Effects of Methanolic and Aqueous Extract of *Cucumis metuliferus* Fruits on Newcastle Disease in Pullets

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ABSTRACT

The study was conducted to determine the effect of aqueous and methanolic extracts of *Cucumis metuliferus* fruit on the prevention and cure of Newcastle Disease (NCD) in pullets. Ninety (90) pullets were used for the experiment. The pullets were divided into 9 groups, 4 prophylactics, 4 curative and one control group. For prophylactic groups, aqueous extract of the fruit at a concentration of 2.5 g and 5.0 g/litre of water and methanolic extract of the fruit at 75 and 50 mg/litre were given to the pullets ad-libitum for two weeks and then challenged with Newcastle disease virus kudu 113 strain via intra-ocular route while for curative groups, the pullets were challenged with the virus and then treated with the aqueous and methanolic extracts of the fruits at the same concentrations. The results showed that methanolic extract of *C. metuliferus* at a concentration of 75 mg/l showed superior prophylactic ability recording lower clinical signs, mortality, postmortem lesions, higher antibody titre and higher white blood cell activity and also exhibited very good therapeutic potential. It was thus suggested that methanolic extract of *C. metuliferus* at 75 mg/l can be recommended for the control of NCD to farmers and that further studies be conducted to isolate the alkaloids of *C. metuliferus* responsible for these findings.

Keywords – Newcastle disease, *Cucumis metuliferus*, pullets, Methanolic extract, Aqueous extract.

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INTRODUCTION

Poultry production is the fastest-growing segment of the agricultural subsector in Nigeria due to the increased demand for animal protein in Nigeria with the ever-growing population [1]. However, one of the constraints to the development of the industry is an outbreak of diseases¹. In poultry, Newcastle Disease Virus (NDV) causes a highly infectious neurological, respiratory or enteric disease.

Newcastle disease remains a serious economic challenge to all segments of the poultry industry because of its high morbidity and mortality rates [2]. The only control method so far remains vaccination, which does not confer 100% immunity in all vaccinated birds. It has also been reported that vaccination often induces a carrier state in birds and also reduces their growth [2]. The immunity conferred on birds due to vaccination has been observed to degrade or wane over time [2], predisposing birds to become vulnerable to re-infection. There is an indication to explore alternative mechanisms to complement the protective effects of vaccinations.

Plants have been used for various purposes since prehistoric times [3,4] and medicinal herbs are being increasingly studied by researchers [5]. Reports of various plants used in the treatment of diseases have been documented in various parts of the world [6, 7, 8.] Indian Ayurveda medicine used herbs as early as 1900 BC describing 700 medicinal plants [9]. According to the World Health Organization (WHO), more than 80% of the world's populations rely on traditional medicine for their primary healthcare with the majority being plants or their active principles [10].

Cucumis metuliferus is locally called 'gautar kaji' by the Hausa tribe in Northern Nigeria [11]. The plant belongs to the family Cucurbitaceae and is a monoecious, climbing, annual herb that can be grown practically anywhere, provided the season is warm [12]. It is commonly known as African horned cucumber, *melano*, Jelly melon, and *kiwano* in the Polish language. The fruits occur in two forms: the bitter and non-bitter forms, which occur mostly in the wild state. The bitter form contains cucurbitacins (triterpenoids), which is a highly toxic compound [11]. The non-bitter form is less toxic and has also been widely cultivated [13]. The plant is endemic to the semi-arid regions of Southern and Central Africa [13]. Its fruits are ovoid berries of 8-10 cm long and 4-5 cm in diameter, reddish-orange at maturity, hanging, covered with strong spiny outgrowths and the seeds are embedded in the mesocarp which is emerald green and consists of juicy, bland-tasting tissues. It is reported that the fruits and seeds of *Cucumis metuliferus* are eaten raw as supplements by local populations of Africa [11, 13]. Reports have also shown that the seeds can be ground into fine flour, made into an emulsion with water, and then eaten to expel parasites (helminths) from the body [14].

Local poultry farmers in the study area lay claim to the efficacy of *Cucumis metuliferus* fruits in the treatment of their birds for various diseases including Newcastle disease by immersing the whole fruits in the drinking water of birds (Personal communication, 2017). The present research is designed, therefore, to evaluate the effects of methanolic and aqueous extract of *Cucumis metuliferus* fruits on Newcastle Disease infection in Pullets.

MATERIALS AND METHODS

Study Area

The study was conducted at the Poultry Unit of the Department of Animal Science Farm, Federal University Dutsinma, Katsina State, Nigeria. Dutsinma lies within the coordinates of latitude 12.45N and longitude 7.49E and also at the altitude of 605 m above the sea level¹⁵.

Study Design

The research was carried out using a Completely Randomized Design with 9 treatments, each treatment/group was made up of 5 birds. Day-old pullets were sourced from a reputable hatchery and brooded under strict biosecurity measures to prevent the entry of pathogens. The birds were vaccinated against Infectious bursal disease on day seven of the arrival but not against Newcastle disease. They were fed with chick mash. Experimental groups consisted of Curative, Prophylactic and Control groups. Aqueous solutions of *Cucumis metuliferus* was made at 2.5 g/l and 5 g/l.

Collection, Identification and Extraction of the Plant Sample

Fresh fruits of *C. metuliferus* were collected from Dutsin-ma in the month of May 2018. The plant sample was authenticated by a taxonomist at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher number (No: 1936) was assigned. The fruits were shade dried. It was reduced to coarse particles using mortar and pestle before being pulverized into fine particles using a laboratory hammer mill. Exactly 1,000 g of the powder was exhaustively extracted in methanol using soxhlet extraction. The extract was filtered using Whatman size 1 filter paper

and the filtrate was concentrated *in vacuo* at 40°C. The extract was stored in a refrigerator at 4°C for further use in the studies.

Experimental Design

The curative groups were T1 and T2 (administered 2.5 g/l and 5 g/l aqueous extracts respectively) and T3 and T4 (administered 50 g/l and 75 g/l methanolic extracts respectively). These groups were initially challenged with the Newcastle disease virus Kudu 113 strain at a dose of 107.8/ 0.1 ml /bird at 8 weeks and treated as indicated depending on the group's *ad libitum* in drinking water as soon as clinical signs were observed. The prophylactic groups were T5 and T6 (administered 2.5 g/l and 5 g/l aqueous extracts respectively) and T7 and T8 (administered 50 g/l and 75 g/l methanolic extracts respectively). These groups were treated with indicated treatments *ad libitum* in water for 2 weeks and then subsequently challenged with Newcastle disease virus Kudu 113 strain at a dose of 107.8/ 0.1ml /bird to observe if the treatments would prevent or reduce the intensity of infection. The control group was challenged with the Newcastle disease virus Kudu 113 strain at the same dose but was not treated with any extracts at 8 weeks of age.

Study Parameters

The clinical signs, mortality and post mortem lesions were observed and recorded daily.

Laboratory Analysis

Blood was collected via the wing vein of birds at the second and fourth weeks of the experiment. Whole blood was collected for

haemogram/blood picture and the serum for Haemagglutination inhibition test.

Data Analysis

Data obtained from the experiment were subjected to analysis using one-way analysis of variance (ANOVA) and Duncan Multiple Range posthoc test. Values of $P < 0.05$ were considered significant. The results were presented in texts, tables and as Mean $\pm$ SEM (standard error of the mean). SPSS version 25.0 was employed for the data analysis.

RESULTS

Clinical Signs

The clinical signs of ND observed included: gasping, sneezing, greenish diarrhoea, anorexia, torticollis, depression, swollen head, incoordination and emaciation. For both curative and prophylactic treatments the groups administered with 75 g/l of methanolic extract (groups T4 and T8) recorded the least clinical signs. Likewise, the groups treated with 2.5 g/l of aqueous extract (groups T1 and T5) showed the most clinical signs (Tables 1 and 2).

Table 1: Clinical Signs for Curative Treatments of birds with *Cucumis metuliferus* extracts

Treatments	Greenish						Swollen	Weight	Incoordination/
	Sneezing	Diarrhea	Torticollis	Anorexia	Gasping	Depression	Head	Loss	Staggering
T1	+	+	+	+	+	-	-	+	+
T2	+	+	+	+	+	-	-	+	-
T3	+	+	-	+	+	+	-	+	-
T4	-	+	-	+	-	+	-	+	-
Control	+	+	+	+	+	+	+	+	+

Key - T1=2.5 g/l aqueous extract; T2= 5 g/l aqueous extract; T3=50 g/l methanolic extract; T4=75 g/l methanolic extract; + = present; - = absent; n =5

Table 2: Clinical Signs for Prophylactic Treatments of birds with *Cucumis metuliferus* extracts

Treatments	Greenish						Swollen	Weight	Incoordination/
	Sneezing	Diarrhea	Torticollis	Anorexia	Gasping	Depression	Head	Loss	Staggering
T5	+	+	+	+	+	+	+	+	+
T6	-	+	+	+	+	+	-	+	-
T7	-	+	-	+	-	-	-	+	-
T8	-	+	-	-	-	-	-	+	-
Control	+	+	+	+	+	+	+	+	+

Key - T5=2.5 g/l aqueous extract; T6= 5 g/l aqueous extract; T7=50 g/l methanolic extract; T8=75 g/l methanolic extract; + = present; - = absent; n =5

Mortality Rates

Treatment 8, administered 75 g/l of methanolic extract amongst the prophylactic groups, was the only group that did not record any mortality. For the curative treatments, T4 (treated with 75 g/l methanolic extracts) recorded the lowest

mortality rate of 1 bird. The control group recorded the highest mortality rate of 10 followed by T5 which was treated with 2.5 g/l of aqueous extract among the prophylactic treatments with 3 mortalities (Table 3).

Table 3: Mortality Rates of birds treated with *Cucumis metuliferus* extracts

Treatments	Mortalities
T1	2
T2	2
T3	2
T4	1
T5	3
T6	2
T7	1
T8	0
Control	10

Key – T1=2.5 g/l aqueous extract; T2= 5 g/l aqueous extract; T3=50 g/l methanolic extract; T4=75 g/l methanolic extract (Curative treatments), T5=2 .5 g/l aqueous extract; T6= 5 g/l aqueous extract; T7=50 g/l methanolic extract; T8=75 g/l methanolic extract (Prophylactic treatments); n =5

Post Mortem Lesions

The post mortem lesions seen during the post mortem examinations included haemorrhage in the intestine, trachea and proventriculus, bile enlargement, cloudy air sack, haemorrhage on glandular surface of the proventriculus and necrotic areas in caecal tonsils (Table 4 and 5).

Table 4: Post Mortem Lesions for Curative Treatments of birds treated with *Cucumis metuliferus* extracts

Treatments	HII	HIT	HOP	BE	ASC	HGSP	NCT
T1	+	+	+	+	+	+	-
T2	+	+	+	+	+	+	+
T3	+	+	+	+	+	+	+
T4	+	-	+	-	-	+	+
Control	+	+	+	+	+	+	+

Key: HII - Haemorrhage in the intestine, HIT – Haemorrhage in the trachea, HOP – Haemorrhage on Proventriculus, BE – Bile duct enlargement, ASC – Cloudy air sac, HGSP – Haemorrhage on the glandular surface of the proventriculus, NCT – Necrotic areas on cecal tonsils

Table 5: Post mortem lesions for Prophylactic Treatments of birds treated with *Cucumis metuliferus* extracts

Treatments	HII	HIT	HOP	BE	ASC	HGSP	NCT
T5	+	+	+	-	+	+	+
T6	-	+	+	-	+	+	+
T7	-	-	-	-	-	+	+
T8	+	-	-	-	-	-	-
Control	+	+	+	+	+	+	+

Key: HII - Haemorrhage in the intestine, HIT – Haemorrhage in the trachea, HOP – Haemorrhage on Proventriculus, BE – Bile duct enlargement, ASC – Cloudy air sac, HGSP – Haemorrhage on glandular surface of the proventriculus, NCT – Necrotic areas on cecal tonsils

Blood Picture/Haematology

Groups T4 and T8 treated with 75 g/l of methanolic extract for curative and prophylactic treatments respectively recorded the highest PCVs, total white blood cell count, as well as a total red blood cell counts for prophylactic and curative treatments respectively (Tables 6 and 7).

Table 6: Blood Picture/Haematology (Curative Treatments)

Parameters	T1	T2	T3	T4	Control	SEM
PCV	31.00 ^a	24.33 ^b	25.33 ^b	36.67 ^a	16.00 ^c	2.62926
HGB (g/dl)	10.20 ^a	8.07 ^a	8.43 ^a	5.37 ^b	5.53 ^b	0.81666
TP	4.73 ^a	3.53 ^b	4.87 ^a	5.27 ^a	3.87 ^b	0.15333
TWBC x 10 ⁹ /l	14.27 ^a	14.33 ^a	11.67 ^b	16.00 ^a	11.13 ^b	0.85076
TRBC x 10 ¹² /l	5.23 ^a	4.23 ^a	4.33 ^a	5.73 ^a	3.27 ^b	0.37389
HETERO (%)	13 ^a	9 ^b	8 ^b	9 ^a	5 ^c	1.03494
LYMPHO (%)	82 ^a	89 ^a	90 ^a	86 ^a	95 ^a	1.47745
MONO (%)	0	2	1	0	0	0.39841
EOSINO (%)	0	0	0	4	0	0.23231
BASO (%)	0	0	0	0	0	0.00000
BAND (%)	3	0	1	1	0	0.40237

Means with the same letter are not significantly ($P>0.05$) different.

Table 7: Blood Picture/Haematology (Prophylactic Treatments)

Parameters	T5	T6	T7	T8	Control	SEM
PCV	24.33 ^a	25.33 ^a	37.00 ^a	44.00 ^b	16.00 ^c	1.11469
HGB (g/dl)	7.97 ^a	8.40 ^a	13.67 ^b	12.53 ^b	5.53 ^a	0.35017
TP	6.53 ^a	4.80 ^b	4.33 ^b	6.67 ^a	3.87 ^b	0.32403
TWBC x 10 ⁹ /l	14.40 ^a	9.87 ^b	11.53 ^a	16.33 ^c	10.13 ^b	1.02003
TRBC x 10 ¹² /l	3.97 ^a	4.07 ^a	7.37 ^b	7.87 ^b	3.27 ^a	0.12794
HETERO (%)	14 ^a	11 ^b	9 ^b	11 ^b	5 ^c	1.02291
LYMPHO (%)	84 ^a	85 ^a	89 ^a	85 ^a	95 ^a	1.37737
MONO (%)	2	3	0	3	0	0.47676
EOSINO (%)	0	0	2	0	0	0.23503
BASO (%)	0	0	0	0	0	0.00000
BAND (%)	0	1	0	1	0	0.16330

Means with the same letter are not significantly ($P>0.05$) different.

Haemagglutination Inhibition

The 75 mg/l groups for both curative and prophylactic treatments (Groups T4 and T8) recorded the highest antibody titres of 1 in 128 and 1 in 256 respectively (Tables 8 and 9).

Table 8: Haemagglutination Inhibition (Curative Treatments)

Treatment	1/1	1/2	1/2	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
T1	+	+	+	+	+	-	-	-	-	-	-	-
T2	+	+	+	+	-	-	-	-	-	-	-	-
T3	+	+	+	+	+	+	-	-	-	-	-	-
T4	+	+	+	+	+	+	+	+	-	-	-	-
Control	+	+	+	-	-	-	-	-	-	-	-	-

Key - T1=2.5 g/l aqueous extract; T2= 5 g/l aqueous extract; T3=50 g/l methanolic extract; T4=75 g/l methanolic extract

Table 9: Haemagglutination Inhibition (Prophylactic Treatments)

Treatment	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
T6	+	+	+	+	-	-	-	-	-	-	-	-
T5	+	+	+	+	+	+	-	-	-	-	-	-
T7	+	+	+	+	+	+	+	-	-	-	-	-
T8	+	+	+	+	+	+	+	+	+	-	-	-
Control	+	+	-	-	-	-	-	-	-	-	-	-

Key – T5=2.5 g/l aqueous extract; T6= 5 g/l aqueous extract; T7=50 g/l methanolic extract; T8=75 g/l methanolic extract

DISCUSSION

Newcastle disease has been reported to be one of the major diseases bothering poultry with its high cost of management driving up the cost of pullet production [16, 17]. The alleviated clinical signs seen could be as a result of the alkaloids present in *Cucumis metuliferus* which several authors have proven to be beneficial for various ailments. Alkaloids have been documented to have antiviral and antileukemic activities [18]. Our findings of fewer clinical signs in 75g/l curative and prophylactic treated groups agree with the findings of Wannang *et al.* [19] who showed that the isolated alkaloids from *C. metuliferus* at a dose of 600 mg/kg of the extract reduced the clinical signs seen in Newcastle disease and reversed the haemorrhagic lesions associated with the disease.

The mortality rates observed were not surprising since the clinical signs reflected similar findings and it is anticipated that higher mortality would occur in groups with more serious clinical signs and vice versa. Wakamatsu [20] worked on a similar isolate of NDV as Kudu 113 strain used in this study and reported 100% mortality post-infection. The result of this study showed that groups administered methanolic extract at 75 g/l recorded fewer clinical signs and this would be translated into less mortality. Wannang [19] recorded a similar finding where extract (600 mg/kg) reversed the haemorrhagic lesions induced by Newcastle disease virus thus reducing clinical signs and mortality. These results suggest that *Cucumis metuliferus* may be effective for use in the management of

Newcastle disease infection in chickens.

Findings from the post mortem lesions followed a similar pattern with groups T4 and T8, both administered 75 g/l of methanolic extract of *Cucumis metuliferus*, showing the least post mortem lesions for the prophylactic and curative groups respectively. The control group exhibited all signs seen followed by groups T1 and T5 treated with 2.5g/l of aqueous extract of *Cucumis metuliferus* recorded the most post mortem lesions for the prophylactic and curative groups respectively.

The severity of the post mortem lesions seen may be attributed to the route of challenge (Ocular) which may enhance the clinical signs and gross lesions observed in most cases as the Newcastle disease virus replicates in the Haderian gland before spreading to other sites [21]. However less severe clinical signs and mortality seen in groups T4 and T8 also translated in the less severe post mortem lesions observed in these groups. There were fewer hemorrhagic lesions observed for these groups and this was probably due to the preventive effect of alkaloids in *Cucumis metuliferus*. Wannang [19] carried out a study that showed that the extract of *Cucumis metuliferus* at 600 mg/kg reversed the haemorrhagic lesions induced by the Newcastle disease virus.

The result highlighted that the plant *C. metuliferus* increases the level of chicken PCV. The higher white blood cell count seen from blood pictures for groups T4 and T8 indicated higher white blood cell activity and thus higher chances of the immune system fighting off the infection when compared with other groups. This could translate to the lower clinical signs and mortality rates observed in these groups.

Usman [22] carried out a study which elucidated that the fruit of *C. metuliferus* was shown to increase the values of blood parameters: packed cell volume, haemoglobin, red blood cell and white blood cell counts. This is in agreement with the work of Wannang *et al.* [23], that the aqueous extract of *C. metuliferus* significantly increased the level of PCV in rats when given at 1000 mg/kg. An increase in the PCV value of rabbits treated with *C. metuliferus* against trypanosomiasis was also reported [24]. Jimam *et al* [25] carried out a study using methanolic extract of *C. metuliferus* and observed that birds treated with 200, 400 and 600 mg/kg of the extract exhibited a significant ($P < 0.05$) increase in PCV, Hb and RBC count on day 14 and 21 after treatment. Similarly, a significant ($p < 0.05$) increase in RBC count was observed in birds treated with the extract at 400 and 600 mg/kg after 7 days post treatment [25]. Usman *et al.* [26] in their study also showed that *C. metuliferus* may be used as haematinic as well as in the treatment of malnutrition. The presence of iron in the fruit may have stimulated the production of RBC and this, therefore, confirmed the folkloric use of *C. metuliferus* as a haematinic agent [26].

Antibody titres for haemagglutination inhibition tests were indicated by the highest titre levels at which agglutination became absent thus indicating no antigen-antibody reaction at that titre. All groups showed an initial positive response. This could be attributed to maternally derived antibodies [27]. Similar results of maternal antibodies conferring protection in all groups have been reported by Agang *et al.*, [28]. The results for HI were not surprising as the groups with the

highest antibody titres had the lowest recorded clinical signs in curative and prophylactic groups respectively as well as mortality and postmortem lesions. The findings from the present study are in agreement with the observations by Anyawu *et al.* [29] that the fruit alkaloids of *Cucumis metuliferus* induced some levels of protective immunity against the lethal effects of Newcastle disease virus-LaSota using embryonated chicken eggs.

Conclusion and Recommendation

The study revealed that methanolic extract of *Cucumis metuliferus* at 75 mg/l showed good prophylactic and therapeutic promise and as such can be recommended for the control of NCD to farmers and that further studies be conducted to isolate the alkaloids of *C. metuliferus* responsible for these findings.

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