



## Efficacy Trials of Crude Extraction from *Artemisia Annul L.* against Newcastle Disease Virus in Vivo in Xinjiang

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### Abstract

In the present paper, bioactive compounds were extracted using the water-boiling method and their effects on the inhibition of newcastle disease virus (NDV) were investigated through the cultivating of chicken embryos and trials of haem agglutination. Results showed that extracts of sweet wormwood (*Artemisia annual L.*) had no side effects, and along with the inoculation of both drug and NDV inhibited the proliferation of NDV in chicken embryos.

**Keywords:** *Artemisia annul L.*, Chicken embryo, NDV, Inhibition

### 1. Introduction

ND is an important disease among poultry, which lead to immeasurable economic loss in breeding industry. As a result, lots of measures have been taken to prevent, control and eradicate ND. However, antibiotic and sulfonamides have no therapeutic effects on ND, and thus in order to investigate the effects of sweet wormwood on ND, inhibition trials of artificial infected ND by its extracts were observed in the present paper.

### 2. Materials and methods

#### 2.1 Materials

##### 2.1.1 Chicken embryos for trial

9 days old embryos were purchased from chicken embryo hatchery in Shihezi old street of Xinjiang.

##### 2.1.2 NDV field strains

ND wild virus was provided by the lab of infectious disease infecting both domestic animals and human of Shihezi University. The HA titers of allantoic fluid in chicken embryo was  $9\log_2$ , diluted into the concentration of  $1 \times 10^{-1}$  by sterilized normal saline before application.

##### 2.1.3 Drugs

Natural sweet wormwood was sampled in the suburb of Shihezi, flowers, leaves and fruits dried in the shade was cooked with water by the traditional decocting method, changed into slow fire after boiling, and replicated twice. Resultant extractions were filtered and then condensed into the solution of the concentration of 1g/mL by the evaporation method (Fu, 2005, P. 246).

##### 2.1.4 1% Chicken red blood cells

Blood was sampled from cocks, rinsed with normal saline for three times, and then diluted into 1%.

##### 2.1.5 Apparatus

Rotary evaporator(MCO-15AC, Japan); imported 96-well cell culture plates; oscillator (China);carbon dioxide incubator (China); super clean performance bench (SB-JS- , Shanghai medical treatment facility factory, Boxun Development Corporation); electric tachometer indicator thermostatic incubating oven(DPX-9272B-1, Shanghai Fuma experimental equipment Co. Ltd); Pulverizer; soxhlet extractor(China); casserole of decocting medicinal herbs; automatic autoclaving pot (SLP-32L, Japan); Low speed large capacity multi-tube centrifuge (LXJ-11B, Shenzhen, China ); electronic balance; oven.

## 2.2 Methods

### 2.2.1 IC<sub>50</sub> Measurement

Allantoic fluids were serially diluted into different concentrations, and then inoculated with chicken embryos. IC<sub>50</sub> was obtained by the traditional methods. The concentration of inoculated allantoic fluid used for official test was diluted into 1:500.

### 2.2.2 Acute toxicity test

Original drug was doubling diluted with sterilized normal saline, and then both stock and dilution solutions were inoculated into five 9-days old chicken embryo allantoic fluids, each 0.2ml, kept in the incubator for 72h at 37°C. Survival condition was observed each day. The most high drug concentration was used as the initial concentration in official test when the whole chicken embryos were alive, normal saline used as control as well.

### 2.2.3 Effects of sweet wormwood extracts on the proliferation of NDV

According to the dilution concentration of allantoic fluid and drug concentration listed in Table 2, in group 1 20 chicken embryos were inoculated with drug solution 0.2ml and NDV dilution solutions 0.1ml; in group 2 used as control, 20 chicken embryos were inoculated with normal saline 0.2ml and NDV dilution solutions 0.1ml; in group 3 20 chicken embryos were inoculated neither NDV nor drug.

Aforementioned chicken embryos were incubated in 37°C thermostat, candling inspection each day for 2~3 times, discarded the dead embryos in 24h, collected and froze the dead ones after 24h, collected survival embryos in 96h and allantoic fluid of dead embryos in 24~96h and stored at low temperature for further investigation, measured the hemagglutination titers respectively.

## 3. Results and discussions

### 3.1 Drug toxicity trails

Through three replicate trials, sweet wormwood with different concentration all had no obvious lethal effects on chicken embryos which was listed in Table 1 in detail. Therefore, the original concentration was used as initial concentration for official tests.

Prevention of NDV have been paid great attention all over the world, however, due to the continuous appearance of very virulent strains and variants and all kinds of vaccine failure factors, NDV are always easily epidemic. In the present paper, toxicity trails of sweet wormwood extracts was undertaken, and results showed that original extracts have on side effects on chicken embryos and could used as initial concentration.

### 3.2 Effects of sweet wormwood extracts on the proliferation of NDV

As seen from Table 3, in group 1 the dead and survival number of embryos were 2 and 1, respectively, which indicated that natural sweet wormwood could significantly inhibit the proliferation of NDV. It has been reported that other Chinese herbs could be used as agents against NDV but their effects were not ideal (Ben, 2007, PP. 33~34). In the present study, we proved that sweet wormwood could be used as an assistant curative against NDV.

## 4. Conclusions

ND is an infectious disease caused by NDV, due to the extensive application of vaccines, which was characterized in a chronic and sporadic manner. There are no efficacious therapeutics for NDV at present. There have been many successful experiences using Chinese herbs against NDV. In the present study, attempt was made to give theoretical basis for further curing NDV clinically. New bioactive compounds screened from plants have been an important way to obtain new drugs which attracted many researchers' attention (Tao, 1991, PP. 7~13). Effects of sweet wormwood on viruses have not been documented till present, less records of this plant used as drugs as well.

## References

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Table 1. Drug toxicity trails of extracts on chicken embryos

Extractions concentration	Inoculum size	Survival condition		
		24h	48h	72h
Original concentration	0.2ml	*5/5	5/5	5/5
1:1		5/5	5/5	5/5
1:2		5/5	5/5	5/5
1:4		5/5	5/5	5/5

Note: \*Denominator represented the inoculated number of embryos; numerator represented the number of survival embryos.

Table 2. Inhibition effects of sweet wormwood extracts

Group	Number of dead embryos in 24~72h	Number of survival embryos in 72h	HA titers
1	4**	15**	0**
2	17	0	10log2
3	0	19	0

\*\* p<0.01.