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Effect of Ethanol Extract of Momordica Balsamina and Some Antioxidants on Biomarkers in Broilers with a Virulent Newcastle Disease Virus

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ABSTRACT

Newcastle disease poses a serious threat to the poultry industry as it has economic and ecological impact on pet, free living as well as domestic birds. The aim of the study was to evaluate the effects of some antioxidants and *Momordica balsamina* on haematological parameters in broilers challenged with ND Kudu 113 Virus. Two-hundred-day-old broilers were procured from a hatchery in Ibadan. The chicks were brooded for 4 weeks then divided into 8 groups (1 ml/L) of 20 chicks each. Each group was housed in a pen on deep litter system with a floor space of 1.14 cm^2 /bird with the exception of G1 which was housed outside the faculty. All the experimental groups except G1 were challenged with NDV Kudu 113 strain but G1 was given 2 ml of distilled water at 5 weeks of age. Birds in G2 were challenged with NDV and not treated with any antioxidant but given 2 ml of water at 5 weeks of age. Birds in G3 were administered vitamin C600 mg tablets in 2L of water at the rate of 2 ml of water per bird orally. Birds in G4 were treated with vit. E 400 mg/ml and challenged. Birds in G5 were treated with selenium-vit Eat 1 ml/L in 2 L of water /bird orally. Birds in G6 were treated with ethanol extract of *M. balsamina* leaves 400 mg/ml in 2 L of water orally. Birds in G7 were administered ethanol extract of *M. balsamina* roots 200 mg/ml in 2 L of water orally. Birds in G7 were administered ethanol extract of *M. balsamina* roots 200 mg/ml in

INTRODUCTION

Poultry production is a very important part of the agricultural subsector in Nigeria. Many species of domestic, semi-domestic and wild birds are susceptible to ND (Arshad *et al,* 1988; Kaleta and Baldouf, 1988; Alexander *et al,* 1997; Aldous and Alexander, 2001). Newcastle disease virus was isolated from apparently healthy ducks in and around Jos, Plateau State, Nigeria but there is no report of clinical ND in the ducks (Majiyagbe and Nawathe, 1981; Echeonwu *et al.,* 1993). Newcastle disease virus is a non-segmented

single stranded RNA genome of negative polarity and replicates in the cytoplasm of cells (Emmerson, 1999; Knipe *et al.*, 2007). The envelope contains lipids and surface glycoproteins (haemagglutinin-neuraminidase (HN) and fusin (F), which surrounded the virion (Alexander, 1988).

Statement of the Research Problems

Newcastle disease is devastating in local and exotic breeds of chicken worldwide (Doyle, 1927). The economic losses due to virulent Newcastle Disease Virus (NDV) pose a threat to the fast-growing poultry industry worldwide. The disease which affects poultry and other birds is an important disease because it causes high morbidity and mortality (Alexander, 1990).

Justification of the Study

Newcastle disease also caused great losses in Nigerian poultry within the last several years and still poses a threat to the poultry farmers. So, there is need for improvement in the poultry industry which should incorporate emphasis on the prevention and control of disease that cause economic losses (Alexander *et al,* 2009). *Momordica balsamina* is one of the medicinal plants that are important source of life saving drugs for the majority of the world's population (Hassan and Umar, 2006). Momordicins and Saponins prevent ND, acquired immune deficiency syndrome and other related viruses (Hassan and Umar, 2006). This idea also led to the work done by Agang, (2014), who worked on effect of Selenium, Lamividine and *M. balsamina* fruits which showed that selenium has ameliorative effect in reducing pathological lesions and clinical signs induced by ND with only 31.7% mortality rate due to NDV recorded in the selenium treated group which was the lowest rate in all the groups. This prompted further work to compare the effect of some antioxidants and *M.balsamina* leaves and roots for the treatment of ND Agang, (2021). Therefore, continuous search for appropriate and cheap drug that will meet up with farmers challenge is still imperative hence the need for this work.

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Aim of the Study

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

Objectives of the Study

To determine the ameliorative effects of vitamin C, E. selenium and *M.balsamina* on;

i. Antioxidants parameters of broilers experimentally infected with ND virus

Research Questions

1) Can vitamin C, E, selenium and *M.balsamina* ameliorate the effect on antioxidants of broilers experimentally infected with VNDV?
2) Can vitamin C, E, selenium and *M.balsamina* singly and in combination ameliorate the effects on antioxidants of broilers experimentally infected with VNDV?
3) Can vitamin C, E, selenium and *M. balsamina* ameliorate effect 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, Nigeria, with the reference number of ABU CAUC/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_{50}). The chicks where inoculated with 0.1ml/bird reconstituted challenge virus after a dilution of 1.9 ratio according to the source recommendations Echeonwu *et al,* 1993.

Broilers

A total of 200 day-old Cobb 500 breed of broilers were procured from a hatchery in Ibadan without ND vaccination and transported to

Zaria. The chicks were housed in the experimental pen of the VTH, ABU, Zaria, On arrival, the chicks were brooded for 4 weeks before they were divided into groups of 20 chicks.
At the end of the brooding period, 20 broilers were assigned to each group but separated under deep litter system with a floor space of 1.14cm^2/ bird at one week of age.

Table 3.1: Experimental design for evaluation of changes in Biochemical parameters of five weeks old broilers challenged with a virulent Newcastle disease 113 virus.

Group (N= 20)	Treatment	Week of treatment
1	Negative contr 2 L of H_2O oral route	Distilled H_2O daily for week 5-8
2	Virus 0.1 ml i/n + posit contr adm 2 L H_2O oral route	Dist H_2O daily for week 5-8
3	Viruso.1 ml i/n +Adm vit.C (600mg) tablets In 2 L of H_2O oral route	Dist H_2O daily for week 5-8
4	Virus 0.1 ml i/n +Adm of vit E 400 iμ solfgels in 2 L of H_2O oral route	Dist H_2O daily for week 5-8
5	5Virus 0.1 ml i/n + Adm of se-vit E at 1 ml/Lin 0.2 L of H_2O oral route	Dist H_2O daily for week 5-8
6	Virus 0.1 ml i/n + Adm of plant leaves Extract 400 mg in 2 L of H_2O oral route	Dist H_2O daily for week 5- 8
7	Virus 0.1 ml i/n +Ad plant root extract 200 mg In 2 L of water oral route	Dist H_2O daily for week 5-8
8	Virus 0.1 ml i/n +Adm of plant leaves extract 400 mg/ml in 2 L of H_2O+Vit C 600 mg tablets Oral route	Dist H_2O daily for week 5-8

Key: Plant leaves= *M. balsamina*, plant root= *M.balsamina* roots, Vit C= Vitamin C, Se-Vit E = Selenium- Vitamin E, Vit E= Vitamin E, i/n= intraocular route and H^{2}o= Water

Feed

Commercial broilers starter (vital feed, grand cereal V.A.C) Nigeria

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Drugs
On arrival, vitalyte® (Anupco company Crudatt, Road Lady Lane Lud estate, UK, England)

Vaccine
Infectious bursa disease 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 bursa disease with the dose rate of 200 doses/2L of water per bird through free chlorinated drinking water and each bird expected to take 10 ml/bird.

Selenium- vitamin E
MIAVIT® (Selenium-VitaminE) GMbH, Robert-Bosch-Strabe 3, D. 49632 Essen (Oldb.) Distributed by Agriculture STD plus NIG. LTD. No36, 7 UP road Oluyole, off-ring road Ibadan Nigeria was obtained from a Veterinary pharmaceutical shop. The dose was 1 ml/L through water.

Vitamin C
Pure Vitamin C (Michalle Lab. Ltd. Nigeria) was sourced from an Agrovet shop at Samaru Zaria, Nigeria. The dose was 600 mg/L through drinking water.

Tween 80® (Surfactant)
Tween 80® a product of William 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 VTH, faculty of veterinary medicine, ABU, Samaru, 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 (b) with a range of temperature of 36-37°c (Demerchi *et al.,* 2008).

Challenge of Birds

At the age of five weeks, the birds were challenged with 0.1 ml of Kudu 113 strain of Newcastle disease virus ($10^{8.5}EID_{50}$) i/n Echeonwu *et al.,* 1993.

Plant Collection

Momordica balsamina leaves and roots were collected from Zaria and taken to Department of 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 socked in 7 litres of 70% ethanol and extract by shaking the container/mixture for 6 hrs on wrist action shaker. Two hundred grames (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 hrs. 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).

Vitamin C

Pure Vitamin C (Michalle Lab. Ltd. Nigeria) was sourced from an Agrovet shop at Samaru-Zaria,. Nigeria. The dose was 600 mg/L through water.

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METHODS

Vaccination procedure for Infectious Bursa Disease (IBD)

A vial of IBD vaccine was sourced from NVRI, Vom, containing 200 doses of IBD vaccine. It was diluted into saline water to dissolve the freezed dried vaccine. This was done after fasting the birds for 2hour. The reconstituted vaccine was given immediately and should be taken within an hour. The leftover should be buried with the empty vial to avoid spreading of the virus since it l s air born. The birds were vaccine when they were 2 weeks of age.

Determination of Reduced Glutathione Peroxidase

The procedure to estimate reduced Glutathione Peroxidase (GSH) level, the method described by Elman, (1959) was followed. Blood was homogenized in 5 ml/mg cold buffer or with a PH7.5 in EDTA. Homogenized blood was centrifuged at 1.8 x g for 15 min at 4°C. The supernatant was removed for assay of glutathione (GPX). Glutathione peroxidase activity was measured using the glutathione peroxidase assay kit (Cayman chemical Company,

USA). The kit measures (GPX) activity indirectly by a coupled reaction with glutathione reductase (GR).
Glutathione (GPX) reduced hydrogen peroxide to oxidized glutathione (GSSG) which recycled to its reduced state by GR. This accompanied a decrease in absorbance at 340 nM.

Determination of Catalase Enzyme.

Catalase activity was determined by the method of Aebi, (1974). Pure blood was homogenized in 1.5 ml/mg cold buffer (50 mM potassium phosphate and 1m M EDTA, pH7.0 and was centrifuged at 1.8 x g for 15 min at 4 °C. The supernatant was used for assay. Catalase assay kit (Cayman Chemical Company, USA) was used. The kit utilized the peroxidatic function of CAT for determination of enzymes activity. The method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2 (Hydrogen Peroxide). The formaldehyde produced was measured spectrophotometrically with 4-amino-3-hydrozeno-5-mercapto-1,2,4-trizazole as the chromagen.

Determination of Superoxide Dismutase

Superoxide dismutase (SOD) was determined by the method of Ismail (2010). Pure blood 5 ml/mg was homogenised in 200 ml buffer. (0.05 mM potassium phosphate and 0.1 mM EDTA, pH7.8) and centrifuged at 1.86 x g for 30 mins at 4 °C.
The supernatant was used for superoxide dismutase. Superoxide dismutase was measured using the superoxide dismutase assay kit (Oxis Research, USA). The BIOXYTECH assay was based on the SOD – mediated increased in the rate of autoxidation of 5,6,6a, 11b-tetrahydro-3,9, 10 – trihydoxybenzo © fluorine R1 in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nM. The kinetic measurement of the 525 nM absorbance change was performed after the addition of R1. The SOD activity was determined.

Determination of the Median Lethal Dose (LD_{50})

A total of 23 broilers of 5weeks old were used for the experiment. The birds were divided into two groups of 2 (A = 9 birds and B = 3 birds).

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Group A was further divided into 3 subgroups of 3 broilers each. The birds in subgroups A1, A2 and A3 were orally threated with *M.balsamina* 10, 100 and 1,000 mg/kg body weights, respectively. The birds were observed for 2 days (48 hrs) for signs of toxicity and mortality. The birds in group B were further divided into 3 groups B1, B2 and B3 of 1 birds 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 Lorke's Method, (1983).

Data Analysis

Data from antioxidants parameters 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 differences between groups to establish the level of significance values of $P \leq 0.05$ were considered significant and results were presented in tables, plates and figures.

RESULTS

Malondialdehyde of Broilers Challenged at 5 weeks old with Newcastle Disease Kudu 113 virus

At week 4, the malondialdihyde of broilers for groups 1, 2, 4 and 8 were 3,000.0 µ/min, 6,800.0 µ/min, 1,500.0 µ/min and 2,800.0 µ/min, respectively. At week 5, the malondialdihyde for group 1,2,4 and 8 were 2,800.0 µ/min, 6,800 µ/min , 2,800 µ/min and 7,200 µ/min, respectively. At week 6, after challenge the malondialdihyde for groups 1, 2, 4 and 8 were 3,000.0 µ/min, 3,100.0 µ/min, 3,000.0 µ/min and 2,800 µ/min, respectively. At week 7, the malondialdihyde for groups 1, 2, 4 and 8 were 3,300.0 µ/min, 2,000.0 µ/min, 4,800.0 µ/min and 3,200.0 µ/min,

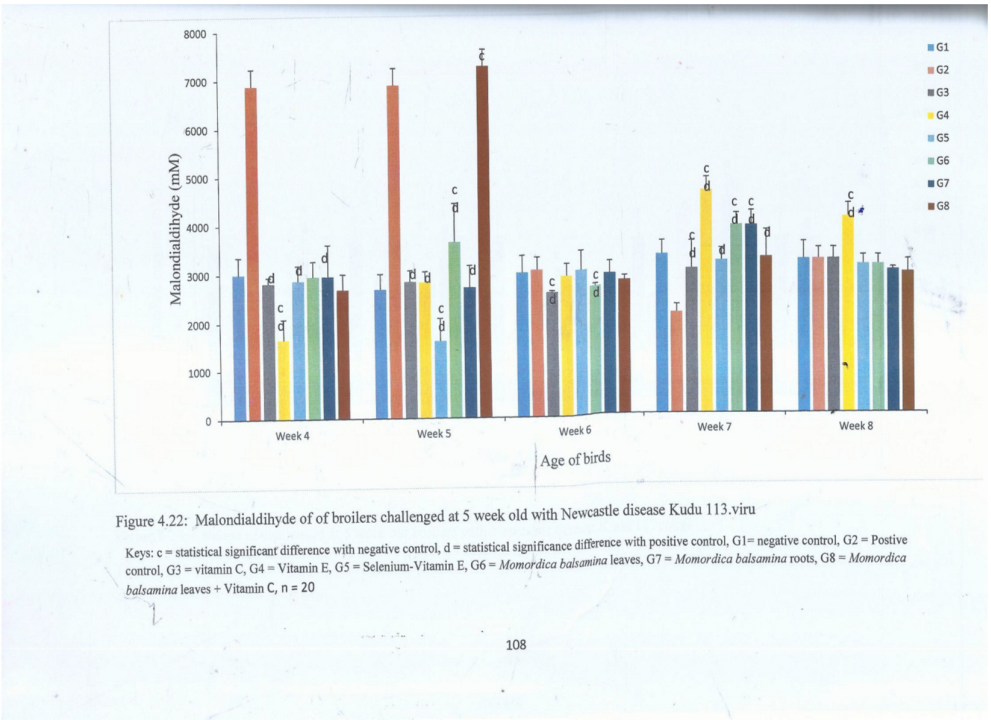

Figure 4.22: Malondialdihyde of of broilers challenged at 5 week old with Newcastle disease Kudu 113.viru

Keys: c = statistical significant difference with negative control, d = statistical significance difference with positive control, G1= negative control, G2 = Postive control, G3 = vitamin C, G4 = Vitamin E, G5 = Selenium-Vitamin E, G6 = *Momordica balsamina* leaves, G7 = *Momordica balsamina* roots, G8 = *Momordica balsamina* leaves + Vitamin C, n = 20

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respectively. At week 8, the malondialdihyde for groups 1, 2, 4 and 8 were 3,200 µ/min, 3,200.0 µ/min, 4,200.0 µ/min and 2,800.0 µ/min, respectively. (Figure 4,23).

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Superoxide dismutase of broilers challenged at 5 weeks old with Newcastle disease Kudu 113 virus

At week 4, the superoxide dismutase enzymes for groups 1, 2, 4, and 8 were 7.0 µ/min, 5.2 µ/min, 5.8 µ/min and 6.2 µ/min, respectively. At week 5, before challenged the superoxide dismutase for groups 1,2,4, and 8 were 7.0 µ/min, 5.2 µ/min, 6.0 µ/min and 4.8 µ/min, respectively. At week 6 the superoxide dismutase enzymes for groups 1, 2, 4 and 8

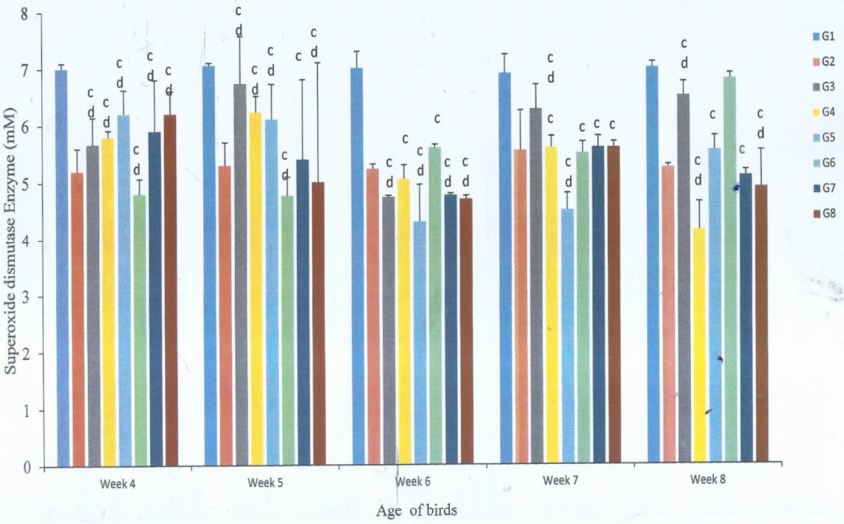

Figure 4.24: Superoxide dismutase of broilers challenged at 5 week old broilers with Newcastle disease Kudu 113 virus

Keys: c = statistical significant difference with negative control, d = statistical significance difference with positive control, G1= negative control, G2 = Postive control, G3 = vitamin C, G4 = Vitamin E, G5 = Selenium-Vitamin E, G6 = *Momordica balsamina* leaves, G7 = *Momordica balsamina* roots, G8 = *Momordica balsamina* leaves + Vitamin C, n = 20

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were 7.0 µ/min, 5.2 µ/min, 6.0 µ/min and 4.8 µ/min, respectively. At week 7 the superoxide dismutase enzyme for groups 1, 2, 4 and 8 were 7.0 µ/min, 5.5 µ/min, 5.5 µ/min and 5.5 µ/min, respectively. At week 8, the superoxide dismutase enzymes for groups 1, 2, 4 and 8 were 7.0 µ/min, 5.3 µ/min, 4.3 µ/min and 5.3 µ/min, respectively. (Figure 4.24).

DISCUSSION

In this study, there was a mark increased in the level of malondialdihyde (MDA) in groups 1 and 8 with values 7,000.0 μ/min and 7,200.0 μ/min, respectively before challenge, but after challenged and treated with drugs malondialdihyde did not show any increase at week 7 for group 2, the value was 2,000.0 μ/min. Superoxide dismutase enzymes (SOD) was 7.0 μ/min at week 8, group 6 which also did not show any appreciable change except at week 6 probably due to the challenge of ND Kudu 113 virus, causing pathology. Biomarkers are always release due to breaking of blood cells and other tissues resulted to enzymes release. They are produce in minute quantities and function as serogate end points, clinical end points, drugs research, drugs trial and projects. Their significance are useful in disease diagnosis. Histogram indicated normal at weeks 4 and 5 but when challenge increased at week 6 probably because of latent period of virus and decreased at week 7 and 8 when the birds started coming back to normal physiological state.

CONCLUSIONS

Biomarker Malondialdihyde of oxidative stress was highest in group 1 6086.22 $\pm$ 801.00 nM before challenge due to effect of drugs administered.

RECOMMEDATIONS

From this work it was recommended that;

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

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

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

(iv) Ethanol extract of *Momordica balsamina* roots subjected to proximate analysis to determine the phytochemical constituents or metabolites.

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

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