RESEARCH ARTICLE

EVALUATION OF ANTIVIRAL ACTIVITY AGAINST NEWCASTLE DISEASE VIRUS AND EMBRYOTOXICITY OF ESSENTIAL OIL AND ETHANOLIC EXTRACT OF *TRACHYSPERMUM AMMI*

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ABSTRACT:

Background: *Trachyspermum ammi* (Ajwain) is a medicinal plant known for its therapeutic properties. Given its bioactivity, it presents a promising candidate for treating viral diseases such as Newcastle disease, a highly contagious and economically significant viral illness of poultry caused by the Newcastle disease virus (NDV). **Objective:** The present study aimed to evaluate the antiviral activity and embryo toxicity of the essential oil and ethanolic extract of *T. ammi* against NDV using an in ovo model. **Methods:** Ten serial dilutions (100 to 0.19 µg/mL) of both *T. ammi* essential oil and ethanolic extract, and five concentrations (40 to 20 µg/mL) of the standard antiviral drug ribavirin were prepared. Each concentration was mixed with a fixed titer (4 HA units) of virulent NDV. The mixtures were inoculated into nine-day-old specific pathogen-free (SPF) chicken embryos, which were then incubated for 72 hours. Antiviral activity was assessed post-incubation using a hemagglutination test, while embryo toxicity was calculated based on the percentage of embryo survival. **Main Outcome Measures:** The primary outcomes were the minimum concentration of each substance that achieved complete inactivation of NDV (antiviral activity) and the concentration at which embryo mortality occurred (embryo toxicity). **Results:** The essential oil of *T. ammi* demonstrated complete inactivation of NDV across a wide concentration range, from 0.78 µg/mL to 100 µg/mL. The ethanolic extract of *T. ammi* showed complete antiviral activity from 1.5 µg/mL to 100 µg/mL. The Embryo toxicity for both the essential oil and the ethanolic extract was observed only at the highest tested concentration of 100 µg/mL. Ribavirin exhibited strong antiviral activity at all tested concentrations (20-40 µg/mL) but was found to be embryo-toxic at the higher concentrations of 40, 30, and 25 µg/mL. **Conclusion:** This experiment demonstrates that both the essential oil and ethanolic extract of *Trachyspermum ammi* possess significant antiviral potential against NDV, with a favorable safety profile showing embryo toxicity only at a high concentration. Their efficacy and lower toxicity compared to ribavirin suggest that *T. ammi* is a promising candidate for further investigation in the development of natural antiviral agents against Newcastle disease

Key words: Antiviral activity, Embryo toxicity, Newcastle Disease Virus, *Trachyspermum ammi* extract and oil.

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1. Introduction

From centuries medicinal plants have been used because of their efficacy to treat various ailments but in past their use was mainly limited to South East Asia and in African countries. In these days many developed nations are also emphasizing on the use of herbal products in their health care systems. Natural medicinal products have commercially become a part of nutritional supplement industry and also in holistic medicine in the North America [1]. Because of increased resistance to antimicrobials the interest is now to develop safe agents derived from plants against micro organisms. The herbal medicines show remarkable role in managing and controlling diseases [2]. *Trachyspermum ammi* also known as ajwain, is a common flavoring agent and also an important medicinal herb and is a part of the family apiaceae. Its habitat is India, Iran, Pakistan and Egypt[3].

Pharmacological properties of *T.ammi* has been investigated and it was found that it has hypolipidemic[4] ,liver defensive[5] ,anti-inflammatory[6], pain-relieving[7], kidney defensive[8], bronchoconstriction preventive[9], spasms reliever of smooth muscles[10], antioxidant[11], antibacterial[12],antifungal [13]and antiviral activities.

Newcastle disease is considered to be one of the most popular viral disease in poultry all over the world which has caused epidemics in poultry populations in third world countries. ND badly destroy the poultry industry and thus affect the economy of countries[14].Newcastle disease virus is a member of the genus Avulavirus of the family Paramyxoviridae in the order of Mononegavirals [15].NDV is an enveloped RNA virus having only one strand [16]. NDV disturbs the breeding and reproduction of birds. Vaccination is used as a tool to control the virus and to decrease the economical damage due to disease. Another problem with NDV is development of resistance due to mutations in viral strains and thus it has now become much difficult to control it . Therapeutically important herbs can be a source in controlling NDV particularly in third world countries. Different glycosides, flavors, spices contained in plants have a good activity against NDV[17]. The antiviral activity of essential oil of *T.ammi* was studied against Japenese encephalitis virus and it was found to be effective against this virus[18]. Present study was conducted to evaluate the antiviral activity of *T.ammi* essential oil and ethanolic extract.

2. Materials and methods

The seeds of *T.,ammi* were purchased from the local market in Lahore and identified from the Herbarium of the Department of Botany, Government College and University (GCU) Lahore. The reference number (Ref. No.GC.Herb.Bot.3449) was assigned to the plant.

The seeds of *T.ammi* were shade dried and grounded into coarse powder.

Extraction of oil was performed by hydrodistillation method. The *T.ammi* oil was purified by rotary evaporator at 70°C. The ethanolic extract of *T.ammi* was preperad by using Soxhlet apparatus.

The NDV was obtained from Microbiology department, UVAS Lahore. It was propagated and Haemeagglutination assay showed virus growth till 1:256 dilution which was taken as 1HA. From this 1HA value 4HA was calculated which was at 1:64. This 4HA NDV was used in the experiment.

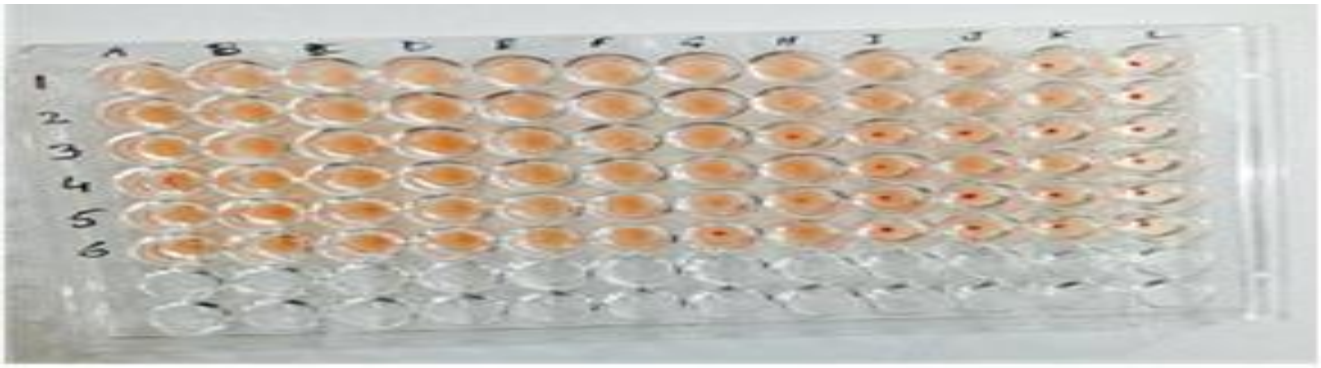

Fig.1: Haemeagglutination test (HA) in 96 well microtitre plate

Ten different concentrations of each of the essential oil and ethanolic extract of *T.ammi* were selected. The concentrations were 100, 50, 25, 12.5 , 6.25, 3.1, 1.5, 0.78,.0, 0.39 and 0.19 µg/mL . Ribavirin was used in five concentrations (40, 35, 30, 25 and 20 µg/mL). DMSO was used as a diluent for essential oil and ethanolic extract of *T.ammi*. For Ribavirin the diluent was normal saline.

Inoculum for antiviral activity was prepared by using 4HA NDV and 100µl of respective concentration of essential oil and ethanolic extract of *T.ammi*. Inoculum for embryo toxicity consisted of 100µl of DMSO instead of virus .The

respective inoculum was given a shot in each of the embryonated egg of the groups designed for the antiviral activity, embryo toxicity. Inoculated embryos were placed in incubator adjusted at 37°C, with relative humidity of 60-70% for 48 hours. Candling was done at interval of 12 hours to check the viability of embryos. After 72 hours of incubation dead and live embryos were separated by candling and chilled at 4°C for overnight. Amnio- allantoic fluid was harvested from each of the eggs and NDV was checked in the groups for the antiviral activity by using the Spot haemagglutination test and Haemagglutination test. The data was analyzed by using One way ANOVA and mean of log HA values were taken. The differences between mean values were called statistically significant if $P < 0.05$. The statistically analyses were carried out by using SPSS 19.0 software.

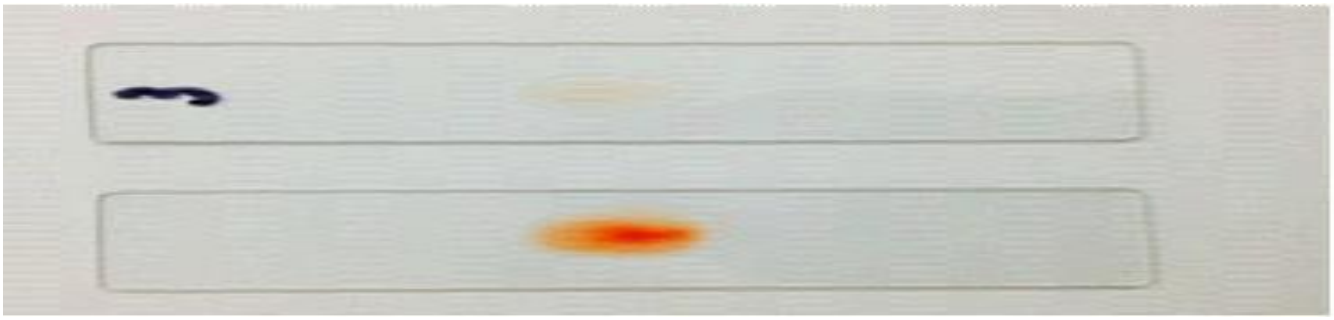

Fig.3: Showing the Spot haemeagglutination test results

Result

The results of antiviral activity of essential oil, ethanolic extract and ribavirin against NDV were compared. When antiviral activity of Ribavirin was studied then it was found that all concentrations of Ribavirin (40, 35, 30, 25 and 20 µg/mL) showed strong activity against NDV. Ribavirin was embryo toxic at 30, 35 and 40 µg/mL while at 25 and 20 µg/mL concentrations of ribavirin were not embryo toxicity A similar study using ribavirin and *Glycyrrhiza glabra* was conducted. In that study antiviral activity of *Glycyrrhiza glabra* was accessed by ovo method. The concentrations of *Glycyrrhiza glabra* were 30mg/100 mL, 60mg/100mL and 120mg/100mL. Ribavirin concentrations for this study were 10µg/mL, 20µg/mL and 40µg/mL. Spot haemagglutination test was performed to check the antiviral activity of *Glycyrrhiza glabra* and ribavirin and toxicity was evaluated by calculating the cell survival percentage. While studying essential oil it was found that at concentrations (100, 50, 25, 12.5, 6.25, 3.1, 1.5, 0.78 µg/mL) essential oil of *T.ammi*

showed maximum activity against NDV. At 0.39 µg/mL of essential oil agglutination was till 1:32 and 1:64 well and at 0.19 µg/mL viral growth was till 1:64 and 1:256 indicating no antiviral activity. The antiviral activity of essential oil of T.ammi has also been evaluated in many studies. Essential oil of T.ammi was also evaluated against Japenese encephalitis virus and findings showed good antiviral activity[19]

Essential oil of *T.ammi*	**Conc./ Group (µg/mL)**	**100 µg/mL**	**50 µg/mL**	**25 µg/mL**	**12.5 µg/mL**	**6.25 µg/mL**	**3.1 µg/mL**	**1.5 µg/mL**	**0.78 µg/mL**	**0.39 µg/mL**	**0.19 µg/mL**
	Mean Log HA ± S.D	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	1.33±2.3[b]	5.67±0.58[c]	7.33±1.15[d]
Ethanolic extract of *T.ammi*	**Conc./group (µg/mL)**	**100 µg/mL**	**50 µg/mL**	**25 µg/mL**	**12.5 µg/mL**	**6.25 µg/mL**	**3.1 µg/mL**	**1.5 µg/mL**	**0.78 µg/mL**	**0.39 µg/mL**	**0.19 µg/mL**
	Mean Log HA ± S.D	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	4.0±0.0[b]	5.66±0.57[c]	7.33±1.15[d]

Table.1 : Antiviral activity of essential oil and ethanolic extract of *T.ammi*

The antiviral activity of ethanolic extract of *T.ammi* against NDV indicated that it had strong antiviral activity at concentrations 100, 50, 25, 12.5, 6.25, 3.1, 1.5 µg/mL. At 0.78 µg/mL agglutination was till 1:16 dilution, at 0.39 µg/mL agglutination was till 1:32 well and at 0.19 µg/mL virus growth was observed till 1:64 well showing moderate to weak antiviral activity against NDV.The results are shown in table 1. These results are in accordance with the the study in which antiviral activity of ivermectin was evaluated against NDV on 9-day old chicken embryos. Different six concentrations 200, 100, 50, 25, 12.5 and 6.25 µg/mL of the drug were assessed. The concentration of ivermectin 200, 100 and 50 µg/mL were having strong antiviral activity[20].

Discussion

Antiviral potential and embryo toxicity of essential oil and ethanolic extract of *T.ammi* was determined using Ribavirin as a standarad. The Percentage yield of essential oil of *T.ammi* by hydrodistillation method was 3%. The % age yield of ethanolic extract of *T.ammi* by using Soxhlet appratus was 19%.

Higher concentration of both essential oil and ethanolic extract which was 100 µg/mL showed embryo toxicity. While all the remaining concentrations were non toxic for the embryos. In results it was found that 60 mg/100 mL of *Glycyrrhiza glabra* aqueous extract showed no embryo toxicity with anti viral activity. In case of Ribavirin the concentration 20 µg/mL was non toxic and had antiviral activity[21].The essential oil of *T.ammi* was accessed for its antiviral activity against Hepatitis C virus protease and results showed the efficacy of essential oil of *T.ammi* against hepatitis C virus protease[22]. These results indicated that essential oil of *T.ammi* has more significant antiviral activity against NDV as compared to ethanolic extract of *T.ammi*. While at concentration of 25µg/mL of essential oil, ethanolic extract and ribavirin there was strong antiviral activity against NDV with no embryo toxicity.

Conclusion

It can be concluded from the experiment that essential oil and ethanolic extract of *T.ammi* has maximum strength of antiviral activity against NDV and show embryo toxicity only at higher concentration as demonstrated by spot haemagglutination test (HA) and embryo survival persontage respectively. It is recommended that active biomolecule

of *T.ammi* should be obtained and in vivo trial should be performed to find out the efficacy of *T.ammi* essential oil and ethanolic extract against NDV in future.

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Conflict of interest

The author declares no conflict of interest.

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