Effects of Ethanol Extract of *Momordica Balsamina* and some Antioxidants on Pathological Changes in Broilers Challenge with a Virulent Newcastle Disease Virus.

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ABSTRACT

Newcastle disease (ND) possess a serious threat to the poultry industry as it has economy and ecological impact on pet, free living as well as domestic birds. The aim of the study was to determine the effects of *M. balsamina* and some antioxidants on pathological changes in broilers challenged with ND Kudu 113 virus. Two hundred day-old broilers were procured from a hatchery in Ibadan without NDV vaccination. The chicks were brooded for 4 weeks then divided into 8 groups of 20 broilers each. Each group was housed in a pen on deep litter system with a floor space of 1.14 cm^2 and birds in G1 were housed outside the faculty for fear of spread. All the experimental groups except group 1 were challenged with NDV Kudu 113 virus strain. Group 11 was given 2 L of distilled water at 5 weeks of age and challenged with NDV and not treated with any antioxidant. Birds in group 3 were administered vitamin C (600 mg tablets in 2 L of water after challenged. Birds in group 4 were treated with vitamin E (400 mg) soft gel capsules in 2 L water orally and challenged with NDV Kudu 113 virus. Birds in group 5 were treated with selenium-Vitamin E (1ml/L) in 2 L of water per birds orally after challenged. Birds in group 6 were treated with ethanol extract of *M. balsamina* leaves 400 mg in 2 L of water orally after challenged. Birds in group 7 were administered ethanol extract of *M. balsamina* roots 200 mg in 2 L of water orally after challenged and birds in group 8 were administered ethanol extract of *M. balsamina* leaves extract 400 mg orally + vitamin C 600 mg orally after challenged. The birds were bled for whole blood and serum weekly at 3, 4, 5, 6 and 7 weeks of age. The gross lesions recorded in groups 2, 3, 4 were congestion, haemorrhages, mucoid production in trachea, cloudy air sacs, and necrosis of the intestinal mucosa and distended gall bladder. In conclusion, ethanol extract of *M. balsamina* has no side effect even when administered at high dose of up to 5,000 mg per bird. Ethanol extract of the roots of *M. balsamina* has ameliorative effect against NDV by reducing mortality rate. Vitamin C also had ameliorative

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properties against NDV by reducing the pathology induced, and reduced mortality rate. Combining vitamin C (Synergistically) with ethanol extract of *M. balsamina* leaves had ameliorative properties against ND and recommended that the crude ethanol extract of *M. balsamina* roots can be used for treatment and control of ND.

Key words: *Momordica balsamina* Linn, pathology,Cobb 500, antioxidants, Newcastle disease

INTRODUCTION

Background of the Study

Newcastle disease was first recognized in Java, Indonesia and Newcastle – Upon – Tyne, England in 1926 and it was named Newcastle (Doyle, 1927). The disease is known in certain regions as Ranikhet disease, Pseudo fowl pest, pseudo poultry plague, avian pest, avian distemper, avian pneumoencephalitis and Korean fowl plague (Roy, 2012). Latter, it was found in various part of the world (Ashraf and Shah, 2014). By 1944, ND has been recognized throughout the world. Since then, the disease of chickens is widely spread throughout Nigeria with annual epidemics being recorded in highly susceptible poultry flocks (Adu *etal.*, 1986; Sa'idu *etal.*, 1994). The first documented outbreak of ND in, Nigeria occurred between December, 1952 and February, 1953 in and around Ibadan (Hill *etal.*, 1953). Since then, the disease became the most important viral disease of chickens, and it is widely spread throughout the country with annual epidemic being in highly susceptible poultry flocks (Adu *etal.*, 1986; Sai'du *etal.*, 1994). Newcastle disease virus is a non- segmented single stranded RNA genome of negative polarity and replicates in the cytoplasm of cells (Emmersion, 1999, Knipe *etal.*, 2007). The envelope contains lipids and surface glycoproteins (haemagglutin inneuraminidase (HN) and fusion (F), which surround the virion (Alexander, 1988). The penetration of the host cell by NDV occurs by fusion and mediated by a protein of the external envelope called fusion protein (F- protein) (Rott and Klenk, 1988). The protein is synthesized as a precursor (Fo) and needs to be cleavred into smaller fragments in order to be active (F1 and F2) (Rott and Klenk, 1988).

The pathogenicity of the NDV is determined by the amino acid sequence at the cleavage site of F-protein (Rott and Klenk, 1988; Lee *etal.*, 2004). There are four forms of ND which are caused by pathotype of different strains of the virus (Doyle's (Viscerotropic Velogenic), Beach's (Neurotropic Velogenic), Baudette's (mesogenic) and Hitchner's (Lentogenic) forms. In the Doyle's form all ages are susceptible. Morbidity rate may reach up to 100% and mortality is very high, usually 90% (Dobson, 1939). In the Beach's form (Neurotropic Velogenic), Morbidity is also high and mortality is variable. About 10% mortality in adult is very common though may be higher by the mesogenic strain (Elam, 1993). Among immature chickens, mortality is as high as 90%. Mortality in the adult in the case of the Beaudette's form (mesogenic) is rare and morbidity is variable (Elam, 1993). The mortality rate in young birds especially when complicated with other infectious diseases can reach 30% (Elam, 1993).

Medicinal plants have been used in the treatment of humans and animals diseases and infections (Mukhter *et al.*, 2008). Traditional medicine has increased in industrialized countries as many prescription drugs have originated from tropical flora (Nelson – Harrison *et al.*, 2002). Nigeria is endowed with many medicinal plants like every other developing country (Oladumoye and Kehinde, 2011) where majority of its population depends on these plants to meet their health needs (Oladunmoye and Kehinde, 2011). Plants have been used all over the world in Folkloric medicine for the treatment of infectious and non-infectious diseases in man and animals and this has led to renewed scientific interest in the use of plants (Oridupa *etal.*, 2011). One of such plants used is *M. balsamina*. The general botanical characteristics of the cultivated member of this family (*Cucurbitaceae*) has been outlined by paurseglove (1968) as follows, dendril climbing or postrate annual herb, climbing vines growing 3-4 mm length. The family has large leaves which are dark green and Kidney shaped with large nodes and entire margins (Cobley and steel, 1977). Its dendrils are fairly stout and divided about half – way along their length into many branches. The leaves are also very hairy on the under surface and simple but often deeply alternate or spirally arranged (Cobley and steel, 1977). The names for the plants in Nigeria are Garahuni (Hausa), Akban ndene

(Igbo) and Ejirin (Yoruba). In Uganda it is known as Bombo and the common name of *M.balsamina* in English is Balsam apple or *Bitter Cuccumber* (Hassan and Umar, 2006). In Nigeria *Momordica balsamin*a leaves are cooked as part of green vegetables soup for lactating mothers to help purify breast milk (Hassan and Umar, 2006).

Due to the contagious nature and seasonal occurance of ND, local people treat local chickens with various plants like, red pepper (*Capsicum frutenscens*) and gauta Kaji (*Solonum modiflorum*). In a Reasearch conducted to compare the ameliorative effects of *M.balsamina*, selenium and lamivudine on ND, selenium was found to have higher (62.90%) survival rate, and ameliorative effect on ND (Agang, 2014). This prompted further study with some antioxidants like selenium – Vitamin E, Vitamin C, ethanol extract of *M. balsamina* leaves and roots as therapeutic agents against ND. Vitamin C (ascorbic acid) is an essential dietary nutrient which has antioxidant property of ascorbic acid that provides an electron to unstable oxygen atom to occupy the oxygen atom orbit and attributed to its ability to reduce potential damaging reactive oxygen species (ROS), forming, instead, resonance – stabilized and relatively stable ascorbate free radical (AFR) (Buettner, 1993). Vitamin E is found naturally in some foods, is added to others, and available as a dietary supplement. Vitamin E is the collective name for a group of fat – soluble compounds with distinctly antioxidant activities that enhances immune system that reduce incidence of cancer (Traber, 2006), Selenium is a key element in antioxidant defence and works in close concert with other antioxidants in particular with vitamin E which also burst immune system.

AIM OF THE STUDY

The aim of the study was to evaluate the effects of ethanol extract of *M. balsamina* and some antioxidants on pathological changes in broilers following challenged with a virulent Newcastle disease virus.

OBJECTIVES OF THE STUDY

To determine the ameliorative effects of Vitamins C. E, Selenium and *M. balsamina* on:

1 Pathological changes in broilers experimentally infected with VNDV.
2 Can Vit. C. E., Selenium and *M. balsamina* singly and in combination ameliorate the effect on pathological changes of broilers experimentally infected with VNDV.

RESEARCH QUESTIONS

1. Can Vit. C. E., Selenium and *M. balsamina* ameliorate the effect on pathological changes of broilers experimentally infected with VNDV.
2 Can Vit. C. E., Selenium and *M. balsamina* singly and in combination ameliorate the effect on pathological changes of broilers experimentally infected with VNDV.

MATERIALS AND METHODS

Ethical Clearance

Ethical Clearance was obtained from the ethical committee on Animal use and Welfare, Ahmadu Bello University, Zaria, with the reference number ABUCAUC/2018/038

Newcastle disease Virus

Virulent NDV strain (Kudu 113 strain) was obtained from the National Veterinary Research Institute (NVRI), Vom, plateau State, Nigeria ($10^{8.5}$EID). The chicks were inoculated with 0.1ml/bird reconstituted challenge virus after dilution of 1:9 ratio according to the source recommendations Echeonwu *etal.*, 1993.

Broilers

A total of 200 days old Cobb 500 breed of broilers were procured from a hatchery in Ibadan without ND vaccination and transported to Zaria. The chicks were brooded for 4 weeks before they were divided into 8 groups of 20 chicks each. At the end of brooding, 20 broilers were assigned to each group but separated under deep litter system with a floor space of 1.14 cm^2/bird at one week of age.

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Table 3.1 Experimental design for evaluation of clinical changes of five weeks broilers Challenged with a virulent Newcastle disease kudu 113 virus.

Group N=20	Treatment	Week of Treatment
1	Negative control 2 litres of water orally	Distilled water daily from week 5 to 8
2	Virus (0.1ml 1/n) + (positive control) continuous administration of 2 litres of water oral route	Distilled water daily from week 5 to 8
3	Virus (0.1ml 1/n) + continuous administration of vitamin C 600mg tablets in 2litres of water oral route	Distilled water daily from week 5 to 8
4	Virus (0.1ml 1/n) + continuous administration of vitamin E 400IU soft gels in 2 litres of water oral route	Distilled water daily from week 5 to 8
5	Virus (0.1ml 1/n) + continuous administration of selenium – vitamin E (1ml/L in 2litres oral route	Distilled water daily from week 5 to 8
6	Virus (0.1ml 1/n) + continuous administration of plant leaves extract (400mg/ml) in 2litres of water oral route.	Distilled water daily from week 5 to 8
7	Virus (0.1ml 1/n) + continuous administration of plant root extract (200mg/ml) in 2litres of water oral route	Distilled water daily from week 5 to 8
8	Virus (0.1ml 1/n) + continuous administration of plant leave extract (400mg/ml) in 2 litres of water oral route + vitamin C (600mg tablets)	Distilled water daily from week 5 to 8

Key: Plant leaves = *M. balsamina* leaves, plant roots= *M. balsamina* roots, vit C= vitamin C, Se – vit E= selenium – vitamin E, vit E=vitamin E, plant leaves + vit C = *M. balsamina* leaves + vitamin C, 1/n = intra nasal route, H_2O = Water.

Feed

Commercial broiler starter feed, Grand cereal U.A.C, Nigeria.

Drugs

On arrival, vitalyte (Anupco company cruckatt, Road lady lane lud esdtate, U/C England).

Vaccine

Infections bursal vaccine (Gumboro) was sourced from NVRI, Vom, Plateau State, Nigeria and it was used to vaccinate birds through water at the age of 2 weeks against infectious bursal disease with the dose of 200 doses/2L of water/bird through free chlorinated drinking water.

Selenium – Vitamin E

MIAVIT (Selenium – VitaminE) GmbH, Robert Bosch – Strabe 3, D-49632 Essen (Oklb), Distributed by Agriculture STD plus Nig. LTD, No. 36, 7up road Oluyole, off ring road Ibadan Nigeria was obtained from a veterinary pharmaceutical shop. The dose was 1ml/L, through water.

Vitamin C

Pure vitamin C (Michelle laboratory LTD, Nigeria) was sourced from an Agrovet shop at Samaru Zaria, Nigeria. The dose was 600mg/L through water.

Tween 80 (Surfactant)

Tween 80, a product of Williams and sons company England, was obtained from a reputable chemical store in Zaria, Nigeria. It was used at 2 – 3 drops to dissolve the ethanol extract of *M. balsamina* leaves of 1 kg and roots 200 g.

Management

Housing: The chicks were housed in the research poultry pen of the veterinary Teaching Hospital, Faculty of veterinary medicine, Ahmadu Bello University, Samuru, Zaria, Nigeria with a floor space of 1.14 cm^2/bird under deep litter system, for one week of age. Heat: Heat was provided using 200 W.bulbs (6) with a range of temperature of 36 – 37°C (Demerchi *etal.*, 2008).

METHODS

Challenge birds

At the age of the five weeks, the birds were challenged with 0.1ml of Kudu 113 strain of Newcastle disease virus ($10^{8.5}$ EID_{52}) intranasally Echeonwu *etal.*, 1993).

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Vaccination procedure for infectious Bursa Disease (IBD)

A vial of IBD vaccine was sourced from National Veterinary Research Institute, Vom, Jos, South Local Government Area, Plateau State, Nigeria containing 200 doses of IBD Vaccine. It was diluted into 2 L of free Chlorinated water by aspirate 2ml of saline water to dissolve the frozen dried vaccine. This was done after fasting or with hold water for 2hr. The reconstituted vaccine was given immediately and should be taken within an h. the leftover should be buried with the empty vial to avoid spreading of the virus since it is air born. Vaccination has to be done when the birds are two weeks of age.

Plant Collection

Momordica balsamina leaves and roots were collected from Zaria environ and taken to Department Botany, Faculty of Life sciences, ABU, Zaria for Identification. The plant leaves and roots were washed and shade dried and pounded to fine powder using mortar and pestle and stored in dry containers until used.

Extraction of Plant

A 1.0 kg of the fine powdered plant leaves were soaked in 7 litres of 70% ethanol and extracted by staking the container/mixture for 6h on wrist action shacker. Two hundred grams (200.0 g) of powdered plant root parts were processed using the same protocol as the leaves. The preparations were left to stand for 24 hr. After filtration through Whatman's paper, samples were concentrated to dryness on a water bath at 40°C for 1 – 2 weeks and packaged in water proof polythene bags and stored in the refrigerator at – 4°C until used (Atawodi, 2005).

Determination of the median lethal dose (LD_{50})

A total of 12 broilers of 5 weeks old were used for the experiment. The birds were divided into two groups of 2 (A = 9 birds and B = 3birds). Group A was further divided into subgroups of 3 broilers each. The birds in subgroups A1, A2, and A3 were orally treated with *M. balsamina* 10,100 and 1,000 mg/kg body weight, respectively. The birds were observed for 2 days for signs of toxicity and mortality. The birds in group B were further subdivided into 3 groups B1, B2, and B3 of 1 bird

each. Based on the initial results, further doses of 1,600, 2,900, and 5,000 mg/kg body weight were administered to the groups, respectively. The procedure was the same for *M. balsamina* roots. The LD_{50} of the leaves and roots were calculated based on Locke's Method, (1983).

DATA ANALYSIS

Data from gross lesions were expressed as analysed using descriptive statistics, Data from gross lesions were reduced to mean and standard error (deviation) and one way ANOVA was applied using statistical package for social services (SPSS) version 20, (2015) to analyse the difference between groups to establish the level of significance. Value of $P \leq 0.05$ were considered significant results were presented in tables, figures and plates.

RESULTS

Plates 1-4 showing: *M. balsamina* leaves, fruits and seeds, group 2, 7 and 8 showing mortality and survival rates.

Plate 1: Photograph showing *Momordica balsamina* leaves, fruits and seed used for the experiment 2019.

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Plate 2: Photograph showing high mortality in group 2 of broilers challenged With ND Kudu 113 virus and administered distilled water 2019

Plate 3: Photograph showing recovered broilers of group 7 challenged with ND Kudu 113 virus and administered *M. balsamina* roots ethanol extract 2019

Plate 2: Photograph showing recovered broilers in group 8 challenged With ND Kudu 113 virus and administered M. *balsamina* leaves ethanol extract plus vitamin C 2019)

Gross Lesions in 5 Weeks Old Broilers Challenged with Newcastle Disease Kudu 113 Virus

The highest percentage of 20% of gross lesions were observed in group 3. The percentage in groups 2, 4 and 5 was 16.7% each while 13% of the lesions were recorded in group 7 (Table 4.4). The most common gross lesions were: haemorrhages of the proventriculus, intestines, caecal tonsils, trachea and skeletal muscle, congestion of the lungs, liver and spleen, necrosis and mucus production in the small intestine (plate1).

Microscopic Lesions of Intestine in 5 Week Old Broilers after Challenged with Newcastle Disease Virus Kudu 113 Virus.

There were chondrocytic degeneration, disruption of the tracheal mucosa leading to sloughing of the epithelial mucosal wall resulting to ulceration. There were haemorrhages and congestion of the tracheal mucosa as shown in (Plate 2).

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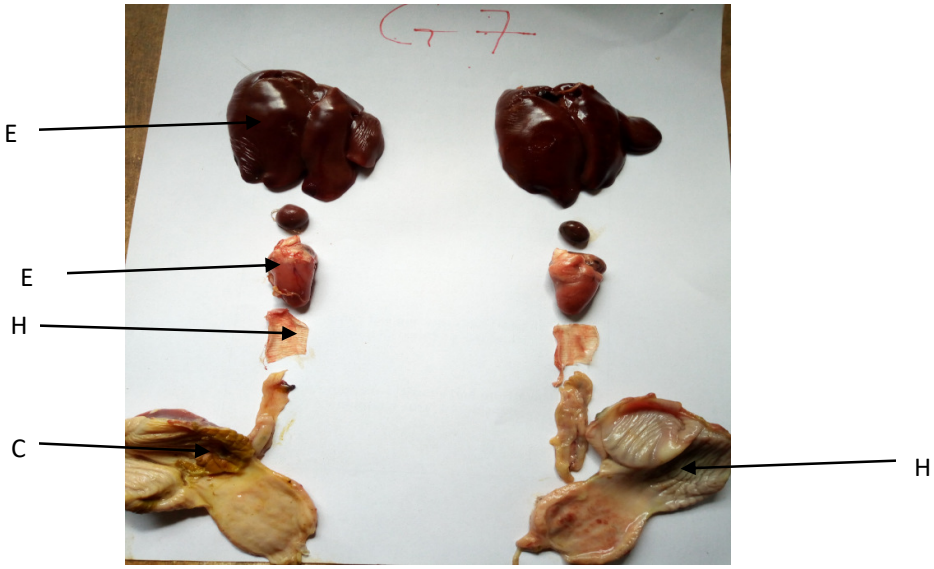
Table 4.4: Gross lesions of broilers challenged with Newcastle disease Kudu 113 virus at 5 weeks. Total number (N) per group abc abc abc

Gross lesions	Group G1	G2	G3	G4	G5	G6	G7	G8
Hemorrhages TR, PR, CA	$\frac{0}{20}$(0%)	$\frac{4}{20}$(20%	$\frac{4}{20}$(20%)	$\frac{4}{20}$(20%)	$\frac{6}{20}$(30%	$\frac{3}{20}$(15	$\frac{5}{20}$(25	$\frac{4}{20}$(20%)
Congestion LI, L H	$\frac{0}{20}$(0%)	$\frac{1}{20}$(5%)	$\frac{2}{20}$(10%)	$\frac{4}{20}$(20%)	$\frac{3}{20}$(15%)	$\frac{2}{20}$(10	$\frac{3}{20}$(15%	$\frac{3}{20}$(10%)
Cloudy air sacs AI	$\frac{0}{20}$(0%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)
Enlargement SP, HE,	$\frac{0}{20}$(0%)	$\frac{3}{20}$(15%	$\frac{4}{20}$(20%)	$\frac{3}{20}$(15%)	$\frac{0}{20}$(0%)	$\frac{2}{20}$(10	$\frac{0}{20}$(0%)	$\frac{0}{20}$(0%)
Necrosis IN	$\frac{0}{20}$(0%)	$\frac{2}{20}$(10%	$\frac{3}{20}$(15%)	$\frac{0}{20}$(0%)	$\frac{3}{20}$(15%	$\frac{2}{20}$(10	$\frac{0}{20}$(0%)	$\frac{2}{20}$(10%)
Emaciation MU	$\frac{0}{20}$(0%)	$\frac{1}{20}$(5%)	$\frac{2}{20}$(10%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%	$\frac{1}{20}$(5%)	$\frac{1}{20}$(5%)
Mucus Production TR	$\frac{0}{20}$(0%)	$\frac{3}{20}$(15%	$\frac{1}{20}$(5%)	$\frac{3}{20}$(15%)	$\frac{2}{20}$(10%	$\frac{2}{20}$(10	$\frac{2}{20}$(10%	$\frac{2}{20}$(10%)
Total %	0%	10%	12%	11%	10%	9%	8%	8%

Key: G1 = No challenge with NDV and gave water, G2 = Challenged with NDV and gave water, G3 = Challenged with NDV and gave vitamin C, G4 = Challenged with NDV and gave vitamin E, G5 = Challenged with NDV and gave selenium- vitamin E, G6 = Challenged with NDV and gave ethanol extract of M. *balsamina* leaves, G7 = Challenged with NDV and gave ethanol extract of M. balsamina leaves and G8 = Challenged with NDV+ M. balsamina leaves +vitamin C

KEY: TR = trachea, PR = proventriculus, CA – caeca tonsils, LI = liver, L = lungs, HE = heart, AI = air sacs, SP =spleen, IN = intestine, and MU = muscles., G = group



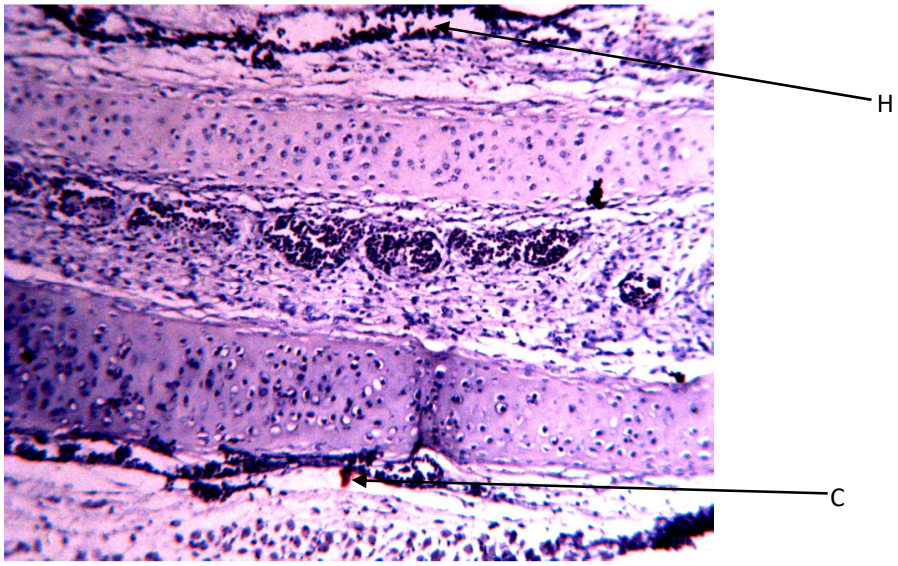


Plate 2 : H & E, Mag × 100 : Chondrocytic degeneration and haemorrhages of the mucosa seen in group 2 H = haemorrhages of the tracheal mucosa and C = chondrocytic degeneration

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DISCUSION

The gross lesions observed in this study were similar to those reported by previous researchers (Kommers *et al.*, 2002; Oladele *et al.*, 2005), who reported excess mucus in the trachea, pin point haemorrhages in the proventriculus, necrosis in the intestinal mucosa, dehydrated muscles, congested lungs, cloudy air sacs, discolouration of the liver and enlargement of the spleen. The findings also agreed with the report of previous workers (Alexander *et al.*, 1997; Kommers *et al.*, 2002; Sai'du *et al.*, 2006), who reported multifocal and linear haemorrhages and ulcers in digestive tract including oral cavity, oesophagus, proventriculus and intestine in chickens infected by NDV Kudu 113 strain. In the present work onset of infection started 3 days post challenge (dpc) with Kudu 113 strain in group 3, 4, 5, 6, 7, and 8, while onset of clinical signs started 4 (dpc) only in group 2 to show the difference in incubation periods probably due to the route of challenge observed. Mortality started 3 days after clinical signs in all the groups except group 2 which had mortality at 4 days of the onset of clinical signs probably due to the route of challenge and administration of the drugs.

CONCLUSIONS AND RECOMMENDATION

Conclusion

From this work it was concluded that:
Ethanol extract of *M. balsamina* roots (200 mg/L) with mortality rate of 35.0% had ameliorative effect against Newcastle disease in reducing clinical signs and pathological lesions of ND.

Vitamin C (600 mg/L) with mortality of 55.0% had good ameliorative properties against Newcastle disease in Lessing pathological lesions of ND.

Combining vitamin C (600 mg/L of water plus ethanol extract of *M. balsamina* leaves 400 mg/L in water) with mortality rate of 40.0% had good ameliorative properties in reducing clinical signs and pathological lesions of ND.

The most commonly observed pathological lesions in this findings were haemorrhages of the thigh muscles, breast muscles trachea and caecal tonsils were less by the administration of the drugs.

Recommendations

From this work, it was recommended that;

Farmers should make use of ethanol extract of *M. balsamina* roots (200 mg/2L of water) for treatment of ND).

Farmers should use the combination of ethanol extract of *M. balsamina* leaves (400mg/2L) plus vitamin C (600 mg/2L of water) for treatment of Newcastle disease outbreak.

Farmers should make use of vitamin C (600 mg/2L of water) to treat birds against Newcastle disease infection.

Ethanol extract of *M. balsamina* roots subjected to proximal analysis to determine the phytochemical constituents.

Further studies are needed to know the real medicinal properties by extraction of the roots of ethanol extract of *M. balsamina* plant

REFERENCES

Adu, f.d., Edo, U. and Sokale, B. (1986). Newcastle disease. The immunological status of local chickens. Tropical Veterinarian, 4:149-159.

Agang, I.D. (2014). Evaluating the effect of M. balsamina Linn, Selenium and lamivudine for the treatment of Newcastle disease in pullets. MSc Thesis, Faculty of Veterinary Medicine, Ahmadu Bello University – Zaria, Nigeria.

Alexander, D. J. (1988). Newcastle disease methods of spread. In: Alexander, D. J. (Ed): *Newcastle Disease. Development of Veterinary Virology,* Kluwer Academic Publishers, Boston, England, Pp, 256 – 272.

Alexander, D.J., Manvell, R.J., Lowngs, J.P., Frost, K.M., Collins, M.S., Russell, P.H. and smith, J.E. (1997). Antigenic diversity

and similarities detected in avian paramyxovirus type1 (Newcastle disease virus) isolates using monoclonal antibodies. Avian pathology, 26:399-418.

Atawodi, S.E. (2005). Comparative in vitro trypanocidal activities of petroleum either, chloroform, methanol and aqeous extracts of some Nigerian Savannah plants. African Journal of Biochetechnology, 4(2):177-182.

Buettner, G.R. (1993). The packing order of free radicals and antioxidants; Lipid paroxidation, alphatocopherol, and ascorbate. Archive Biochemistry and Biophysics, 300:535-543.

Cobley, C.S. and steel (1977). An introduction to the botany of tropical crop. Longman, Harlow, P.19.

Demerchi, G., Chiozzi, G. and Fasola, M. (2008). Solar incubation cuts down parental care in a burrow nesting tropical shore bird. The crash plover dromas areola. *Journal Avian Biology,* 39(5): 484-486.

Dobson, N. (1939). *Newcastle disease*. In: P*roceeding of the 7th World Poultry Congress,* 7: 250 – 257.

Doyle, T.M. (1927). A hitherto unrecorded disease of fowls due to a filter passing virus. Journal of comparative pathology, volume 40. Pp.144-169.

Echeonwu, G.D.N., iroegbu, C.W. and Emerunsa, A.C.(1993). Recovery of Velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. Avian pathology, 22:383-387.

Elam, M.K. (1993). The influence of season, type, breed, age and type of vaccine on the morbidity due to Newcastle disease after vaccination in poultry zaria, Nigeria. PhD Thesis, faculty of Veterinary medicine, Ahmadu Bello university.

Emmerson, P. (1999). Newcastle disease virus (Paramyxovidae). In: Granoff, A.U. and Webster, R. CHrsg), Encyclopedia of virology, 2nd (ed). Academic press. San Diego California, USA. Bd2, Pp. 1020-1025.

Hassan, L.G. and Umar, C.J. (2006). Nutritional values of balsam apple leaves. *Pakistan Journal of Nutrition,* 5(6): 522-529.

Hill, C.D., Davie, O.S. and Wilde, J.K. (1953). Newcastle disease in Nigeria. New British veterinary Journal, 6:381-385.

Kommers, G.D., King, D.J. and Seal, B.S. (2002). Pathogenesis of six pigeon-origin isolates of Newcastle disease virus for domestic chickens. *Veterinary Pathology,* 39: 353-362.

Knipe, D., Peter, M. and Itowleg, N. (2007). Fields of virology, section II: specific virus families. Wolters-kluwer Lippincolt Williams & Wikkins, Philadelphia, Pa, London, PA, 1450-1496.

Lee, Y.J., Sung, H.W., Choi, J.G., Kim, J.H. and Song, C.S. (2004). Molecular Epidemiology of Newcastle disease viruses isolated in south Korea using sequencing of the fusion protein cleavage. Site region and phylogenic relationships. Avians pathology, 33(5):482-491.

Lorke, D. (1983). A new approval to practical toxicity testing. *Archives of Toxicology,* 53: 275-289.

Mukhtar, M., Arshad, M., Pomerantz, R.J. and Wigdahl, B. (2008). Antiviral potential of medicinal plants. *Virus Research,* 131(2):111-120.

Nelson-Harrison, S.T., King, S. R., Limbach, C., Jackson, C., Galiwango, A., Kato, S.K. and Kanyerezi, B.R. (2002). Ethnobotanical research into the 21th century. In: Iwu, M.M., Wootton, J. C.(Eds). *Ethnomedical Drug Discovery*. Elsevier, Amsterdam, *Neurology,* 59: 1161-1169.

Oladele, S. B., Nok, A. J., Esievo, K. A. N., Abdu, P. A. and Useh, N. M. (2005). Haemagglutination inhibition antibodies, rectal temperature and total protein of chickens infected with local Nigerian isolate of velogenic Newcastle disease virus. *Veterinary Research Communications,* 29: 171-179.

Oladunmoye, M.K. and Kehinde, Y.F. (2011). Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South West Nigeria. *African Journal of Microbiology Research,* 5(19):2991-3004.

Oridupa, O.A., Saba, A.B. and Suleiman, L.K. (2011). Preliminary report on the antiviral activity of the ethanolic fruit extract of lagenaria breviflora reports on Newcastle disease virus. Tropical veterinarian, 29(1):22-23.

Pauseglove, J.W. (1968). *Tropical Crops*: Dicotyledons, Longman, harlow,. Pp.196.

Rott, R. and Klenk, H.D. (1988). Newcastle disease: Molecular basis of infectivity and pathogenicity of Newcastle disease virus, In: Alexender, D. J. (Ed). *Newcastle Disease Development of Veterinary Virology*, Kluwer Academic Publishers, Boston, USA, Pp. 98 -113.

Roy, P. (2012). Diagnosos and uninfected of Newcastle disease in developing countries. World poultry Science, 68:700-703.

Sai'du, L., Abdul, P.A., Umoh, J.U. and Abdullahi, U.S.,(1994). Disease of Nigeria indigenous chickens, Bulletin of Animal Health and Production in Africa, 42:19-23.

Sai'du, L., Abdu, P.A., Tekdek, L.B., Umoh J.U., Usman, M. and Oladele, S. B. (2006). Newcastle disease in Nigeria. *Nigerian Veterinary Journal*, 27(2): 23-32.

Traber, M.G. (2006). Vitamin E. In: Shils, M.E., Shike, M., Ross, A.C., Caballero, B. and Cousoins, R. eds. <odern Nutrition in Health and Disease. 10th ed. Batimore, M.D. Lippincott Williams and Wilkins, U.S.A. Pp.396-411.