DETERMINATION OF ANTIVIRAL EFFECT OF NERIUM OLEANDER DISTILLATE ON SOME VIRUSES

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INTRODUCTION

Bovine Herpesvirus-1 (BHV-1) is a major viral agent in cattle. BHV-1 belongs to Alphaherpesvirinae subfamily and Herpesviridae family. Virus can be transmitted from latent infected animals after different factors (stress or corticosteroid applications). Different clinical symptoms in upper respiratory [(Infectious Bovine Rhinotracheitis, IBR) and genital system (Infectious Pustuler Vulvovaginitis, IPV; Infectious Balano Postitis, IBP)] infections can be reported in BHV-1 infections [1].

Herpes Simplex Virus-1 (HSV-1) is a DNA virus which has a 180-250 nm diameter, and belongs to family of Herpesviridae. HSV-1 is a pathogenic agent which generally affects face, ocular, mouth, central nervous systems [2]. It can be results as a latent infection such as other Herpesviruses [3].

Bovine Adenovirus-1 (BAV-1) is a major viral agent in cattle which affects both digestive and respiratory systems. BAV-1 is a double strangled DNA virus [4], and belongs to Mastadenovirus genus and family of Adenoviridae [5], not includes a peplos, has an icosahedral symmetry.

Nerium oleander (NO) is a member of the *Apocynaceae* family. NO is a toxic plant after digestion. Nerium oleander distillate (NOD) and chemical extract was found beneficial to cancer, diabetes and cholesterol [6,7]. Furthermore, NO chemical extract has antimicrobial and antifungal activity [8,9]. But antiviral effect of NO has not been detailed investigated. It has been reported that chemical extract of NO has no antiviral effect against *Autographa Californica nuclear polyhedrosis virus* [10].

Effect of NOD against BHV-1, HSV-1 and BAV-1 is hypothesized that is antiviral effect of its distillate may be determined when NO hot water distillate and different virus are used. The aim of this study was to determine the effect of NOD against BHV-1, HSV-1, and BAV-1 in Vero and MDBK cell lines in vitro.

MATERIAL AND METHODS

Preparation and cytotoxicity of Nerium oleander distillate: Lyophilized NO distillates were dissolved at different concentrations (0.02, 0.04, 1, 2, 4, 8, 10, 12, 14, 16 and 20 mg/ml) in sterile distilled water and sterilized with filtration (0.22 µm membrane filter). Vero and MDBK cells (250.000/ml) were exposed to different concentrations of NOD.

Cell cultures, viruses, MTT and titration: Vero (African green monkey kidney), Madin Darby Bovine Kidney (MDBK) monolayer cell lines, and BHV-1, HSV-1, and BAV-1 were used. 50 *µl* of NOD was treated with 50 *µl* 100$TCID_{50}$ diluted BHV-1, HSV-1, and BAV-1 in 96-well plates. Other wells were evaluated to cell control (CC), NOD control (NODC), and virus control (VC). After treatments, Vero and MDBK cells ($3.5x10^5$/m*l*) in DMEM supplemented with 10% FBS were seeded into well of 96-well plates at 4^{th}, 8^{th}, 12^{th}, 24^{th} and 36^{th} hr, respectively and cultured for 72 hr at 37°C. All wells were observed for cytopathogenic effect (CPE) under inverted microscope on a daily basis. The extent of the cell proliferation and cell viability was determined by thiazolyl blue test (MTT, 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

Antiviral activity of NOD: Dilutions of NOD (10 mg/m*l*) and viruses were incubated with Vero and MDBK cells in 96 wells micro plates for 24hr at 37°C in 5% CO_2 atmosphere.

After treatments 50 µ*l* Vero and MDBK cells ($3x10^5$/m*l*) were seeded into well of 96-well plates and cultured for 72 hr at 37°C. All wells were observed for cytopathogenic effect (CPE) under cell culture microscope on a daily basis.

Statistical Analysis: Absorbance of OD values were compared by ANOVA and Duncan test as posthoc (SPSS 19.0). Test results are presented as mean±SEM. $P<0.05$ level was accepted as statistically significance level.

RESULTS

Cytotoxicity of NOD: Over 10 mg/m*l* and 2.5 mg/m*l* concentrations of NOD had cytotoxic effects on Vero and MDBK cells, respectively. As a result 10 mg/m*l* and 2.5 mg/m*l* concentrations of NOD were used in the antiviral assays.

Antiviral activities of NOD: NOD values of designed groups are shown in Table 1. NOD showed significant ($P<0.05$) antiviral activity against BHV-1 and BAV-1 in MDBK and HSV-1 in Vero cells. Inoculated Vero and MDBK cells treated with NOD at concentrations of 10 mg/m*l* and 0.01 mg/m*l* did not show any detectable CPE in comparison with the VC wells. No CPE was observed in NODC and CC in Vero and MDBK cell lines after 24^{th}, 48^{th} and 72^{th} hr; however CPE was identified in all of BHV-1, HSV-1, and BAV-1 treated with NOD and VC.

Table 1. OD Results of experiment groups (mean±SEM).

BAV-1			HSV-1			BHV-1		
	N	Mean± SEM		N	Mean± SEM		N	Mean ± SEM
CK	5	0.94 ± 0.02^{a}	CK	7	0.87 ± 0.03^{a}	CK	4	0.68 ± 0.05^{a}
NODK	5	0.71 ± 0.03^{bcde}	NODK	7	0.57 ± 0.01^{b}	NODK	4	0.43 ± 0.02^{bc}
VK	5	0.52 ± 0.04^{g}	VK	7	0.38 ± 0.02^{c}	VK	4	0.36 ± 0.1^{c}
5 mg/ml+V	5	0.77 ± 0.02^{b}	10 mg/ml+V	7	0.28 ± 0.01^{c}	10 mg/ml+V	4	0.36 ± 0.03^{c}
2.5 mg/ml+V	5	0.74 ± 0.02^{bc}	5 mg/ml+V	7	0.30 ± 0.03^{c}	5 mg/ml+V	4	0.68 ± 0.05^{a}
1.25 mg/ml+V	5	0.73 ± 0.009^{bcd}	2.5 mg/ml+V	7	0.32 ± 0.03^{c}	2.5 mg/ml+V	4	0.76 ± 0.05^{a}
0.62 mg/ml+V	5	0.72 ± 0.03^{bcde}	1.25 mg/ml+V	7	0.35 ± 0.02^{c}	1.25 mg/ml+V	4	0.72 ± 0.08^{a}
0.31 mg/ml+V	5	0.70 ± 0.01^{bcdef}	0.62 mg/ml+V	7	0.33 ± 0.03^{c}	0.625 mg/ml+V	4	0.62 ± 0.06^{ab}
0.15 mg/ml+V	5	0.64 ± 0.01^{ef}	0.31 mg/ml+V	7	0.31 ± 0.02^{c}	0.312 mg/ml+V	4	0.66 ± 0.03^{ab}
0.07 mg/ml+V	5	0.63 ± 0.01^{f}	0.15 mg/ml+V	7	0.36 ± 0.04^{c}	0.156 mg/ml+V	4	0.65 ± 0.04^{ab}
0.03mg/ml+V	5	0.66 ± 0.01^{def}	0.07 mg/ml+V	7	0.36 ± 0.04^{c}	0.078 mg/ml+V	4	0.78 ± 0.04^{a}
0.01 mg/ml+V	5	0.66 ± 0.02^{cdef}	0.03 mg/ml+V	7	0.36 ± 0.03^{c}	0.0390 mg/ml+V	4	0.68 ± 0.07^{a}

a, b, c, d, e, f, g: values marked with different letters in the same line are statistically significant ($P<0.05$. Duncan test).

DISCUSSION

Nerium oleander has a wide distribution and used for medicine [11]. NO parts and chemical extracts were investigated antimicrobial and antifungal [8,12] and antiviral [13].

In this study, although NOD had antiviral effect against to BAV-1 and BHV-1 it had not against to HSV-1. NOD (10 mg/m*l*) was determined to be cytotoxic effect on Vero and MDBK cell lines. NOD has not toxic effect on 5 mg/m*l* dose in double dilutions.

NO is same family that is *Nerium indicum* has antiviral activities against *herpes simplex virus* [14]. Whereas. NO chemical extract was any discovered antiviral activity [10]. It has been reported that NO can be used as an antifungal [8]. Also many studies have suggested that the NOD used in this study beneficial to cancer, diabetes and cholesterol in vivo studies [6, 7]. But potential antiviral effects of NOD have not yet been examined. In the present study, NOD was investigated that no effect against to HSV-1 with this method. There is not enough literature information about NO and NOD that is antiviral effects. Previous study marked antileukemic effects of 1000. 500 and 50 *µ*g/m*l* concentrations from each extract possess [15]. In the current study, there were not determined effects of NOD against to HSV-1 but it has antiviral effect to BHV-1 and BAV-1. This information proposes that NOD can be ineffective to against HSV-1 with this method; however different methods can be tested in vitro or in vivo.

In conclusion, NOD may be useless against to HSV-1 but further studies of the activity of NOD associated with the different virus types are necessary about in vitro effects of NOD. However, NOD should test different methods and may provide useful comparative information in the future.

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