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Short Communication
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Immunomodulatory effects of aqueous extracts of *Azadirachta indica* and Piper *Guineense* on Newcastle disease vaccination in cockrels

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ABSTRACT

The increased incidence of Newcastle disease (ND) outbreaks in the country even amongst vaccinated flocks, has given rise to the search for other means of boosting the immune system besides vaccination alone. A lot of plants have been shown to possess antimicrobial, antipyretic and anticoccidial effects. This study was designed to evaluate the immunomodulatory effect of the aqueous extracts of the leaves of *Piper guineense* and *Azadirachta indica* (Neem) on immune response to Newcastle vaccination in birds. A total of 110 cockrels were used in this study (30 for acute toxicity test and 80 for immuonomodulatory effects of extracts). The 80 cockerels were divided into four groups (A, B, C and D), each consisting 20 birds. Group A and B were treated with 100mg/kg of the aqueous extracts of *Azadirachta indica* and *Piper guineense* respectively for the first three weeks. All the birds in groups A to C were vaccinated with Newcastle disease vaccine Lasota (NDV-L) at day 21, while Group D was given distilled water only. Blood samples were taken from all groups on day 1, 7, 14 and 21 post-vaccination. Serum antibody level against ND virus were measured by HI test and antibody titre was found to be significantly ($p \leq 0.05$) higher in the group treated with *Azadirachta indica* but low in those treated with *Piper guineense*. There was no significant ($p \geq 0.05$) difference in the immune response seen in Group A compared to the positive control (Group C), but significantly ($p \leq 0.05$) higher compared to the negative control (Group D). It was therefore concluded that the aqueous extract of *Azadirachta indica* may enhance immunomodulatory effects against Newcastle disease virus, unlike that of *Piper guineense.*

KeyWords: *Azadirachta indica,* Cockrels, Immunomodulatory, Newcastle disease, *Piper guineense*.

INTRODUCTION

Newcastle disease is one of the important viral diseases of poultry associated with heavy economic losses in the poultry industry (Elangovan et. al., 2000). Newcastle disease is a contagious, viral disease of wild and domestic avian species (Alexander, 2003). It is caused by an Avula virus of the Paramyxoviridae family (Okoroafor *et al*., 2015), causing high morbidity and mortality of birds (Garba *et al*., 2013). Another effect of Newcastle disease on birds is immunosuppression (Kapczynski *et al*., 2013) which results in increased susceptibility to various diseases and poor responses to vaccinations leading to poor performance by these animals (Durrani *et al*., 2008).

For a long time, vaccination has been the most employed means of curbing the disease but amidst vaccinations, reports of Newcastle disease outbreaks have been recorded. This has facilitated the search for alternative immune boosters that can improve outcome of vaccination

The value of ethnopharmacology in medicine is on a rapid increase, especially in Africa endowed with a lot of herbs. Many plants have been shown to have both therapeutic and prophylactic values (Elangovan et. al, 2000). Neem (*Azadirhachta indica*) commonly called Indian lilac and also known traditionally as Doganyaro belongs to the family Meliaceae (Girish & Shankara, 2008). It has been shown to possess medicinal as well as nutritive value for poultry and has been reported to contain chemicals such as Azadiractin and Nimbin (Makeri *et al*., 2007; Jawad *et al*., 2013). All parts of the Neem plant has been found useful in treating various ailments (Biswas *et al*., 2002; Subapriya & Nagini, 2005). It has been reported to exhibit immunomodulatory effects (Arivazhagan *et al*., 2000). Ahsan *et al*., (1999) reported improved immune response to Newcastle Disease vaccination when neem was incorporated into feed. Aqueous extracts of neem have been reported to exhibit antiviral properties against Newcastle disease virus, Infectious Bursal

disease virus and other viruses (Elangovan *et al*., 2000; Durrani *et al*., 2008 and Jawad *et al*., 2013). *Piper guineense* commonly called West African black pepper (Akudike *et al*., 2016) and locally known as Uziza in the south-east or Iyere in western, Nigeria belongs to the family *Piperaceae* (Olonisakan *et al*., 2006). It has been demonstrated to possess antioxidant (Nwinyi *et al*., 2009), antimicrobial (Ahn *et al*., 2007) properties, and increase uterine contractions (Udoh, 1999). In this study, the immune modulatory effects of the aqueous extracts of the two plants; *Azadirachta indica* and *Piper guineense* were studied.

MATERIALS AND METHODS

STUDY AREA

This study was carried out at the poultry farm of Michael Okpara University of Agriculture, Umudike (MOUAU) and Veterinary Microbiology laboratory, College of Veterinary Medicine, MOUAU.

PLANT MATERIALS:

Fresh leaves of *Piper guineense* and *Azadirachta indica* were collected in Umudike, Abia State, Nigeria. Both leaves were properly identified in the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were air dried. The dried leaves were ground with a miller to obtain a refined powder. In order to obtain the plant extracts, 60g of each dried plant material powder was dissolved in a beaker and distilled water added (1:5 w/v) and mixed thoroughly. The suspension obtained was carefully filtered.

ACUTE TOXICITY TEST

30 birds were randomly grouped into three (for each extract) with 5 birds per group. The birds were given different doses of the extract (10mg/kg, 100 mg/kg and 200mg/kg) respectively. The birds were then observed for 24 hours for signs of toxicity such as anorexia, weakness, nervous signs or death.

EXPERIMENTAL BIRDS

Eighty day-old cockrels were used for this study. They were kept under deep litter system and fed *ad-libitum* with feed and water. They were treated with the extracts from day 1 for three weeks, given Gumboro vaccine on day 10 and Newcastle disease vaccine Lasota (NDV-L)
at 21 days. All vaccines used were procured from the National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria. Ethical Approval was received from the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike with Ethical Approval Number MOUAU/CVM/REC/202305.

ANTIGEN PRODUCTION

Three freshly laid embryonated eggs were incubated for 9 days. 0.2ml of the inoculum (LaSota vaccine) was inoculated into the embryo via the allantoic route and incubated for 4 days, after which the virus was harvested via the allantoic fluid into sterile bijou bottles and preserved at - 20^0 C.

EXPERIMENTAL DESIGN

The birds were divided into four different groups labelled A-D; with 20 birds per group. Groups A was vaccinated with NDV-L and treated with 0.2 ml of the *Azadirachta indica* leaf extract contained 30 birds respectively while Groups C and D had 15 birds respectively. Groups A were given 0.2 ml of the *Azadirachta indica* leaf extract, birds in Group B were also vaccinated and given 0.2 ml of the *Piper guineense* extract. The birds in groups C were vaccinated with the NDV-L only (positive control) while those in Group D were given distilled water only.

SEROLOGY

Blood samples were taken via wing vein from each group at day 1, 7, 14 and 21 post vaccination. The sera were stored at -20 C until the end of the experiment. The assessment of NDV- specific antibody levels were made by haemagluttination- inhibition (HI) tests. Haemagluttination (HA) titre of the antigen was determined as described by Thayer and Beard (1998). The reciprocal of the highest dilution of the antigen causing 100% agglutination of an equal volume of standardized red blood cells (RBC) was taken as HA titre of the antigen. This concentration was used for the HI test. Haemagluttination-inhibition titres of the sera were expressed as the reciprocal of the last dilution showing no agglutination of the RBC as also described by Thayer and Beard (1998).

STATISTICAL ANALYSIS

Data was analysed by ANOVA. Significant differences between the groups were determined using the Duncan's New Multiple Range Test and tests were considered significant at a probability of $P<0.05$ using the Statistical Package for Social Sciences (SPSS)

RESULTS

ACUTE TOXICITY TEST

Results showed extracts to be safe at all doses. There was no clinical sign or death recorded.

SEROLOGY

The results are expressed in Table I. Results obtained showed a significant difference ($p<0.05$) in the mean antibody titre between the Groups A and C compared to the control group but there was no significant difference between the mean titres of Groups A and C. At day 1 post vaccination, only the cockerels in Group D show minute

Table 1: Effect of aqueous extracts of *Piper guineense* and *Azadirachta indica* leaves on the antibody response of birds against Newcastle disease virus.

GROUPS	DAYS			
	1	7	14	21
A	0.010 ± 0.036^{a}	0.043 ± 0.054^{b}	0.100 ± 0.027^{a}	0.125 ± 0.004^{b}
B	0.002 ± 0.018^{b}	0.007 ± 0.000^{b}	0.040 ± 0.006^{b}	0.060 ± 0.003^{c}
C	0.005 ± 0.001^{b}	0.016 ± 0.000^{b}	0.063 ± 0.000^{a}	0.125 ± 0.003^{a}
D	0.060 ± 0.000^{b}	0.060 ± 0.000^{b}	0.060 ± 0.000^{b}	0.061 ± 0.000^{b}

*a, b, c within row= antibody titre means without common superscript differ significantly ($P < 0.05$). *GROUP A= A*zadirachta indica* +Vaccine; GROUP B= *Piper guineense* +vaccine; GROUPC= Vaccine ONLY; GROUP D = Distilled water ONLY.

detectable antibodies. At day 7 post vaccination, immune response was seen in Groups A, B and C; as antibody titres were detected and continued to increase up until day 21. Antibody titre in Group D remained constant. The Group A showed significant ($p<0.05$) increase in antibody titre compared to the control but was not significantly ($p>0.05$) higher than the vaccinated group. Group A also elicited immune response faster (at day 7) than in other groups. Result showed that *A. indica* elicited better immune response than *P. guineense,* but not greater than the vaccine itself.

DISCUSSION

Following challenge of birds with the viral antigen, the significant increase in the antibody titre of Group A, vaccinated and treated with *Azadirachta indica* is in agreement with the reports of Okoroafor *et al.* (2015) who recorded increase in HI titres upon challenge with ND virus. It shows that *Azadirachta indica* has an immune boosting potential, which may likely be due to the presence of saponins which aid in inducing both humoral and cellular immunity (Oda *et al.*, 2003). The level of antibody titre produced in group A (vaccine and neem extract) birds was greater than that produced by those in group C (vaccine only) at days 7 and 14. This elucidates the ability of the extract to elicit rapid humoral immune response than the vaccine. This may be due to the effect of this leaf extract on B cells or other cytokine-producing cells that may lead to rapid activation of B cells. At day 21, there was no significant difference between the immune responses generated by *A.indica* and that elicited by the vaccine. Our findings are congruent with the work of Jawad (2013) who fed neem leaf powder to birds and reported a significant increase in antibody titre against both Newcastle disease and infectious bursal disease.

The group B (vaccinated and treated with *Piper guineense)* showed the lowest antibody titre compared to that of those in Groups A and C. This could mean that it has little or no immunomodulatory effects or could be as a result of the extraction method used in this study. This can be noticed in the work of Osho *et al.* (2016) who reported and increased antibody titre in experimentally infected broilers treated with methanolic extract of *P.guineense*. Other methods of extraction for *Piper guineense* should be studied. Although there has been a lot of work done on the antimicrobial effect of this plant, there is paucity of information on its immunomodulatory effect and there seems to be a lot of research to be carried out on *Piper guineensee*.

In general, the present study shows that *A.indica* stimulates the immune system of the birds and works in synergy with the vaccine to boost the immune system.

CONCLUSION

Azadirachta indica has shown immune boosting potential and should be given alongside vaccines to aid protection against Newcastle disease virus. Further studies on the action of this extract on immune cells should be carried out in order to have a complete understanding of its mechanism of action. This will lead to full exploitation of this herbal plant in immunology and medicine. Other methods of extraction of *Piper guineense* should be exploited in order to have a better understanding of its immune-stimulating properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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