

ANTIVIRAL EFFECT OF AQUEOUS NEEM EXTRACT FROM BRANCHES OF NEEM TREE ON NEWCASTLE DISEASE VIRUS

ONG G.H.*, SYAMSIAH A.S., M.HASRUL A.H., ZUNAIDA B., MAIZATUL Z., M. JIHAN R., M. REDZWAN S., LEOW B.L., FAIZUL F.M.Y., CHANDRAWATHANI P. AND RAMLAN M.

Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh

* Corresponding author: ongghuai@yahoo.com

ABSTRACT. Antiviral effect of crude aqueous extracts of Neem leaves and Neem bark (*Azadirachta indica*) belonging to the family Meliaceae against velogenic Newcastle Disease virus was studied. Maximum non-toxic dose and determination of antiviral activity by *in vitro* and *in vivo* virus inhibition assay was carried out using embryonated SPF chicken eggs and SPF chickens. Different concentration content of the aqueous neem extract from branches of neem tree storing at 4°C reacted against velogenic ND virus was conducted. Determination of antiviral activity by *in vivo* assay in SPF chickens was compared to the group of untreated with Neem extract.

Keywords: *Azadirachta indica* (Neem), ND virus, embryonated SPF eggs, SPF chickens.

INTRODUCTION

Neem or Margosa tree or in scientific classification *Azadirachta indica*, is a famous researched medicinal plant belonging to the family Meliaceae. It had been used in Indian Ayurvedic medicine since ancient times and now being used

in modern medicinal, cosmetics and pharmaceuticals as global scenario is changing towards the use of nontoxic plant products. Its medicinal values comes from the fruits, seeds, leaves, roots and bark. This traditional ancient medicinal plant with a valuable natural product can be developed against various diseases (Biswas *et al.*, 2002). Many reports had proven of its biological active compounds were antiseptic, antiviral, antiinflammatory and antifungal. The two major compound of neem extract are Isoprenoids and Non-isoprenoids. Isoprenoids consists of diterpenoids and triterpenoids consisting of protomeliacins, limonoids, azadirone with its derivatives of gedunin, vilasinin, nimbin, salanin and azadiractin. Non-isoprenoids are proteins, carbohydrates, sulphurous compounds etc. (Koul *et al.*, 2006). Since neem plants had been in traditional medicinal use, it is important to emphasize on the usage of controlling various avian diseases. The aim of this study is to proof its antiviral activity towards avian viruses of Velogenic Newcastle disease strain. The study involved were determination of maximum non-toxic dose and determination of

antiviral activity of both the aqueous neem bark and neem leaves extract. Maximum non-toxic activity of aqueous neem extract from the leaves and bark were conducted using SPF embryonated chicken eggs at different concentration content. Antiviral activity via virus inhibition assay of neem aqueous extract from the leaves and bark of different concentration on velogenic Newcastle disease virus was conducted in SPF embryonated chicken eggs (*in vitro*) and in SPF chickens (*in vivo*).

MATERIALS AND METHODS

Preparation of aqueous extract mixture

Fresh healthy neem leaves and neem bark from branches of neem tree were collected, washed and dried in shade. They were grind, weighed and mixed with distilled water and store as concoction at 4°C (Parida *et al.*, 2002).

Maximum-non toxic dose

Different concentration of aqueous neem extract from the leaves and bark from branches of neem tree were used for the maximum-non toxic dose assay. The range of both neem extract used was from 0.1 mg/ml to 15 mg/ml. Different neem extract range from leaves and bark were inoculated into 10 days old embryonated SPF chicken eggs via intra allantoic route respectively. Eggs were incubated at 37°C and candled daily for toxicity assay to find

out the maximal dose which could be non-toxic to the chicken embryos (Parida *et al.*, 2002).

In vitro virus inhibition assay

Different range of non-toxic dose from neem bark and neem leaves extract that were obtained from Maximum-non toxic dose of toxicity assay were used in the *in vitro* antiviral assay. Ten fold dilution of local velogenic Newcastle disease virus (ND) were prepared in the range containing 10^1 EID₅₀ to 10^4 EID₅₀ in PBS. Different doses of neem bark and neem leaves extract were mixed with different concentration of virus in equal volume and incubated at 37°C for 1 hour. 100 µl of each respective mixture of neem bark extract and neem leaves extract with different virus concentration were inoculated into 10 days old SPF embryonated eggs via intra allantoic route. Different range of ND virus concentration, different range of neem bark extract and neem leaves extract dose were inoculated into embryonated eggs as control were performed concurrently. They were incubated at 37°C for 5 days and candled daily for viability to determine the *in vitro* antiviral activity (Parida *et al.*, 2002).

In vivo virus inhibition assay

Different concentration of neem bark extract of antiviral activity via *in vitro* assay was mixed with 100 EID₅₀ dose of local velogenic Newcastle disease virus in

equal volume and incubated at 37°C 1 hr 500 µl of mixture was inoculated orally per chicken. Virus without the mixture of neem bark extract were used as virus control and neem bark extract control without virus were inoculated orally at 500 µl per chicken respectively. They were then observed daily for 10 days for clinical sign of Newcastle disease. Pooled organs from dead chickens were pooled and reisolation for Newcastle disease virus were carried out in SPF embryonated eggs.

RESULTS

In this study of neem extract toxicity, both neem extract from the leaves and bark were found not toxic to the embryonated eggs with minimum concentration of 0.1 mg/ml to maximum concentration of 15 mg/ml. See Table 1.

Antiviral activity of neem bark extract via *in-vitro* virus inhibition assay as shown in Table 2 had antiviral activity at the concentration of 5.6 mg/ml where it can inhibit the growth of velogenic Newcastle disease virus at the maximum

Table 1: Determination of neem extract toxicity via Maximum non-toxic dose of neem bark extract (NB) and neem leaves extract (NL) in SPF embryonated eggs at final concentration in mg/ml. (A) indicates alive eggs after 5 days incubation.

Neem extract concentration mg/ml	0.1	0.25	0.43	0.5	0.7	1.0	1.5	2.5	4.2	5.6	10.0	11.3	15.0
Neem bark (NB)	A	A	A	A	A	A	A	A	A	A	A	A	A
Neem leaves (NL)	A	A	A	A	A	A	A	A	A	A	A	A	A

Table 2: Antiviral activity via *in-vitro* virus inhibition assay of neem bark extract (NB) in mg/ml against different Newcastle disease virus concentration in SPF embryonated eggs. (+) indicates no antiviral activity of neem bark extract by HA assay denoting presence of virus. (-) indicates presence of antiviral activity of neem bark extract by HA assay denoting absence of virus.

Neem bark concentration mg/ml	0.1	0.25	0.43	0.5	0.7	1.0	1.5	2.5	4.2	5.6	10.0	11.3	15.0
10 EID ₅₀	+	+	+	+	+	+	+	+	+	-	-	-	-
100 EID ₅₀	+	+	+	+	+	+	+	+	+	-	-	-	-
1000 EID ₅₀	+	+	+	+	+	+	+	+	+	-	-	-	-
10000 EID ₅₀	+	+	+	+	+	+	+	+	+	-	-	-	-

concentration of 10^4 EID₅₀ in embryonated eggs. See Table 3.

Neem leaves extract were found not to have antiviral activity where it did not inhibit the growth of velogenic Newcastle disease virus. See Table 4.

Determination of antiviral activity via *in vivo* virus inhibition assay, neem bark extract at 15 mg/ml was found to protect all the chickens from velogenic ND virus after 10 days post inoculation in this study.

DISCUSSION

In toxicity study, both the neem leaves and neem bark extract from branches

of neem tree were found not toxic to the embryonated eggs with different concentration ranging from 0.1 mg/ml till 15 mg/ml.

There is no antiviral activity of neem leaves extract as shown in table 3 via *in vitro* virus inhibition assay against velogenic Newcastle disease virus in this study. For neem bark extract the antiviral activity was at 5.6 mg/ml against the whole concentration range of velogenic Newcastle disease virus from 10EID₅₀ to 10000EID₅₀ used as shown in Table 2.

Antiviral activity via *in vivo* assay for neem bark extract was at 15.0 mg/ml where all chickens were alive 10 days post inoculation.

Table 3: Antiviral activity via *in-vitro* virus inhibition assay of neem leaves extract (NL) in mg/ml against different Newcastle disease virus concentration in SPF embryonated eggs. (+) indicates no antiviral activity of neem leaves extract by HA assay denoting the presence of virus.

Neem leaves concentration mg/ml	0.1	0.25	0.43	0.5	0.7	1.0	1.5	2.5	4.2	5.6	10.0	11.3	15.0
10 EID ₅₀	+	+	+	+	+	+	+	+	+	+	+	+	+
100 EID ₅₀	+	+	+	+	+	+	+	+	+	+	+	+	+
1000EID ₅₀	+	+	+	+	+	+	+	+	+	+	+	+	+
10000 EID ₅₀	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4: Antiviral activity via *in-vivo* virus inhibition assay of neem bark (NB) extract in mg/ml against different Newcastle disease virus concentration in 4 weeks old SPF chickens. (A) indicates a positive antiviral activity in alive chicken after 10 days post inoculation. (D) indicates dead chicken which has no antiviral activity.

Neem concentration mg/ml	4.2	5.6	10.0	15.0
100 EID ₅₀	2A & 3D	2A & 3D	3A & 2D	5A

CONCLUSION

In this study aqueous neem bark extract from branches of neem tree was found to be able to inhibit velogenic Newcastle disease virus as compared to neem leaves extract where it could not inhibit the virus. Clinical chickens condition of neem bark treated group was more healthy compared to untreated group. Therefore supplement of neem bark extract can be given to chickens as one of the neem extract constituents is antiviral where it will protect chickens against Newcastle disease in this study.

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