated orally with 300mg of aqueous ethanol extract of *M. balsamina* / bird for 1 week before challenge with NDV Kudu 113. Birds in group II were inoculated with NDV Kudu 113 and then treated immediately with 300mg of ethanol extract of *M. balsamina* fruits for 3 weeks. Birds in group III were inoculated with NDV Kudu 113 and immediately treated with lamivudine with 300mg/ 1 litre of water and group V and VI were negative and positive control groups respectively. Both positive and negative control groups were treated with drinking water for 3 weeks orally. Birds were bled in weekly basis: on day-old, 4th week, 5th week, 6th week and 7th week. Serum was obtained by allowing the blood collected from each bird to stand for 3hrs at room temperature. Clinical signs of ND recorded in groups due to ND in pullets were: Group I: inactive, emaciation, dollness, ruffle feathers, anorexia, weakness, coughing, sneezing, greenish white diarrhea, salivation, mortality 5,9,8,1,7. Group II: inactive, emaciation, dollness, ruffle feathers, anorexia, weakness, coughing, sneezing, greenish white diarrhea, salivation, mortality 12,13,3. Group IV: inactive, emaciation, dollness, ruffle feathers, anorexia, weakness, coughing, sneezing, greenish white diarrhea, mortality 4,3,2,2.

INTRODUCTION
Background of the Study
Newcastle disease virus (NDV) is an avian paramyxovirus that causes significant economic damage to the poultry industry. It is a specific, highly contagious disease primarily of chickens and turkeys, other domestic poultry, various species of wild birds, and people are susceptible to it, but in man it is usually mild and is characterized by inflammation of one eye, seldom both (Knipe *et al.;* 2007 and Aldous and Alexander, 2001). Among young chickens the loss may be as high as 100%. Chicks that survived an outbreak are retarded in growth and efficiency of feed utilization. It also has potentials for expanding its host range in nature (Brandly, 1950). The virus itself belongs to the order mononegarirales, family paramyxoviridae, subfamily paramyxovirinae, genus avulavirus. The avulavirus genus includes serologically distinct avian paramyxovirus (APM V-1-APM V-9) (Alexander, 1997; Bread and Hanson, 1997; Knipe *et al.;* 2007; Alexander and Jones, 2008). Over 200 avian species are naturally or experimentally susceptible to ND. Isolates are of single serotypes, but have a wide range of naturally occurring pathogenicities from avirulent (lentogen) to mildy virulent (mesogen) and highly virulent (velogen) (Alexander and Senne, 2008).

Many species of domestic, semi domestic and wild birds have been found to be susceptible (Khan, 1968 and Arshad *et al.;* 1988). The death loss among laying birds usually is quite low but occasionally may be as high as 80 % of the flock (Alexander, 2000). Production and quality of eggs usually drop sharply (Alexander, 2000). The disease in breeding flocks also results in lower fertility and hatchability. Additional losses to the poultry industry arise from the restriction on exportation of live and dressed fowl across the globe (Alexander, 2000).

The simplest and most logical control measure against ND and other infections is to prevent contact of the virus with susceptible birds and to vaccinate (Brandly, 1952). Ethnoveterinary medicine, the scientific term for traditional animal health care, encompasses the knowledge, skills, methods, practices, and beliefs about animal health care found among the members of a community (McCorkle ,1986). The knowledge base differs not only from region to region but also among and within communities. It has been developed through trial and error and deliberate experimentation. Therefore, it is less systematic, less formalized, and not universally recognized as a valid method of disease control in animals. In many countries, there has been little documentation of traditional knowledge; rather, it has been transmitted across generations by an oral tradition and therefore is in danger of extinction. While traditional healers have less to offer in the treatment and control of epidemic and endemic infectious diseases like foot and mouth disease, rinderpest, septicemia, anthrax, and acute life-threatening bacterial diseases and viral diseases e.g Newcastle disease, but they can cope with a reasonable spectrum of common diseases such as diarrhea, wounds, colds, worms, coccidiosis, and reproductive disorders (McCorkle, 1986). Traditional medicine has increased significantly in industrialized countries due to the fact that many prescription drugs have originated from the tropical flora (Nelson-Harrison *et al.;* 2002). Nigeria is endowed with many medicinal plants both domesticated and wild like every other developing country, where majority of its population depend on these plants to meet their health needs (Oladunmoye and kehinde, 2011). Several plants have been used in Folkloric medicine for the treatment and prevention of infectious diseases and non- infectious diseases in man and his animals, and this has led to renewed scientific interest in the use of plants for this purposes (Oridupa *et al.;* 2011). One of such plants finding applications in this respect is *Momordica balsamina.*

The general botanical characteristic of the cultivated member of this family (*Cucurbitaceae*) have been outlined by Purseglove. (1968) as follows: dendril-climbing or postrate annual occasionally rapidly growing perennials, annual herb, climbing vines growing 3-4 m in lengh. The family has large leaves which are dark green and kidney shaped with large nodes and entire margins (Cobley, 1977). Its dendrils are fairly stout and are divided about halfway along their length into many branches. The leaves are also very hairy on the under surface and are simple but often deeply alternate or spirally arranged (Cobley, 1977). Due to the nature of ND, local people developed the habit of treating their local chickens with various items viz: Red pepper (*Capsicum frutenscens*), gauta kaji (*Solonum modiflorum*) and experiment conducted using *Momordica balsamina*

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(*Balsam apple*), Selenium and lamivudine in which Selenium was found to be more effective (62.90 %) ameliorating the effect of ND (Agang, 2014).

MATERIALS AND METHODS

Materials

Ethical Clearance

Ethical clearance certificate was obtained from Ethical Committee on animal use and welfare, Ahmadu Bello University, Samaru, Zaria, Nigeria with number ABUCAUC/2018/038.

Source of Newcastle Disease Virus

Newcastle disease virus Kudu 113 strain was sourced from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The virus was transported to Zaria using a flask containing ice blocks. The titre of the NDV Kudu 113 was 10 8.5 ETD, dilution of NDV Kudu 113 virus was 1:99ml of phosphate buffered solution and 0.1ml administered per bird intra nasally and the number of viruses were 31,622, 776.6 million.

Pullets

Day-old pullets (Dominant black) were purchased from a reputable commercial hatchery at Ibadan, Nigeria.

Feeds

Commercial chick mash from Vital feed Grand cereal composed of (300-400kg) maize, (1.0%) calcium, (4.0%) bone meal, (23%) crude protein, (10%) fat, (10%) crude fibre, (0.46%) available phosphorus, (3000 kcal/kg metabolisable energy).

Drugs

Vitalyte (Anupco,company, England) a preparation containing vitamins and minerals was given at a dose rate of 5g/4 litres of water. Neomycin sulphate (Neotreat®) at a dose rate of 60mg/200 litre of water was administered to the birds during the brooding period. Birds were allowed to stay only on clean drinking water for one week after the last drug and vitamin administration for the birds to stabilize.

Infectious Bursal Disease Vaccine

The second week of age of the broilers. Georgia strain (Intermediate strains vaccine) manufactured by (Indovax-India) 2 vials of IBDV (200 doses/vial) were constituted in 4 litres of diluents (saline) for administration through drinking water (orally). Vaccination was carried out using milk which traped debrise and was administered in the morning.

Source of Lamivudine

Lamivudine, a product of Danadams pharmaceutical company, Accra Ghana was also obtained from reputable pharmaceutical store in Samaru, Zaria, Nigeria and given at a dose rate of 150mg/kg (2 tablets/40 chickens) in drinking water (7.5mg/chicken).

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Management

Housing

The chicks were housed in the experimental animal house of the Department of Public Health and Preventive Medicine and Veterinary Anatomy for the control group in the Faculty of Veterinary Medicine, ABU Zaria, Nigeria. The chicks (350) on arrival were brooded together. At the end of the brooding period, 40 pullets were assigned to every challenged group in the same house while group I and control group were separated. The chicks in all the groups were raised on deep litter system with a floor space of 0.14sqcm^2/bird.

Source of Heat

The chicks were brooded together for 4 weeks to enable them obtain source of heat. Heat was provided using six (6) 200 watt electric bulbs with a range of temperature of 36-37°C was applied (Demarchi *et al.;* 2008).

Chemicals and Reagents

Source of Selenium

Selenium, a product of Biovac Company in Israel was purchased at Rabiah Company Nigeria Ltd in Jos, Plateau State, Nigeria. Ingredient: Selenium was given at a dose rate of 1ml/litre of drinking water.

Source of Tween 80 (Diluent)

Tween 80, a product of William and Sons Company England was obtained from a reputable chemical stone Kwangila, Zaria, Nigeria. It was used at 2-3 drops to dissolve the *M. balsamina* fruit ethanol extract.

Methods

Collection of Plant

Fresh fruits of *M. balsamina* were collected between the month of February to April, 2013 at the National Veterinary Research Institute (NVRI)-Vom in Jos South Local Government Area of Plateau State, Nigeria (Plate I and plate II). The collected fruits were transported to Zaria, Kaduna State, Nigeria. The identification of the plant fruits was done and confirmed by Mallam Musa Muhammed, a taxonomist and a voucher specimen number, 01137 was deposited at the department of Biological Sciences, Ahmadu Bello University, Zaria Nigeria. The fruit was air dried in the laboratory at room temperature, pounded using mortar and pestle and stored in a plastic container until needed.

Lork's Method (LD_{50})

Three pullets each in the groups: A_1, A_2 and A_3 were orally treated with 10, 100 and 1000mg/kg body weight respectively. The birds were observed for 2 days (48h) for signs of toxicity and mortality. The birds in group B were also subdivided into 3 groups B_1, B_2 and B_3 of one bird each. Based on the initial results, further doses of 1600, 2900 and 5000mg/kg body weights were administered to calculate an LD_{50} as described by (Lorke, 1983).

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Table 2.1: Experimental Design of Groups and Ages (in weeks) of pullets inoculated and treated

Group	(Weeks) of Pullets or Inoculation Treatment			
	4	5	6	7
T_1	*M.b*	Kdv	*M.b*	*M.b*
T_2	kdv+*M.b*	*M.b*	*M.b*	*M.b*
T_3	kdv+Lam	Lam	*Lam*	*Lam*
T_4	kdv+Se	Se	Se	Se
T_5	Solv	Solv	Solv	Solv
T_6	Kdv	Solv	Solv	Solv

Key: Lam = Lamivudine (antiretroviral drug), *M.b* = *Momordica balsamina*, kdv = Newcastle disease virus (Kudu 113), Solv = Solvent, Se = Selenium.

Collection of Blood Sample

In day-old chicks about 2ml of blood was collected from the heart of each day-old chick using 2ml syringes and 25G needles were used. matured cocks were also sampled 2ml of fresh blood for Haemagglutination inhibition test (HI). The blood samples were transfered into 5ml plastic tubes and left overnight in a refrigerator at a temperature of -4°C. Serum samples were extracted using a plastic micropipette and transferred into sample bottles without EDTA (Anticoagulant)(Ethyline TetraAmine) and were frozen under -20°C (Deep freezer) until tested. The sera were used for haemagglutination inhibition test (HI) as described by OIE (2009).

Haemagglutination Test

Haemagglutination test was used to determined antibody titres to Newcastle disease using methods described by OIE, 2009. This was done for all the groups. Newcastle disease virus La-sota obtained from National Veterinary Research Institute (NVRI), Vom, Nigeria was used as the antigen for the HI test.

Preparation of 1% Chicken Red Blood Cell

An equal volume of 2ml of alsever's solution was added to 2ml of blood that was collected from matured cocks. The red blood cells were washed 3 times with phosphate buffered saline (PBS). Thereafter, 1ml of the washed RBC was added to 99ml of PBS to obtain 1% RBC solution.

Haemagglutination Inhibition Test

Sera collected from pullets were tested for NDV specific antibody by the HI as described by Allan and Gough (1974) and OIE (2009). The HI test was performed using beta technique (constant virus and varing serum) against 4HA units of the virus computed from the HI titration. Two fold serial dilution of 25ml serum was made with phosphate buffered saline (PBS) in V-bottomed microtitre plates up to 10^{th} well. Twenty five micro litres of 4 haemagglutinating (HA) units of NDV virus or antigen (La-sota) was added up to 11^{th} well. The plates were kept at room temperature for more than 30 minutes to facilitate antigen-antibody reaction. Then 50ml of 1% (v/v) chicken RBC suspension was added to each well. The 11^{th} well contains antigen and RBCS as the positive control and

the 12th well contains only RBCS as the negative control. After gentle mixing the RBCS were allowed to settle at room temperature for 40 minutes and agglutination was assessed by tilting the plates.

Data Analysis

Mean antibody titre values were expressed as mean ± S.E.M and were subjected to two way analysis of variance (ANOVA) followed by Turkey's post-hoc test using graph pad prism version 4.0 for windows from graph pad software, San Diego, California, USA, values of $P \leq 0.05$ were considered significant. Mortality rate was calculated (number of dead (n) ÷ total number (N) x 100.

RESULT

Clinical Signs

Common Clinical Signs were: ruffled feathers, seclusion, dollness, inactive, anorexia, weakness, coughing, greenish watery diarrhea, torticolis, salivation, leg paralysis, emaciation, rales and mortality 13,9,9,7. The morbidity rate recorded after challenged for groups I, II, III, IV and VI were: 25%, 27.5%, 47.5%, 20%, 32.5% while mortality recorded in groups I, II, III, IV were: 88.10%, 68.10%, 73.7% and 37.10% respectively. Post Mortem lesions observed in groups I, II, III, IV, V and VI were: generalized heamorphages in the proventrivulus, intestine, trachea and caecal tonsils. There was congestion of the visceral organs: liver, lungs, intestine, while the air sacs were cloudy. At day-old, there was no statistical significant difference ($P > 0.05$) in the level of antibody titre in all the birds. Similarly, at 4 weeks of age post challenge, there was no significant difference CP = 0.02626) in the level of ND antibody titre in challenged birds. Therefore, it may concluded that selenium can be used to control ND in pullets. The study recommended further research on the leaves, stems, roots and whole plants to determine the effect or benefit of *M. balsamina* in the control of ND and their mode of actions on the eggs (Alexander, 2000).

Gross Lesions

Gross Lesions were:

T_1: Congestion of the lungs, haemorrhages of the intestinal lining, liver, thymus and spleen. Haemorhagic bands in the proventriculus, necrosis of the intestinal lining and the trachea, mucoid in the trachea, severely haemorrhagic proventriculus, cloudy air sacs, emaciation of the muscles, blood clot in the thorac and heart. Pus in the proventriculus, distended gall bladder, and pale kidney.

Haemagglutination Inhibition Titre

Generally, the HI titre was significantly higher in group I compared to the other groups. Table 3.1 showed the level of HI titre, which appeared higher in group I. The sample showing peculiar central button shaped settling of RBC's were recorded as positive and maximum dilution of each sample causing haemogglutination inhibition was considered as the end point, which was used to estimate the HI titre. The HI titre of each serum sample was expressed as reciprocal of the serum dilution and most conveniently expressed

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as the logarithm to the base 2. The HI test is based on the principle that the haemagglutinin on the viral envelope can bring about the agglutination of chicken red blood cells (RBC) and this can be inhibited by specific antibodies. In the absence of any antibody against the virus haemogglutination occurs appearing as a diffused red colour at the bottom of the well. In the wells where the antibody against the virus is of a sufficient level, haemagglutination is inhibited and the red blood cells sediment and appear as a small pellet at the bottom of the well. The presence or absence of agglutination is accurately assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing RBCs and PBS only) should be considered to show inhibition.

Table 3.1: Antibody Titre

Group		Age in Weeks			
Group	Day-Old	4 Weeks	5 Weeks	6 Weeks	7 Weeks
	P=0.2626	P=0.01506	P<0.01	P<0.01	P=0.287
T1	2.20±0.09	0.00±0.00	10.00±0.00	5.38±1.39	7.56±1.25
T2	3.14±0.34	3.00±0.58	4.00±1.16	9.56±0.24	9.50±0.34
T3	3.17±0.54	3.50±0.50	4.60±0.68	9.50±0.31	9.00±0.21
T4	2.00±0.45	2.75±1.18	8.29±0.18	6.22±0.78	10.00±0.00
T5	2.38±0.33	2.00±0.00	1.00±0.00	8.13±0.81	9.25±0.75
T6	2.43±0.37	3.00±1.00	5.17±0.91	6.00±0.00	8.50±1.50

Table 3.2 mortality rate in four weeks old pullets following administration with virulent NDV Kudu 113 group I-VI.

Table 3:2 Mortality Rate

Group	Number Infected	Number Dead	Mortality Rate %
T1	10	37	88.1
T2	14	27	68.6
T3	23	29	73.7
T4	11	15	37.1
T5	0	0	0.0
T6	4	31	78.9

Mortality Rate % $= \frac{n}{N} \times \frac{100}{1}$

DISCUSSION

Rearing of pullets could be attributed to the desire to raise money to supplement the family nutrition. This agrees with the report of Amballi *et al.;* (2005) and Ajala *et al.;* (2007) where it was indicated that most poultry farmers do so to raise income. In Asia, similar results have been reported among poultry farmers in rural areas of India, Nepal, Pakistan, and Sri Lanka. (Herath, 2008) analysis results showed that ND to be the major poultry disease affecting pullets. Management and high cost of orthodox drugs lower pullets production too. These findings were in agreement with results of similar studies in other parts of the country Nigeria (Ibrahim and Tanya, 2001; Bukar – Kolo *et al.;* 2006).

Group 1 pretreated with *M. balsamina* for 1 week had increased in feed consumption and the pullets were active probably due to high amount of calcium, phosphorus and vitamin in ash (11.10%) of *M. balsamina*. These minerals and vitamins were required for increased bone density and muscles. This agreed with the work of Hassan and Umar. (2006) that *M. balsamina* has high calcium, phosphorus and vitamins.

In this study, mean antibody titre increased from day-old up to the 7th week. At day-old mean ND antibody titre range from 2.00±0.45 to 3.17±0.54 log 2 while after challenge mean antibody titre range increased to 10.00±0.00 log 2 for group 1. The mean HI antibody titres measured for all the groups were above the cutoff point of 4 log 2 and above, indicating protection of the chickens against ND. This is similar to what was reported previously by (Allan *et al.*, 1978; Verma *et al.*, 1985) that HI antibody titre of 4 log 2 or higher is considered as protective. This titre obtained at day-old could be ascribed as maternal derived antibodies (MDAS). The significantly increased mean HI titre obtained at first and second weeks for all the experimental groups could be attributed to the first active doses of IBD vaccine administered. This is because specific immunity against ND developed within a week of age or older (Brandly, 1952). Moreover the mean antibody titre for group 1 was significantly higher than that of group II 10.00±0.00 and 4.00±1.16 respectively. This is in agreement with the finding of Kouwenhoven, (1993) and Alexander, (1995). This study conducted that *M. balsamina* was found to be nontoxic to 4 week old pullets which dosed up to 5000mg/kg body weight, Selenium was effective in control of ND than *M. balsamina*. And it was recommended that further research is needed on other parts of the plant and that selenium has good potentials for the control of Newcastle disease in pullets.

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