Tropical Journal of Applied Natural Sciences
Trop. J. Appl. Nat. Sci., Vol 2 Issue 1, (2024)
ISSN: 2449-2043
https://doi.org/xxx/TJANS/XXXX.X.X.X

BOOSTING THE ANTIVIRAL ACTIVITY OF *BAPHIA NITIDA* LEAVES EXTRACT IN BROILER CHICKS USING VITAMIN C

Iheukwumere, C.M.[1], Iheukwumere, I.H.[2], Obianom, A.O.[1], Unaeze, B.C.[3], Ejike, C.C [4], Igiri, V.C.[5] and Okereke, F.O.[2]

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria[2]

Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria[1]

Department of Medical Laboratory Science, Faculty of Health Science and Technology, Nnamdi Azikiwe University Awka, Anambra State, Nigeria[3]

Department of Medical Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria[4]

Microbiology unit, Department of Biological Sciences, University of Agriculture and Environmental Sciences, Umuagwo, Imo State, Nigeria[5]

Corresponding author:cm.iheukwumere@unizik.edu.ng

ABSTRACT

Newcastle disease is a highly contagious and devastating viral disease of the poultry with worldwide distribution and resulting in large economic loss among the poultry farmers. The aim of this is to evaluate the role of vitamin C in enhancing antiviral activity of *Baphia nitida* in broiler chicks. A total of sixteen (16)14 day old broiler chicks were used in this study which was divided into 4 groups. Group 1 and 2 were protected with the extract before being challenged with the virus whereas group 3 received only the virus and group 4 served as the control. The protective effect of the extracts was determined by monitoring the changes in body weight, organ weights, pathological featuresand lesions in broiler chicks. The study showed that Baphiavic significantly ($p<0.05$) improved the weight gain, minimize clinical features and lesions in protected chicks. Hence this study has demonstrated that a mixture of *Baphia nitida* and vitamin C (Baphiavic) can protect birds from Newcastle virus

Received: Jan., 2024
Accepted: Jan., 2024
Published: Feb., 2024

KEYWORDS:

Antiviral-activity, *Baphia nitida* ,Newcastle disease, Protective -effect

INTRODUCTION

The use of plants as traditional medicine against viral diseases in the production of animals have been described and practiced worldwide. The use of herbs and their extracts as antiviral agents began following World War II in Europe, and the research was later developed worldwide (Ahmad *et al*., 2014). The poultry industry is one of the most important agricultural industries, providing food to almost 7 billion people worldwide. The demand for chicken meat has been steadily increasing and is expected to reach 131,607.3 thousand tonnes in the year 2026 (Abd- Alla *et al*., 2012).

Disease causing microorganisms in the poultry industry includes various virus, bacteria and protozoa. The most challenging pathogens among these, is the virus pathogen which continue to emerge through various genetic modification such as mutations, recombinations or co-evolution with vaccines. The most destructive avian viral diseases are Newcastle disease virus (NDV), avian influenza virus (AIV), infectious bursal disease virus (IBDV), infectious bronchitis virus (IBV), egg drop syndrome avian adenovirus, and fowl pox virus (Yasmin *et al*., 2020). Newcastle disease virus (NDV) is the etiological agent for Newcastle disease (ND), which is a viral disease of birds (Lakshmi, 2014). The virus belongs to the paramyxovirus (PMV) which is of public health importance and it is significant in poultry as it constitutes one of its major threats. Velogenic strains of Newcastle disease virus (NDV) can cause conjunctivitis in humans, usually when the person has been exposed to the virus consistently in large quantities (Bakari *et al*., 2012). Vaccination programmes against these viruses has been applied in many countries worldwide. However, the problems arise from backyard-reared chicken infections, which are normally not vaccinated, but still prevalent, leading to the spread of the virus that eventually causes outbreak in the community (Yasmin *et al*., 2020). Modern treatments of the infected avianspecies are laborious and expensive. Treatments with medicinal plants have been practicedtraditionally to overcome the virus infection.

Baphia nitida (Leguminosae- Papilionoideae) is one of the species of *Baphia*, known locally as 'okazi' in the Igbo tribe of Nigeria. It is a shrub which grows to a height of about 9 m, geographically, it is found in the wetter parts of the coastal regions, the rain and secondary forests and on abandoned farmland from sea-level up to 600 m altitude. Various parts of *B. nitida*has been used by indigenes of many West African countries for a wide range of ethno-medicinal purposes and often also used for ornamental purposes. *B. nitida* is used to treat constipation, ringworm, sprains and swollen joints, parasitic skin diseases, wounds, ulcers, boils, venereal diseases, and gastrointestinal problems. This study was thus aimed at evaluating the neutralizing potentials of *Baphia indica* augmented with vitamin C against this virus

MATERIALS AND METHODS

Preparations of Plant Materials: The leaves of *Baphia nitida* were collected from Onitsha, Anambra State, Nigeria. The plant material was authenticated appropriately Dr B. Garuba, in Botany Department, Michael Okpara Federal University of Agriculture, Umudike. The plant material was washed and dried under shade at room temperature for 14 days. The dried plant material was ground to powder form using sterile electric grinder. (Iheukwumere *et al.*, 2020).

Extraction of the Extract: A 2000 mL Soxhlet extractor that has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble was used for process. Twenty grams (100 g) of the plant material to be extracted was placed inside the thimble. The thimble was then loaded into the main chamber of the Soxhlet extractor. Then 1000 mL of ethanol was placed in a 1000 mL distillation flask. The flask was placed on the heating mantle (2000 mL, 220 V, 500 W). The Soxhlet extractor was placed at the top of the flask. A reflux condenser was placed at the top of the extractor. When theethanol was heated to reflux, the solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapour cooled,and dripped back down into the chamber housing the solid material. The chamber containing the solid material slowly filled with warm solvent. When the Soxhlet chamber was almost full, the

chamber was emptied by the siphon. The solvent then returned to the distillation flask. The thimble ensured that the rapid motion of the solvent did not transport any solid material to the still pot. This cycle was allowed to repeat many times for 12 h. After extraction, the solvent is removed, typically by means of a rotary evaporator to collect the extract.

Preparation of Extract: The plant extract and vitamin C were each reconstituted with phosphate buffer saline (PBS). One (1.0) g of each respectively was separately dissolved in 10ml of PBS to form 0.10 ppm using sterile conical flasks. They were evenly homogenized and stored in clean sterile containers for use.

Viral Sample Preparation: The lyophilized viral stock (LaSota strain of the Newcastle virus) was prepared by dissolving each vial in 2.5 ml of phosphate buffer saline (PBS). Each were thoroughly homogenized and used immediately after the preparation.

Broiler Chicks: A total of sixteen 14 day old broiler chicks were used for this study. The chicks were purchased from Dr C. Udechukwu poultry farm at Ojoto in Idemili South L.G.A, Anambra State, Nigeria. The chicks were kept in a cleaned and disinfected house and fed with growers mash and water *ad libitum*. They were grouped into 4 and their weight was recorded using an electronic weighing balance.

Sample Administration for *In Vivo* Study: Groups 1 and 2 were administered 0.5 ml of preparations *Baphia nitida* extract and a mixture of the extract and vitamin C respectively for 3 days after which 0.2 ml of the viral sample was administered. The extract and mixture administration was continued for more 3 days for each group. Group 3 was administered only 0.2 ml of the viral sample. The control group was giving only feed and water. The chicks were weighed daily through the period of the experiment to determine their change in weight.

Examination of Experimented Chicks: The chicks were carefully observed daily for pathological signs such as anorexia (loss of appetite), greenish diarrhea, nasal discharge,

sneezing, ruffled feathers, paralysis and death for a period of 2 weeks. The protective rate of the plant extract and mixture was determined. Post mortem examination of the liver and spleen as well as their weight measurement was carried out. Abnormalities such as swollen proventriculus,liver and spleen hemorrhage, spleen congestion were noted (Fawzy *et al*., 2021).

Statistical Analysis: The da ta generated from this study was presented in form of mean ± standard deviation (SD), percentage and also in Tables and figures. Significance of the study wasdetermined using one way Analysis of Variance (ANOVA) at 95% confidence limit. Pair wise comparism was done using student 't' Test (Iheukwumere *et al*., 2018).

RESULTS

The present study revealed progressive increase in the weights of the experimented chicks as shown in Table 1. The increase in body weights were most pronounced among the chicks protected with *Baphia nitida* extract supplemented with vitamin C (Baphiavic), followed by those chicks protected with *Baphia nitida* extract, and these were retarded among the infected chicks without any protection as compared to the control group in Table 1. It was also observed that the progressive weight increase were statistically non significant ($p > 0.05$). There were significant ($p < 0.05$) reduction in pathological features of Newcastle disease virus among the chicks protected with *Baphia nitida* extracts, and these were most pronounced when the extract was mixed with vitamin C as shown in Table 2. It was also observed that the mixture of *Baphia nitida* extract and vitamin C reduced the pathological features of the study virus to that of the normal control group (Table 2).

There was increase in the weights of the liver and spleen of the protected chicks when compared to that of the infected and control groups, but these increase where statistically non significant ($p > 0.05$) as shown in Table 3. The liver harvested from those chicks protected with the mixture of

the extract and vitamin C was higher than that of the control group whereas their spleen were almost equivalent (Table 3).

The postmortem examination of the experimented chicks revealed significant ($p < 0.05$) reduction of pathological features associated with the liver and spleen of the chicks as shown in Table 4. Liver hypertrophy, liver haemorrhage, spleen hypertrophy and spleen haemorrhage were completely reduced to zero level among the protected chicks. Spleen congestion was reduced to zero level only on those chicks protected with *Baphia nitida* leaves extract supplemented with vitamin C (Baphiavic).

Table 1: Weights of the experimented chicks

Experimented chicks	Day 0 (g)	Day 1 (g)	Day 2 (g)	Day 3 (g)
Infected chick	124.56	126.77	128.21	129.92
Baphia nitida	124.22	133.11	143.81	152.96
Baphiavic	124.44	134.51	145.02	155.07
Control	125.06	133.61	144.43	153.73

Table 2: Pathological features of the experimented chicks

Feature	Infected chick	*Baphia nitida*	Baphiavic	Control
Sneezing	4	1	0	0
Nasal discharge	3	0	0	0
Watery stool	4	1	1	0
Greenish stool	4	0	0	0
Anorexia	4	2	1	0
Paralysis	3	0	0	0
Death	3	0	0	0

Table 3: Weights of liver and spleen of the experimented chicks

Experimented chick	Liver (g)	Spleen (g)
Infected chick	4.01	0.75
Baphia nitida	4.60	1.12
Baphiavic	5.75	1.14
Control	5.50	1.15

Table 4: Morphological features of liver and spleen of the experimented chicks

Feature	Infected chick	*Baphia nitida*	Baphiavic	Control
Liver hypertrophy	4	0	0	0
Liver congestion	4	1	1	0
Liver haemorrhage	2	0	0	0
Spleen hypertrophy	4	0	0	0
Spleen congestion	4	1	0	0
spleen haemorrhage	3	0	0	0

DISCUSSION

The increase in body weight of the protected groups is in line with the findings of Eze *et al*. (2012) who observed increased feed intake with subsequent weight gain in treated groups. Hieu*et al*. (2022) also reported that the addition of vitamin C to the feeds of chicken improved their weight gain but this contradicts the findings of Bakari, (2013) who reported initial weight loss onthe treated groups but with later increase after 14 days.

The leaf extract of *Baphia nitida* and vitamin C had more effect on the Newcastle disease virus by reduction in pathological features associated with the virus in protected chicks, this is in agreement to the findings of Chambial *et al*. (2013), who reported that vitamin C protects the immune system, reduces the severity of allergic reactions and helps fight off infections. Regarding mortality Newcastle disease virus has been a serious problem in the poultry industry worldwide. The Newcastle disease caused by the velogenic strain may cause mortality up to 100% or vary from 80% to 90% in the case of the mesogenic strains, mortality can reach 25%. The significant reduction in the clinical parameters including mortality and pathological lesionsin the protected groups is in line with the findings of Bakari, (2013), who observed similar result using *C. swynnertonii* resin extract. The increase in the weights of the protected organs similar tothat of the control is in agreement with the findings of Hashemi *et al*. (2008), but disagrees with the reports of Nouzarian *et al*. (2011) who observed reduction in weight of these organs intreated groups.

CONCLUSION

This study has shown that ethanolic extract of *Baphia nitida* shows antiviral effects against Newcastle disease virus which is accompanied by a decrease in virus infectivity and pathological

lesions associated with this virus in the protected groups. These effects are most pronounced when the extract is supplemented with vitamin C. Hence could be used in the management of this virus by the poultry farmers.

REFERENCES

Abd-Alla, H.I., Abu-Gabal, N.S., Hassan, A.Z., El-Safty, M.M., Shalaby, N.M.(2012)). Antiviral activity of Aloe hijazensis against some haemagglutinating viruses infection and its phytoconstituents**.** *Arch. Pharmacol. Res*. **35**:1347-e1354.

Ahmad, W., Ejaz, S., Anwar, K., Ashraf, M.(2014). Exploration of the in vitro cytotoxic and antiviral activities of different medicinal plants against infectious bursal disease (IBD) virus. *Cent. Eur. J. Biol*. **9** (5):531-e542.

Bakari, G.G., Max, R.A., Mdegela, H.R., Phiri, E.C.J., Mtambo, M.M.A. (2013). Efficacy of resinous extract from Commiphora swynnertonii (Burtt) against Newcastle infection in chickens. *Int. J. Methods Psychiatr. Res*. **2**: 156-e161.

Bakari, G.G., Max, R.A., Mdegela, H.R., Phiri, E.C.J., Mtambo, M.M.A.(2012). Antiviral activity of crude extracts from Commiphora swynnertonii against Newcastle disease virus in ovo. *Trop. Anim. Health Prod*. **44:** 1389-e1393.

Chambial, S., Dwivedi, S., Shukla, K.K., John, P.J., and Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry* **28**(4): 314–328

Eze D. C., Okwor E. C., Okoye J. O., Nnabuike O. D. and Olu S. S. V. (2012). Effects of *Moringa oleifera* methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain. *Journal ofMedicinal Plants Research* **6**(27): 4443–4449.

Fawzy, M., Afify, M., Youssef, Youssef, I. (2021). Pathological identification of Egyptian local velogenic Newcastle disease virus genotype viid in layer chickens. *Advances in Animal and Veterinary Sciences* **9**(12): 2054–3331.

Hashemi S. R., Zulkifli I., Hair Bejo M., Farida A. and Somchit M. N. (2008). Acute toxicity study and phytochemical screening of selected herbal aqueous extract in broiler chickens. *International Journal of Pharmacology* **4**(5): 352–360.

Hieu, T.V., Guntoro, B., Qui, N.H., Quyen, N.T.K., and Al-Hafiz, F.A. (2022). The application of ascorbic acid as a therapeutic feed additive to boost immunity and antioxidant activity of poultry in heat stress environment. *Veterinary World* **15**(3): 685–693.

Iheukwumere, I.H., Chukwura, E.I. and Chude, C. (2018). *In vivo* activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds on broiler layers. *Journal of Biology, Agriculture and Healthcare* **9**: 21–36.

Iheukwumere, I.H., Dimejesi, S.A., Iheukwumere,C.M., Chude, C.O., Egbe, P.A.,Nwaolisa,C.N., Amutaigwe, E.U., Nwakoby, N.E., Egbuna, C., Olisah, M.C. and Ifemeje, J.C.(2020). Plasmid curing potentials of some medicinal plants against citrate negative motile *Salmonella* species. *European Journal of Biomedical and Pharmaceutical Sciences* **7**(5); 40 -47.

Lakshmi, T.S. (2014). Growth Promoters and Novel Feed Additives improving Poultry Production and Health, Bioactive Principles and Beneficial Applications: The Trends and Advances-A Review. *International Journal of Pharmacology* **10**: 129–159.

Nouzarian, R., Tabeidian, S.A., Toghyani, M., Ghalamkari, G. And Toghyani, M. (2011). Effect of turmeric powder on performance, carcass traits, humoral immune response and serum metabolites in broiler chickens. *Journal of Animal and Feed Sciences* **20**: 389–400.

Yasmin, A.R., Chia, S.L., Looi, Q.H., Omar, A.R., Noordin, M.M. and Ideris, A. (2020). Herbal extracts as antiviral agents. *Feed Additives* 115–132.