

Effect of methanolic stem bark extract of *Azadirachta Indica* on morbidity and mortality of chickens experimentally infected with velogenic Newcastle disease virus (kudu 113).

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Abstract

Newcastle Disease (ND) is a dangerous disease of poultry all over the world. Economically, ND is characterized by mortality which may reach up to 100% in affected poultry farms. This study was designed to evaluate the protective properties of crude methanolic extract of *Azadirachta indica* in chickens experimentally infected with a velogenic strain of ND virus. A hundred-day old cockerel was brooded for three weeks before randomly divided into five equal groups (I, II, III, IV and V). They were not vaccinated with ND vaccine LaSota. At six weeks of age, each bird in groups I-IV was inoculated with 0.2mL of the live ND viral inoculum intramuscularly while group V was not inoculated with the virus. Thereafter, groups I, II and III were daily given in drinking water 200mg/kg, 400mg/kg and 600mg/kg of *Azadirachta indica* extract, respectively whereas, groups IV and V were not treated with the extract. All the groups were monitored for the onset of clinical signs, morbidity and mortality rate. In all the groups inoculated with the ND virus, about 80% birds showed signs of depression, gasping, coughing, increased thirst, complete inappetence, huddling, diarrhoea, partial/complete paralysis on day 2 post inoculation (PI). A hundred percent (100%) mortality rate was recorded for groups I and IV by day 5 PI and in groups II and III on day 6 PI. Therefore, under the conditions of this study, oral administration of *Azadirachta indica* extract does not protect birds from ND.

Keywords: *Azadirachta indica* stem bark; methanolic extract; chickens; Newcastle disease

Introduction

Newcastle disease (ND) is a highly contagious viral disease affecting wild and domestic avian species (Seal *et al.*, 2000, Alexander, 2003). The ND is caused by an avian paramyxovirus serotype of the genus Avulavirus belonging to the family paramyxoviridae. The disease is distributed worldwide (Alexander *et al.*, 1997). The ND is still one of the most important diseases of chickens in Nigeria since the first outbreak at Ibadan in 1952 (Ezema *et al.*, 2009), not only due to the high flock morbidity and mortality but because of the incessant reoccurrence of the disease in vaccinated flocks, hence the need for an

alternative remedy to prevent the devastating activity of this disease on the poultry industry. Ethnoveterinary medicine is the animal health care which encompasses the knowledge, skills, methods, practices and beliefs about animal healthcare found among the members of a community (McCorkle, 1986). There is an increasing awareness on the use of various plants in treatment and control of animal diseases (Atawodi and Spiegelhalter, 1994), since herbal medicine is no longer viewed as a myth or an ungodly practice (Wanzala *et al.*, 2005). Neem (*Azadirachta indica* A. juss), a Meliaceae family tree, is a hardy evergreen

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tree commonly found in South Asia and some parts of Africa, have been found useful in the treatment of various conditions in man and animals (Schmutterer, 2002; Subapriya and Nagini, 2005). During harmattan in the south-eastern Nigeria, the stem of Neem tree is traditionally used in prevention of Newcastle disease by soaking in the drinking water for birds and it is believed that neem bark has both protective and curative property against Newcastle disease. Neem seed extract was reported to be effective in inhibiting NDV and IBV replication in Vero cells and chicken embryo (Waafa *et al.*, 2007). Hence, the need for an in-vivo study, to evaluate the effects of methanolic neem stem bark extract on the morbidity and mortality of chickens experimentally infected with the velogenic strain (Kudu 113) of Newcastle disease virus.

Materials and methods

One hundred-day-old white Harco cockerels were procured from CHI Ltd., a commercial breeder farm in Ibadan, south-west Nigeria. They were housed in the Poultry Disease Research Unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The birds were reared in deep litter system and were given feed and water *ad libitum*. They were not vaccinated against ND. The bark of *Azadirachta indica* was collected and authenticated by a taxonomist and extract prepared using standard methods. The velogenic Newcastle Disease virus (VNDV) strain, duck/Nigeria/903/KUDU-113/1992 (Echeonwu *et al.*, 1993), was obtained from the National Veterinary Research Institute Vom, Jos, Plateau State, Nigeria. The virus is a genotype XVII NDV (Shittu *et al.*, 2016). The inoculum had a median embryo infective dose (EID₅₀) of 10^{6.46} per ml. The birds were brooded for 3 weeks after which they were randomly divided into five groups (20 chicks each) I,

II, III, IV and V. Group V was isolated. At 6 weeks of age, groups I, II, III and IV were inoculated with 0.2m challenge dose of VNDV (Kudu 113) while Group V was inoculated with 0.2mL of phosphate buffered saline (PBS) intramuscularly and thereafter, groups I, II and III were daily given 200mg/kg, 400mg/kg and 600mg/kg body weight of the *Azadirachta indica* extract, respectively through drinking water while groups IV and V were not treated with the extract. The birds were clinically monitored twice daily. Morbidity and mortality were recorded. On days 3 and 6 PI, three birds from each group were humanely euthanized and necropsied along with dead birds (King *et al.*, 2003) lesions in the proventriculus, bursa, thymus and spleen were observed and recorded.

Statistical analysis

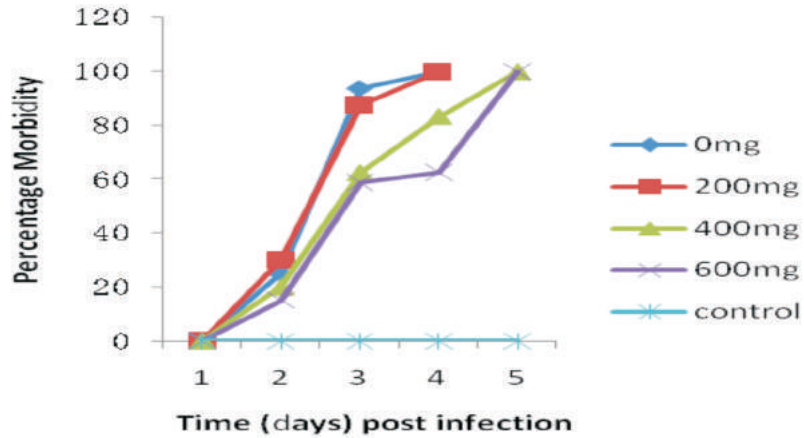
Body weights were subjected to analysis of variance (ANOVA) statistics using the Statistical Package for the Social Sciences version 16.00 for windows (SPSS Inc, Chicago, Illinois). Significant means were separated using the Duncan's new multiple range test and tests were significant at a probability of P<0.05.

Results

The effect of the extract on the birds challenged with the VNDV was observed for six days post challenge. Clinical signs were first observed on day two post inoculation (PI) in groups I, II, III and IV. The clinical signs observed included ruffled feathers with depression, coma, lethargy, prostration inappetence, whitish-greenish diarrhoea with soiling of the vent. Coughing with frothy sounds and serous ocular discharges were evident in 20%, 10%, 10% and 20% while nervous signs of jerking of head and paralysis were observed in 30%, 20%, 15% and 25% of the experimental birds in groups I, II, III and IV respectively. By day three PI morbidity was 87.5%,

62.5%, 58.5% and 93.7% of the birds in experimental groups I, II, III and IV respectively, by day four PI, birds in experimental groups I and IV had 100% depression with clinical signs prominent in

most of the birds, while groups II and III had morbidity rate of 83.3% and 62.5%, respectively. By day five, 100% morbidity was also observed in birds of the experimental groups II and III (Fig. 1).

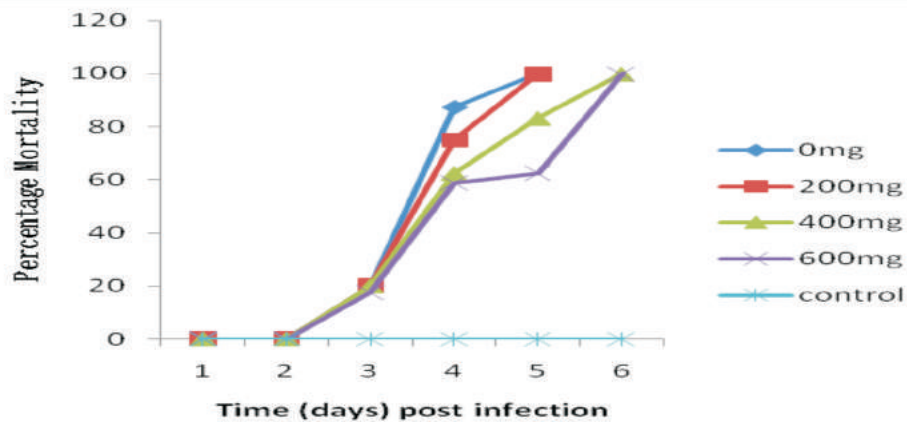


*GRP I- Inoculated/treated with 200mg, GRP II- Inoculated/treated with 400mg, GRP III- Inoculated/treated with 600mg, GRP IV- Inoculated/untreated with 0mg, GRP V (control)- Uninoculated/untreated

Figure 1: Morbidity profile following inoculation with NDV

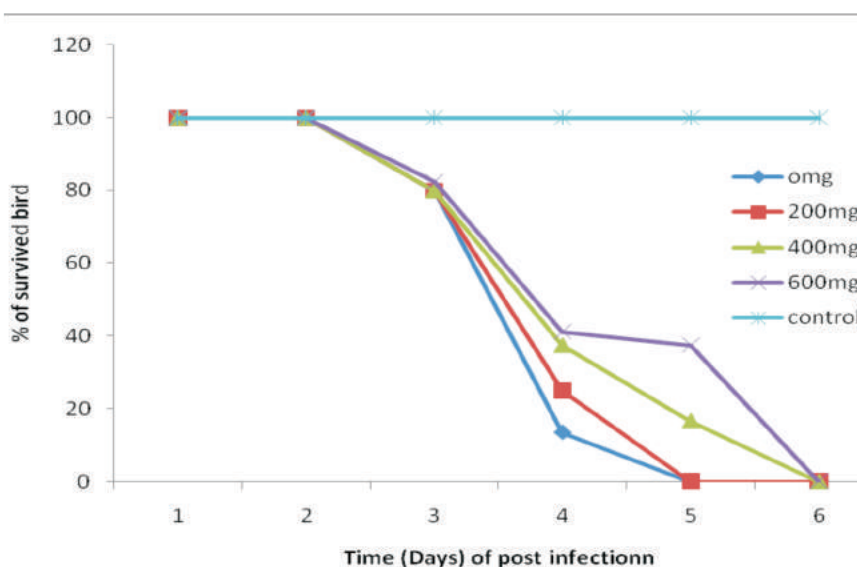
Mortalities of 20%, 20%, 17.6% and 20% were first observed in group I, II, III and IV, respectively on day three PI. Peak

mortalities of 100% occurred on day five PI in groups I and IV, while it occurred in groups II and III on day six PI (Fig.2).



*GRPI- Inoculated/treated with 200mg, GRP II- Inoculated/treated with 400mg, GRP III- Inoculated/treated with 600mg, GRP IV- Inoculated/untreated with 0mg, GRP V (control)- Uninoculated/untreated.

Figure 2: Mortality profile of birds following inoculation with NDV



GRP I- Inoculated/treated with 200mg, GRP II- Inoculated/treated with 400mg, GRP III- Inoculated/treated with 600mg, GRP IV- Inoculated/untreated with 0mg, GRP V (control)- Uninoculated/untreated

Figure 3: Survival profile following inoculation with NDV

There was a reduction in the mean body weights of birds in groups I-IV by day four PI (Table 1), however, the mean body weight of birds in group IV (inoculated /untreated) was significantly ($p < 0.05$) lower than groups I, II and III (inoculated/treated), while the mean body weight of group I with a lower dose of the extract was significantly

($p < 0.05$) lower than the mean bodyweight of birds in group III which was given a higher dose of the extract. The mean body weight for group V (526 ± 12.4) was significantly higher than body weights of group I (409 ± 8.7), group II (419.2 ± 10.1), group III (437 ± 12.6) and group IV (387.1 ± 21.3).

Table 1: The mean body weight of cockerels, infected and treated with different concentrations of *Azadirachta indica* extracts (g)± SEM

GROUPS	I	II	III	IV	V
Days PI	200mg/kg	400mg/kg	600mg/kg	0mg/kg	Control
0	454 ± 17.9	454 ± 17.9	454 ± 17.9	454 ± 17.9	454 ± 17.9
4	409 ± 8.7^b	419.2 ± 10.1^{bc}	437 ± 12.6^c	387.1 ± 21.3^a	526 ± 12.4^d

GRPI- Inoculated/treated with 200mg, GRP II- Inoculated/treated with 400mg, GRP III- Inoculated/treated with 600mg, GRPIV- Inoculated/untreated with 0mg, GRP V (control)- Uninoculated/untreated

*Different superscripts in a row indicate significant difference between the groups ($p < 0.05$).

Grossly, the *post-mortem* examination showed congested breast and thigh muscles (Fig.4) which occurred in birds, in all the groups (I-IV). Severe intestinal hemorrhagic enteritis and ulcers (Fig.5) were seen in 16:20, 14:20, 13:20 and 18:20

birds in group I, II, III and IV respectively. About (50%) of birds in group IV had enlarged caecal tonsils whereas none was enlarged in other groups. Hemorrhages on the proventricular mucosa (Fig. 6) was seen in all (20:20) the birds of groups I and IV,

while 18:20 birds in groups II and III had the lesion. The thymus was atrophic in 18:20 birds in group I, 19:20 birds in group IV, 15:20 birds in groups II and III (Fig. 7). Atrophy of the spleen (Fig.8) occurred in

17:20, 15:20, 13:20 and 19:20 birds in groups I-IV respectively. The bursa was atrophic in 19:20 birds in groups I and IV (fig. 9), while it occurred in 17:20 birds in groups II and III.



Figure 4: Congestion of the breast muscles in infected chickens treated with 400mg/kg and 600mg/kg of *Azadirachta indica* extracts on day 4PI

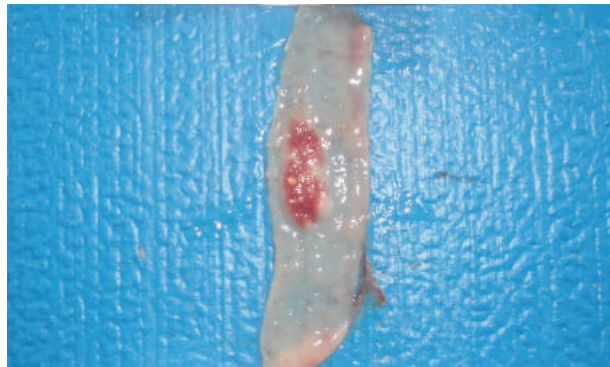


Figure 5: Haemorrhagic intestinal ulcers in infected chickens treated with 200mg/kg of *Azadirachta indica* extracts on day 4 PI



Figure 6: Proventricular haemorrhage in infected chicken treated with 200mg/kg of *Azadirachta indica* extracts on day 4PI



Figure 7: Atrophy of the Thymus in infected birds treated with 600mg/kg of *Azadirachta indica* extract on day 4PI



Figure 8: Atrophy of the spleen in infected birds treated with 600mg/kg of *Azadirachta indica* extract on day 4PI

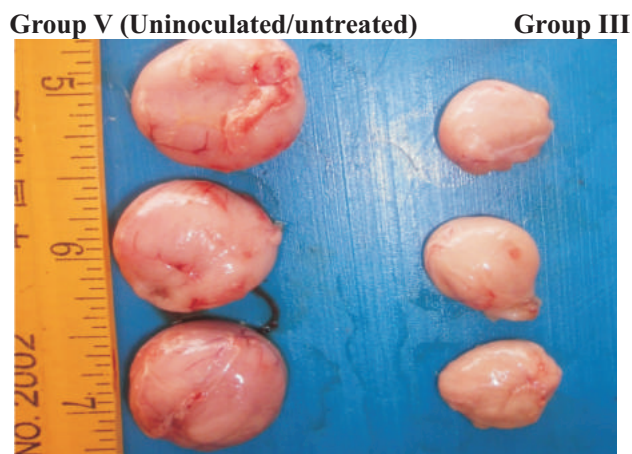


Figure 9: Atrophy of the bursa in infected birds treated with 600mg/kg of the *Azadirachta indica* extract on the day 4 PI

The kidneys were haemorrhagic, swollen and mottled in 16:20 birds in groups I and II whereas it occurred in 15:20 and 18:20 birds

in group III and IV respectively. The liver was equally congested in all the groups (Table 2).

Table 2: Type and frequency of post mortem lesions found in the different groups

GROUP	Total number of chickens in group after challenge	Number of chickens posted	Enlarged caecal tonsils	Intestinal Haemorrhagic ulcers	Proventricular haemorrhage	Atrophic thymus	Atrophic spleen	Congestion of breast and thigh muscles	Atrophic bursa	Haemorrhagic Kidney
200mg/kg	20	20	-	16/20	20/20	18/20	17/20	20/20	19/20	15/20
400mg/kg	20	20	-	14/20	18/20	15/20	15/20	20/20	19/20	6/20
600mg/kg	20	20	-	13/20	18/20	15/20	13/20	20/20	18/20	15/20
0mg/kg	20	20	10/20	18/20	20/20	19/20	19/20	20/20	19/20	18/20

GRP I- Inoculated/treated with 200mg, GRP II- Inoculated/treated with 400mg,, GRP III- Inoculated/treated with 600mg,, GRP IV- Inoculated/treated with 0mg

Discussion

The two days incubation period of experimental ND observed in this study has also been reported by other researchers (Ezema *et al.*, 2009; Wan *et al.*, 2004; Okoye *et al.*, 2000) showed that despite the treatment with the extract, the manifestation of the disease was not prolonged, however, Eze *et al.* (2012) reported an incubation period of three days in birds infected with VVNDV but treated with *Moringa oleifera* methanolic leaf extract, The slight difference in incubation period can be attributed to the immune status of the host (Hamid *et al.*, 1991) and differences in the plant bioactive component in both herbs used. The clinical signs observed in groups I-IV were notable and similar to the clinical signs of those already described for VNDV by Alders and Spradbrow (2001), who observed depression, partial /complete paralysis, inappetence, listlessness and huddling followed by greenish diarrhoea as an indication of Gastro Intestinal tract (GIT) lesion. Severe nervous signs which included ataxia, paralysis, torticollis was also observed in groups I-IV. This suggests that the treatment with the plant extract could not reduce the clinical manifestation of the disease may probably be due to the highly virulent nature of the virus (Alexander, 2003). The disease had a marked reduction of weight in chickens in groups I, II, III and IV. The changes in body weight are common occurrence in septicemia or viraemic diseases, due to reduction in food and water consumption (Okoye *et al.*, 2000). The significant ($p < 0.05$) reduction in weight observed in all the groups was also reported by Okoye *et al.* (2000), Ezema *et al.* (2009) in both immunized and non-immunized chickens infected with VVNDV virus. Reduction in weight was more severe in the untreated/inoculated groups than the treated/inoculated groups, and this could be attributable to the immune boosting effect

of the *Azadirachta indica* extracts (Upadhyay *et al.*, 1993; Arivazhagan *et al.*, 2000) in the treated group which lead to a slight reduction in food and water hence the slight reduction in body weight observed. Despite treatment with *Azadirachta indica* extract, Morbidity rate and mortality rate were up to 100% with a survival rate of 0% on day 4PI in group I and groups II and III on day 5PI is consistent with the reports of Okoye *et al.* (2000), who stated that morbidity and mortality rate of VVND outbreak could be up to 100% in non-immunized birds. However, birds in groups II and III with the higher doses of the extract died later than those in group I with a lower dose of the extract, an indication that higher doses of methanolic extract of *Azadirachta indica* may prolong the survival rate of the birds. The *post-mortem* lesions seen in groups I-IV are characteristic for ND and they are similar to the gross lesions observed in VVNDV challenged birds reported by Brown *et al.* (1999) and Okoye *et al.* (2000). The observable lesions are due to tropism, virulence of the virus, target species and their immunity (Alexander, 2003). The VVND strain is typically associated with a haemorrhagic intestinal lesion, which was severe in both treated and untreated groups. The extract could not reduce the destructive ability of the virus on target organs maybe due to the highly virulent nature of the virus and the period of dosing of the plant extract.

Conclusion

The study showed that the methanolic extract of *Azadirachta indica* stem bark did not reduce the severity of the clinical signs and *postmortem* lesions of the disease in the experimentally infected birds. Therefore, the extract may not be used in prophylaxis or treatment of Newcastle disease caused by velogenic Newcastle disease in poultry.

Ethical approval

Ethical approval for this research was given by the Experimental Animal Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

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