Antiviral Effect of *Andrographis paniculata* Ethanolic Extract Against Newcastle Disease Virus

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**ABSTRACT**

*Andrographis paniculata* ethanolic extract (APE) was evaluated for its in vitro antiviral activity against Newcastle disease virus (NDV). The in vitro antiviral activity assay was performed by incubation of NDV (100 EID\textsubscript{50} / 0.1 ml) with three different concentration of APE (5 µg/ml, 10 µg/ml, and 15 µg/ml) for 1 hour at 37°C, prior to inoculation via intra-allantoic cavity into 9 day old embryonated specific-pathogen-free (SPF) chicken eggs. The antiviral activity was confirmed by the absence of hemagglutination (HA) activity in the infected allantoic fluid. The absence of HA activity was observed at APE concentration of 5 µg/ml, 10 µg/ml and 15 µg/ml. This activity was compared to the presence of HA activity in the virus replication control (phosphate buffer saline (PBS)-NDV) group. Furthermore, APE at a concentration of 15 µg/ml concentration was found to be non-toxic to the embryo as no death was recorded in the three APE-PBS control groups.

Keywords: Newcastle disease virus, anti-Newcastle disease activity, *Andrographis paniculata*

**INTRODUCTION**

The Newcastle disease (ND) which is caused by the Newcastle disease virus posed an economically significant threat because of the huge mortality and morbidity associated with the disease (Alexander 2011, Ganar et al, 2014). This highly pathogenic viral disease of avian species causes high mortality among infected animals, hemorrhagic intestinal lesions, severe respiratory distress, decrease egg production, and nervous disorders (Al-Garib et al, 2003). At present, there is no specific treatment for ND and the disease is widely controlled by vaccination using live or killed ND vaccines; but despite vaccination, sporadic outbreaks of ND do occur in vaccinated flock worldwide (Mori H., et. al, 1994). Therefore, there is a necessity to find an alternative approach for the control of ND, one of which is the use of plant products as an antiviral source. Among these plants is *Andrographis paniculata* (AP), a member of the family of Acanthaceae, which has been used as a traditional herbal medicine in many parts of Asia and Europe (Jarukamjorn and Nemoto, 2008). Extracts of this plant and its bioactive compound, andrographolide, has been shown to exhibit a wide range of pharmacological activities (Akbar 2011). Among these, the antiviral activities of this plant had been investigated against various viruses with positive results. Both the extracts and active isolate has been shown to have antiviral activities against the human immunodeficiency viruses, herpes simplex virus, Ebstein-barr virus, dengue virus and hepatitis C virus (Chang et al. 1991, Calabrese et al. 2000; Wiart et al. 2005, Seubsasana et. al., 2011; Lin et al. 2008; Tang et al. 2012 and Lee et al, 2014). However, no antiviral activity has been reported against virus infecting farm or wild animals. The purpose of this study is therefore to investigate the antiviral potential of the ethanol extracts of *A.paniculata* against the Newcastle disease virus by inhibition assay in embryonated SPF chicken eggs.
MATERIALS AND METHODS

Preparation of Plant Extract
AP plants (sourced from the Batu Gajah Herbal Garden, Perak, Malaysia) was extracted as described by Cowan (1999) and Puri et al. (1993). A stock solution was prepared by the resuspension of extract in warm food grade ethanol at a concentration of 50 μg/ml. Three different concentrations 5 μg/ml, 10 μg/ml and 15 μg/ml of APE working solutions were prepared for this study.

In Vitro Antiviral Activity assay
The in vitro antiviral activity of the APE against NDV was evaluated by virus inhibition assay in embryonated chicken eggs. Briefly, different concentrations (5 μg/ml, 10 μg/ml, and 15 μg/ml) of APE were incubated 1:1 with 100 EID₅₀/0.1 ml of NDV 1174/08 lentogenic strain (APE-NDV). As controls of virus replications, parallel experiments were conducted in which NDV (100 EID₅₀/0.1 ml) was mixed with an equivalent amount of PBS (PBS-NDV); and for APE toxicity, each of different working solution of APE was mixed with an equivalent of PBS (APE-PBS). All the mixtures were incubated for 1 hour at 37°C prior to inoculation. The mixtures were then inoculated into the allantoic cavity of each of five 9-day-old embryonated SPF chicken eggs, which were then incubated at 37°C. The eggs were examined twice daily for 4 days, and the antiviral activity was confirmed by Hemagglutination (HA) test using 0.8% chicken RBC as described in the OIE, 2008. The experiments were repeated five times.

RESULTS AND DISCUSSION
In this study, the antiviral activity studies were conducted against NDV 1174/08 lentogenic strain by virus inhibition assay in embryonated chicken eggs. The NDV inoculated in embryonated eggs could grow in cells lining the allantoic cavity and when the cells burst, the virus is then released in the allantoic fluid, reaching high titres in approximately 24 hours (Al-Garib et al, 2003). In this study, the in vitro inhibitory effect of APE on 100 EID₅₀/0.1 ml of NDV infectivity was confirmed by the absence of HA activity in the infected allantoic fluid. Our earlier study has shown that the APE was not toxic below 20.48 μg/ml concentration against chicken embryo fibroblast (CEF) and at concentration of > 25 μg/ml against Vero cell line (Suriani M.N., et al., 2013). Therefore, based on the maximal non-toxic dose or CC₅₀ of APE, three different concentrations 5 μg/ml, 10 μg/ml, and 15 μg/ml of APE working solutions were prepared for this study. The results of HA test showed the absence of HA activity in the infected allantoic fluid. The presence of high HA activity in the virus replication group (PBS-NDV). The presence of high HA activity in the PBS-NDV control group indicates that without APE, the 100 EID₅₀/0.1 ml of NDV was successfully replicated. The results suggested that pre-incubation of 100 EID₅₀/0.1 ml of NDV with a concentration as low as 5 μg/ml of APE effectively inhibited the infectivity of NDV. This concentration is far lower than the 25 μg/mL of APE reported by Lin et al., 2008, against Epstein-Barr virus. Furthermore, our study also showed that the APE at a concentration of up to 15 μg/ml display no toxic effect on the embryo as no death was recorded in the three APE-PBS control groups during the period of incubation. The consistency of the results were observed in five independent experiments. This preliminary study is the first that observed anti-Newcastle disease activity of APE.
CONCLUSION
This study finds that the ethanol extract of *A. paniculata* has antiviral activity against the Newcastle disease virus at a concentration of as low as 5µg/ml. Furthermore, our study also showed that the extract was able to exert its effect at a concentration of as high as 15µg/ml without displaying any toxic effect against the chicken embryo. Further studies to isolate the bioactive compound and its mode of action can be conducted to provide insight into its antiviral action.

REFERENCES


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