Qatar Univ. Sci. J. (1994), 14 (C) : 18-20

STUDIES ON SOME ASPECTS OF ANTIVIRAL ACTIVITY. 1-INFLUENCE OF PROPOLIS ON NEWCASTLE DISEASE VIRUS

By

AHMED G. HEGAZI*, F. EL BERDINY**, S. EL ASSILY**, E. KHASHABAH**, N. HASSAN** AND S. POPOV***

* Department of Parasitology and Animal Diseases, National Research Centre,
**Veterinary Serum and Vaccine Research Institute, Egypt and
***Institute of Organic Chemistry, Bulgarian Academy of Sciences.

دراسات على بعض مظاهر نشاط مضادات الفيروسات

١ - تأثير البروبوليس على فيروس مرض النيوكاسيل

أحمد حجازي - ف. البيردينى - س . العسيلي - إى . خشبة - ن. حسن
س . بايوف

تم دراسة تأثير البروبوليس على السلالات اللقاحية المختلفة لفيروس مرض نيوكاسيل وفيروس ڤيرولينت . وقد إتضح أن اضافة البروبوليس إلى فيروس مرض نيوكاسيل أدى إلى تناقص ملحوظ في متوسط المعيار الحجمي لإمكانية حدوث العدوى . ولقد كان التأثير واضحاً جداً في لقاحات لاسوتا وكولون - ٣٠ وكذلك في فيروس فيرولينت . وأتضح أن هناك تناقصاً ملحوظاً أيضاً في المعيار الحجمي لتكوين هيمواجلوتينين في حالة كل من فيروسات كوماروڤ وفيرولينت . وتقدم هذه النتائج بعض المعلومات الجديدة عن نشاط البروبوليس كمادة مضادة لفيروس مرض نيوكاسيل وتشير أيضاً إلى أهمية هذا المنتج الطبيعي وإمكانية تطبيقة كعقار مضاد لفيروس مرض نيوكاسيل .

Key Words: Antiviral, Propolis, Virus

ABSTRACT

The effect of popolis on different-Newcastle disease virus (NDV) vaccinal strains as well as virulent virus were studied. It was clear that the addition of propolis to NDV induced a significant reduction of infectivity mean titres. The effect of propolis was pronounced in Lasota, Colon-30 vaccines and virulent virus. The haemagglutination titers of Komarov (K) and virulent virus were reduced significantly. These data obtained offer a certain amount of new information on anti-Newcastle disease virus activity of propolis and point to be the significance of such natural product for practice as a possible anti Newcastle disease drug.

INTRODUCTION

Propolis is a resinous hive product collected by bees. The versatile biological activities of propolis were studied and its activities as antibacterial [3,9 and 1] antismall pox [5], antiinfluenza virus[6], fungicidal[7and 12] and antiprotozoan activity[10] had been reported.

The data in the literature on the experimental study of propolis related to its antiviral are still few in number. Thus the aim of the present communication was conducted to study the effect of propolis on different vaccinal strains of Newcastle disease virus (NDV).

MATERIAL AND METHODS

I-Material:

1. Propolis: was kindly supplied by Prof. Dr. S. Popov from Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Science in a dry form. The propolis was extracted according to the method described by Bankova et al[3].

2-Fertile chicken eggs: 9-10 days old embryonated chicken eggs were obtained from General Poultry Company, Egypt.

3-Newcastle disease virus (NDV): Five batchs of Hitchner B_1 vaccine (locally prepared in Veterinary Serum and Vaccine Research Institute) their infectivity titer (EID_{50}) were between $10^{8.65}$ to $10^{10.33}$/ml; 5 batchs of Lasota vaccine (Commercian vaccine from Intervet International B. V. Boxmeer, Holland) their EID_{50} were between $10^{8.3}$ and 10^{10}/ml; 3 batchs of Colon-30 (Nabilis) vaccine (Intervet International) their EID_{50} were between 10^{8} to $10^{9.85}$/ml and 4 local field isolates of velogenic viscerotropic NDV (VV-NDV) their EID_{50} were $10^{7.12}$ to $10^{7.74}$/ml, were used.

4-Chicken red blood cells: Red blood cells were collected on heparine in dose of 20 IU/ml. The red blood cell suspension was used for haemagglutination (HA) test.

II-Methods:

1-Virus propagation: The virus propagation in embryonated chicken eggs was done according to Allan[1].

2-Virus infectivity titration: Different virus strains were titrated in 9-10 day old embryonated chicken eggs. Serial ten fold dilution 10-1 to 10-10 of the virus were prepared in sterile saline to which 50 IU polymxin, 500 IU penicillin and 250 mg streptomycin were added. 0.2 ml of each dilution from 10^{-5} up to 10^{-10} was inoculated in the chorioallantoic cavity (CAC) using 5 eggs/dilution for calculation of the EID_{50} according to the method adopted by Reed and Muench[8].

3-Haemagglutination (HA) test: The HA was done on allontoic fluid as the method described by Anon [2].

EXPERIMENTAL DESIGN

Propolis (300 mg/ml) was ten fold diluted in phosphate buffer saline (PBS) pH 7.2. Each dilution was injected into chorioallantoic cavity (CAC) in a dose of 0.2 ml/egg to evaluate its lethal dose fifty as well as the effect of propolis on embryonated chicken eggs. The dilution gave the minimum lethality was undertaken to study the effect of propolis on different ND viral strains. The haemagglutination test was done on the fluid of inoculated embryonated chicken eggs by rapid slide haemagglutination test with 10% chicken red cell suspension[2]. The egg fluid of embryonated chicken eggs inocualted with VV-NDV was used for detection of haemagglutinating properties of NDV by rapid slide HA test and β procedure using 1% red cells suspensions to determined its HA titer.

RESULTS

Determination of the minimum lethal dose of the propolis in embryonated chicken egg revealed that the dilution of 1/100 of original (300 mg/ml) propolis gave less mortality, no pathological changes in chicken embryonated eggs as well as no change in the egg fluid.

The effect of propolis on the infectivity titers of different NDV vaccinal strain as well as VV-NDV is illustrated in Tables 1 & 2. From Table (1), the effect of propolis on lentogenic virus was clear. Diluted propolis (1/100) of the original dilution (300 mg/ml) revealed reduction in the infectivity mean titers ($10^{8.4}$) of B_1 strain if compared with the control ($10^{9.96}$); Lasota strain was also reduced from $10^{9.37}$ to $10^{6.94}$. The Colon-30 also decreased in infectivity titer from $10^{9.2}$ to $10^{6.58}$. On the other hand, F strain infectivity titer was decreased to $10^{7.74}$ if compared with the control ($10^{9.73}$). It was clear that the effect of propolis was more pronounced in case of Lasota and Colon-30.

Table 1

Effect of propolis on lentogenic strains of NDV.

	HB1		Lasota		Colon 30		F-strain	
No. of batchs	Without propolis	With propolis	Without propolis	With propolis	Without propolis	With propolis	Without propolis	With propolis
1	9.90*	7.70	8.30	5.90	9.30	6.25	10.00	8.20
2	9.95	7.84	9.60	8.00	9.50	6.00	9.75	7.60
3	10.10	9.00	9.50	7.90	8.80	7.50	9.80	8.10
4	9.85	8.50	9.30	7.75			9.80	6.70
5	10.30	8.95	9.75	8.50			9.60	8.40
6			8.70	5.00			9.10	8.00
7			10.00	8.33			9.30	6.85
8							10.50	7.80
Mean	9.96	8.39	9.37	6.94	9.20	6.58	9.73	7.74

* = Log 10

Table 2

Effect of propolis on mesogenic and velogenic strains of NDV

	Mesogenic K strain		Velogenic VV-NDV	
No. of Batchs	Without Propolis	With propolis	Without Propolis	With Propolis
1	8.21	7.12	7.32	4.21
2	8.21	7.33	7.12	4.00
3	8.49*	8.00	7.74	5.10
4	8.25	6.70	7.13	4.39
5	9.85	6.90		
6	9.00	6.33		
7	8.75	6.25		
8	8.00	6.00		
9	8.50	6.00		
10	8.60	6.80		
Mean	8.58	6.74	7.33	4.42
HA titer	1/1024	1/265	1/1024	1/128

* = Log 10

The influence of propolis on mesogenic and VV-NDV is demonstrated in Table 2. and Fig. 1.

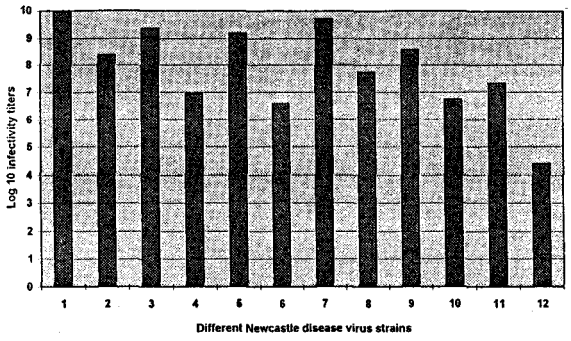

Fig. 1. Effect of propolis on different Newcastle disease virus strains.

1 = HB1 only 2 = HB1 + propolis
3 = Lasota only 4 = Lasota + propolis
5 = Clone 30 only 6 = Clone 30 + propolis
7 = F strain only 8 = F strain + propolis
9 = K strain only 10 = K strain + propolis
11 = Velogenic strain only
12 = Velogenic strain + propolis

It was clear that the infectivity mean titers of K strain and VV-NDV were $10^{8.58}$ and $10^{7.33}$ respectively. While the infectivity mean titers of K and VV-NDV with propolis were markedly reduced to $10^{6.74}$ and $10^{4.42}$ respectively. HA titers of K and VV-NDV were 1/1024 while the uses of propolis induced significant reduction in HA titers reached to 1/256 and 1/128, respectively.

DISCUSSION

The effect of propolis on CAC was studied by injection of 0.2 ml. The less effect of propolis was observed as no changes in the nature and colour of egg fluid. There was no detectable effect on embryoes. These results may be due to the nature of the propolis being dissolved in aqueous solution and did not induce a harmful effect on the embryoes.

The influence of the propolis on reproduction of different viral strains of NDV is measured by the most pronounced inhibitory effect on the log reduction of infectivity titer of NDV. The different strains showed reduction in their infectivity titers reached one log in B_1; 2 logs in F & K strain; 3 logs in Lasota, Colon and VV-NDV. These results were observed by Manolova et al.[6] who found inhibitory effect of influenza virus by different fractions of propolis. Krivoruchko et al[5] found a sharp reduction of infectiveness of small pox vaccine virus within 15 minutes at 20 °C on using an aqueous extract of propolis in vitro. They observed that the concentration of the virus was reduced by 10^{-5}, 10^{-4} and its infectivity was 21-29 times less than that of the control.

Haemagglutinating properties of K and VV-NDV were reduced from 1/1024 to 1/256 and 1/128, respectively. These results may be due to subsequent reduction of infectivity mean titers of NDV. Manolova et al.[6] found a considerable reduction of influenza haemagglutinating activity on using 3 mg/ml propolis.

The data obtained offer a certain amount of new information on anti-NDV activity of propolis. These results contributed towards the decoding of the active principle of the propolis effect and emphasized the significance of such natural product for practice as a possible anti Newcastle disease drug.

REFERENCES

[1] **Allan, W. A., 1974**. Vaccination against NDV with an inactivated oil emulsion vaccine at day old followed by aersol application of Lasota vaccine at 3 weeks. Vet. Rec. 94(3): 54.

[2] **Anon, 1971**. Methods for examining poultry biologics and for identifying avian pathogen Acad. Sci. Washington D.C. 270-294.

[3] **Bankova, V., A. L. Dyulgerov, S. Popov** and **N. Marekov, 1987**. A GC/Ms study of the propolis phenolic constituents. Z. Naturforsch 42: C: 147-151.

[4] **Korsun, V. P., 1983**. The uses of propolis in treating trophic ulcer. Vestnik Dermatoloii i venerologii, ii, 46-48.

[5] **Krivoruchko, V. F., V. I. Degtyarenko, T. I. Zaitseva** and **M. G. Ganchenko, 1975**. Antiviral effect of propolis. Virusy i Virusnye Zabolevaniya 3: 61-64.

[6] **Monolova, N. H., A. Maximova, G. A. Gegova, Y. P. Serkedjieva, S. T. Uzunov, N. Y. Marekov** and **V. S. Bankova, 1985**. On the influenza action on fraction from propolis. Comtes redus de L, Academie Bulgare des Sciences Tome 38(6): 725-738.

[7] **Millet, C. J., D. Michel, J. Simeray** and **J. Chaumont, 1987**. Preliminary study of the antifungal properties of propolis compared with some commercial products. Plante Medicinals et phytotherapie 21(1): 3-7.

[8] **Reed, L.V.** and **H. Muench, 1938**. A simple method of estimating of fifty percent end point. Am. J. Hyg. 27: 493-494.

[9] **Shub, T. A., K. A. Kagramonova, G. Y. Tikhonov** and **V. I. Gritsenko, 1978**. Antimicrobial activity of propolis extracts. Pharmaceutical Chemistry J. 11(9): 1242-1244.

[10] **Starzyk, J., S. Scheller, J. Szaflarski, M. Moskwa** and **A. Stojko, 1977**. Biologic properties and clinical application of propolis. II-studies on the antiprotozoan activity of ethanol extract of propolis. Arzneimittel Forschung 27(6): 1198-1199.

[11] **Tothne, P. V., 1987**. Propolis and its medicinal properties. A propolisziol es gyogyhatasairol Egeszsegugyi Munka 34(11): 325-329.

[12] **Woznisk, K. D.** and **W. Braun, 1972**. First results of treatment with propolis solution against fungal infection and eczema. Medicamentum 13(4): 114-117.