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INHIBITORY EFFECT OF ALOE VERA ON TWO VACCINAL STRAINS OF NDV IN EGGS

(With 3 Tables)

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التأثير التثبيطى لنبات الصبار على عترتين لفيروس النيوكاسل فى البيض

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من المعروف أن المواد الطبيعية مفضلة على العقاقير العلاجية المختلفة كمثبط فيروسي، ولذلك كان الغرض من هذه الدراسة هو البحث في مجال خصائص نبات الصبار والتي يمكن ان يكون لها بعض الأنشطة المثبطة للفيروسات. تم تحديد الجرعة الآمنة من نبات الصبار عندما حقن تخفيفات مختلفة منه في بيض الدجاج الملقح عمر ٩-١١ يوم ثم خلطت مع تركيزات مختلفة لعترتين مختلفتين لفيروس مرض النيوكاسل المستخدمة في تحضير لقاحات المرض وذلك لتحديد تأثيرها الضدي كمثبط فيروسي عن طريق الحقن في البيض. تم اجراء تجربة اخرى استخدم فيها تخفيفات متتالية لفيما وراء الجرعات الآمنة من الصبار والتي تم خلطها بتخفيف ثابت للعترات المختلفة لفيروس مرض النيوكاسل وذلك لتحديد أعلى تخفيف لكل مستخلص له نشاط مثبط للفيروسات.

SUMMARY

Natural products are preferable over synthetic therapeutic drugs as source for antiviral agents (reduce the infectivity titer). The use of Aloe vera is being promoted for a large variety of conditions. In the present investigation, Aloe vera was tested for monitoring its antiviral and/or the inhibitory effect on two vaccinal strains of ND Aloe vera in ECE. The safety dose of Aloe vera was determined and then mixed with different dilutions of the vaccinal strains of NDV to determine the effect of Aloe vera on NDv in ovo. Moreover, another experiment was carried out by using serial dilutions downward the safety dose of Aloe vera and mixing with a constant dilution of of the two vaccinal strains of NDV.

Key words: *Aloe vera, vaccine, Newcastle disease, virology*

INTRODUCTION

Plant extracts were used as competitive inhibition of some enzymes (Osawa *et al.*, 1991; Lopez *et al.*, 2001; and Yagi *et al.*, 2003); also some of plant extracts has antiviral activity against influenza virus (Nagai *et al.*, 1990), herpes virus (Naessens and De Clercq, 2001; Malvy *et al.*, 2005 and Zandi *et al.*, 2007), adeno virus, vesicular stomatitis virus, rota virus and poliovirus (Hussan *et al.*, 1991 and Semple *et al.*, 2001).Aloe species have been valued since prehistoric times as medicine for the treatment of burns, wound infections and other skin problems (Subramanian *et al.*, 2005). The latest reviews of Alo species clearly indicates their antimicrobial, antifungal, anticancer, anti-inflamatory, antiviral and immunomodulatory properties (Vanden-Berghe *et al.*, 1986; Bisset 1994; Reynolds and Dweks, 1999; Djeraba and Quere 2000; Lopez *et al.*, 2001 and Pugh *et al.*, 2001).Aloe vera is one of herbal plant species that has been and continues to be extensively studied. The plant has many consistently identifiable active compounds as anthraquinone and its derivatives which are glycosides (Gjerstad 1971; Budavari 1989 and Semple *et al.*, 2001). Anthraquinone derivatives include anthracenes such as aloe-emodin, which is 1,8-dihydroxy-3 (hydroxyl-methyl)-9, 10-anthracenedione. These water-soluble glycosides were separated from the water-insoluble resinous material (Grindlay 1986). Sydiskis *et al.* (1991), reported that the anthraquinon and its derivatives as alo-emodine-9-anthron (which were identified as being active component of Aloe vera) directly affected both DNA and RNA-containing enveloped viruses but had effect on naked (unenveloped) viruses

Newcastle disease is one of major disease problems that is continually plaguing the poultry industry in Egypt. Newcastle disease virus is protype of a large and important group of paramyxoviruses of man and animals. However, the use of Aloe vera species on poultry diseases has so far not been evaluated. Thus the aim of the present work was to through more light on the inhibitory effect of Aloe vera against two vaccinal strains of NDV in embryonated chicken eggs.

MATERIALS and METHODS

1- Aloe vera: it was kindly supplied by Department of Pharmacology, Faculty of Veterinary Medicine, Beni-Suef University in dried form. Twenty mgs from Aloe vera were dissolved in 20ml of normal physiological saline in sterile labeled caped bottle and then was tested for sterility.

2- Fertile chicken eggs: 9-10 days old specific pathogen free embreyonated chicken eggs were obtained fron Kom Oshim Project. They were used for virus titration and determination of safety dose for Aloe var used in this study.

3- Newcastle disease virus:

a- LaSota strain vaccine: Commercial LaSota vaccine was obtained from Intervet International B.V. Boxmer, Holland. The EID_{50} was $10^{8.30}$/ml.

b- Clone 30 vaccine: Commercial Clone 30 vaccine (TAD, Lot No. 9153.G35) was used in this study. The EID50 was $10^{8.80}$/ml.

4- Chicken red blood cells: They were collected on heparin in a dose of 20IU/ml. The red blood cell suspension was used for haemagglutination test.

5- Virus titration: The vaccinal strains of Newcastle disease virus (LaSota and Clone 30) used in this study were titrated in 9-10 days old specific pathogen free embreyonated chicken eggs.

6- Haemagglutination test: The HA test was done using allantoic fluid as the method described by Anon, 1971.

7- Infectivity titration of NDV vaccinal strains in ECE. Estimation of 50% end point was carried out by the method of Reed & Muench, (1938).

8- Determination of the safe dose of Aloe vera for ECE: serial ten fold dilutions from 10^{-1} to 10^{-6} of Aloe vera in steril normal physiological saline were prepared. Each dilution was inoculated into five ECE; 0.2 ml for each into the allantoic chamber. The inoculated eggs were incubated at 37°C; candled daily for five days, the embryos dying within the first 24 hours post-inoculation were discarded as non-specific mortalities, while the next dead embryos were removed and recorded. The lowest dilution of the Aloe vera at which all the inoculated ECE remained alive; was determined and considered as the safe dose.

9- Study the effect of safety dose of Aloe vera together with different virus dilutions on the infectivity titers of NDV vaccinal strains: One milliliter of the safe dose (10^{-4}) from Aloe vera was added to equal amounts of different dilutions of used viral vaccines from 10^{-5} to 10^{-10}. These mixtures were left for about one hour at room temperature before inoculation. Sterility test on the nutrient agar was carried out before inoculation of eggs. Each dilution was inoculated into five ECE; 0.2 ml for each into the allantoic chamber. Then the EID_{50} of each vaccinal strains together with Aloe vera were calculated according to Reed and Munch. The previously determined titer of each vaccinal strain used was

compared with that of the same vaccinal strain mixed with Aloe vera to detect the effect in the virus titers.

10- Determination of the minimum inhibitory concentration (MIC) of Aloe vera: A constant dilution from different NDV vaccinal strains which was suggested to be 10^{-6} were mixed with equal volumes of different subsafe dosages of Aloe vera. These mixtures were left for an hour at room temperature, after which they were inoculated into 9-11 day old ECE; incubated at 37°C for five days. The eggs were tested for HA and the percentages of positive eggs were recorded.

RESULTS

Table 1: Effect of Aloe vera on macroscopic picture of ECE

Different dilution of Aloe ver	Mortality rate of embryos		Macroscopic pictures of embryos in ECE					
	Number	Percentage	Control	Size	Congestion	CAM	Spleen	liver
10^{-1}	5/5	100%	Normal	Small	+++	Thick and macerated	Congested and atrophy	Necrosis and enlarged
10^{-2}	4/5	80%	Normal	Small	+++	thick and opacity	Congested	Necrosis and enlarged
10^{-3}	2/5	40%	Normal	Small	++	Thick	Congested	Congested and billed
10^{-4}	0/5	0	Normal	Normal	+	Thick	Normal	Congested and billed
10^{-5}	0/5	0	Normal	Normal	+	Thick	Normal	Normal
10^{-6}	0/5	0	Normal	Normal	-		Normal	Normal

Table 2: Effect of Aloe vera on two vaccinal strains of NDV in ECE

NDV vaccinal strains	Infectivity titer (EID_{50}/ml) log^{10}		HA titers/ log^2	
	Virus control	Virus with Aloe vera	Virus control	Virus with Aloe vera
LaSota	8.30	6.25	7	4
Clone 30	8.80	7.75	8	6

Table 3: MIC of Aloe vera

Subsafe dilutions	Positive HA/Negative HA	
	LaSota	Clone 30
10^{-5}	2/3	4/1
10^{-6}	3/2	5/0
10^{-7}	5/0	4/0
10^{-8}	5/0	5/0
10^{-9}	4/0	5/0

DISCUSSION

Viral diseases in general are difficult in their treatment owing to their intracellular tropism of the infected cells. The term antiviral agent means a substance other than virus or specific antibody; which can produce either protective or therapeutic effect on viral infected host. Generally, there is no useful therapy for viral infection in any way similar to antibiotic therapy for bacterial diseases. The current available antiviral compounds of synthetic, chemicals and antibiotics have major drawbacks such as narrow spectrum of activity, limited therapeutic usefulness and variable degrees of toxicity. Natural products are preferable over synthetics as a source for antiviral agents, that could overcome some or all of disadvantages of other substances. So, many traditional medicinal plants have been reported to have strong antiviral acivity and some of them have already been used to treat animals and human who suffer from viral diseases (Hudson 1990; Venkateswarane *et al.*, 1987; Thyagarajan *et al.*, 1988 and 1990; Vogler and Ernst, 1999 and Tan and Vanitha, 2004). Recently, a broad screening program has been initiated by many authors, especially for antiviral and other pharmacologically active substances in higher plants. Such substances were selected on the basis of literature data and medicinal native flora reports. In veterinary medicine, a prophylactic antiviral agent that is inexpensive, well tolerated, can be incorporated into animal feed would be a desirable drug.

In this work, the toxic effect of Aloe vera was studied. Determination of the minimum lethal dose of Aloe vera on ECE (Table 1) revealed that the dilution of of (10^{-4}) of the original Aloe vera gave no mortality, less or no pathological changes in chicken embryos as well as no changes in the egg fluid. These results may be due to the nature of Aloe vera which dissolved in aqueous solution that not induce harmful effect on chicken embryos (Grindlay 1986 and Shelton, 1991 and Saccu *et al.*, 2001).

The influence of Aloe vera on reproduction of ND (LaSota and Clone 30) viruses as measured by the infectivity mean titres were illustrated in Table (2). It was noticed that the safe dose of Aloe vera revealed reduction in the infectivity titers of LaSota strain ($10^{-6.25}$) if compared with the control ($10^{-8.30}$-virus without Aloe vera). The Clone 30 also decreased in its infectivity titers from $10^{-8.80}$ to $10^{-7.75}$. These results were in agreement with of Rashan *et al.*, 1989 who found that the aqueous extract of some medicinal plants has antiviral activity to herpes

virus and two types of influenza virus. Also Waihenya *et al.* (2002), found that the Aloe species could be a potential candidate on the management of Newcastle disease in chickens. Most of the reported antiviral and antitumor effects of Aloe vera likely are due indirectly to the stimulation of the immune system (Pugh *et al.*, 2001; Lee *et al.*, 2001 and Im SA *et al.*, 2005). However, one study reports that anthraquinones, which are present in aloe latex, have direct virucidal effects. The anthraquinone aloin was shown to inactivate various enveloped viruses, such as herpes simplex, varicella-zoster, and influenza (Zandi *et al.*, 2007). Some of plant extracts has antiviral activity to influenza virus (Nagai *et al.*, 1990). Although anthraquinones only appear in AG as a contaminant, low concentrations present in some preparations could have significant antiviral activity (Sydiskis *et al.*, 1991). Also, kemp *et al*, 1990 showed that the glycoside acemannan inhibited the replication and pathogenesis of HIV-1, HSV, and Newcastle disease virus.

The haemagglutination property of ND viruses were reduced when mixed with Aloe vera if compared with the original HA titer (Table 2). The reduction varied according to the type of the vaccine that used. The HA titers of LaSota and Clone 30 were reduced from 7 to 4 and from 8 to 6 respectively. These may be a subsequence of a reduction in the infectivity titers of NDv.

The inhibitory effect of Aloe vera was shown to be dose dependent. Different subsafe dosages of Aloe vera were mixed with equal volumes of constant dilution from different NDV vaccinal strains. The minimum inhibitory concentration (MIC) of Aloe vera was illustrated in Table 3. The results obtained in Table 3 showed higher efficacy on the inhibiting the haemagglutination capacity of LaSota strain than Clone 30. Aloe vera abolished the haemagglutinating capacity of LaSota and Clone 30 at dilution of 10^{-6} and 10^{-5} respectively.

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