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Nerium Oleander Distilatı'nın Blue Tongue Virus Üzerine in vitro Etkisi

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Özet

Nerium oleander (NO)'in kimyasal ekstraktının in vitro yapılan araştırmalarda antibakteriyel ve antifungal etkinliği belirlenmiştir. Fakat NO'nun Blue Tongue Virus (BTV)'una karşı antiviral etkinliği ile ilgili herhangi bir çalışma bulunmamaktadır. Bu çalışmanın amacı NO distilatı (NOD)'nın BTV'ye karşı in vitro etkinliğini değerlendirmektir. Toplanan NO bitkisi tanımlandı, doğrulandı ve distile edildi, sonra NOD liyofilize edildi. Liyofilize NOD distile su ile 10 mg/mL konsantrasyonunda çözdürüldü ve filtre edildi. Vero hücreleri %10 FCS, 10 mg/mL streptomisin, 10000 U/mL penisilin G ve 1250 U/mL nistatin içeren Dulbecco's Modified Eagle's Medium içerisinde 37 ^{o}C'de çoğaltıldı. 96 gözlü pleytde 50 µL NOD, 50 µL 100 $DKID_{50}$ oranında dilue edilen BTV ile inkubasyona bırakıldı. İnkubasyondan sonra sırası ile 4, 8, 12, 24 ve 36. saatlerde gözlere Vero hücreleri ($3x10^5$/mL) ilave edildi. Diğer gözler hücre kontrol (HK), virus kontrol (VK) ve NOD kontrol olarak değerlendirildi. Pleyt %5 CO_2'li inkubatörde 72 saat bekletildi. Bütün gözler sitopatojenik etki (CPE) oluşumu yönünden invert mikroskop ile günlük olarak değerlendirildi. NOD kontrol ile HK'de CPE oluşumu tespit edilmezken, VK ve NOD ile inkube edilen BTV gözlerinin tamamında CPE oluşumu belirlendi. NOD'un bu çalışmada kullanılan yöntem ile BTV'ye karşı antiviral etkinliği bulunmadığı belirlendi. Sonuç olarak NOD'un in vitro şartlarda BTV'ye karşı antiviral etkinliğe sahip olmadığı ifade edilebilir. Ancak NOD'un antiviral etkinliğinin belirlenmesi için farklı viruslar ve metotlar araştırılmalıdır.

Anahtar Kelimeler: BTV, *Nerium oleander* distilatı, Vero

In vitro Effect of *Nerium Oleander* Distillate on Blue Tongue Virus

Abstract

Chemical extract of *Nerium oleander* (NO) is identified as antibacterial and antifungal in vitro researches. But there is no any study about antiviral effect of NO on the Blue Tongue Virus (BTV). The aim of this study was to evaluate the effect of NO distillate (NOD) on BTV in vitro. Collected NO plant was identified, verified and distillated, after NOD was lyophilized. Lyophilized NOD was dissolved at concentration of 10 mg/mL with distillated water and filtered. Vero cells were grown at 37^{o}C in Dulbecco's Modified Eagle's Medium containing 10% FCS, 10 mg/mL of streptomycin, 10000 U/mL penicillin G and nystatin 1250 U/mL. 50 µL NOD was incubated with 50 µL 100 $TCID_{50}$ diluted BTV in 96-well plates. After treatments, Vero cells ($3x10^5$/mL) were added to wells at 4th, 8th, 12th, 24th and 36th h, respectively. Other wells were evaluated as cell control (CC), virus control (VC) and NOD control. Plate was incubated in 5% CO_2 incubator at 72 h after cells were seeded. All wells were examined for cytopathogenic effect (CPE) daily with an inverted microscope. CPE was not observed in NOD control and CC, whereas CPE was determined in all of NOD treated with BTV and VC. It was determined that NOD had no antiviral activity against to BTV when evaluated method used in this research. In conclusion, it may be stated that NOD has no antiviral effect on BTV in vitro. However, different viruses or methods should be investigated for determine the antiviral effect of NOD.

Key Words: BTV, *Nerium oleander* distillate, Vero

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INTRODUCTION

Blue Tongue (BT) is an economically important, infectious, non-contagious, arthropod borne disease in species of sheep, cattle, domestic and some wild ruminants (4, 21). It is caused by Blue Tongue Virus (BTV), a member of the *Orbivirus* genus in the family Reoviridae (18). Twenty-six immunologically distinct serotypes of the virus have been identified in the worldwide (23). BTV was first defined in South Africa and divided two main origins which are circulated in Africa, the Caribbean and the Americas to western origin and another are endemic in Asia, Indonesia and Australia (7). BT disease is asymptomatic and characterized by prolonged viremia. Furthermore, infected cattle have impaired erythrocyte membrane. Pathogenesis of the disease is similar among sheep, cattle and other ruminants. After infected *culicoides* vector bites to ruminants, BTV moves to regional lymph node. It begins to replication and spreads to different tissues. Also BTV replicates in mononuclear phagocytes and endothelial cells (16). Signs of the disease are hemorrhage, congestion and edema. Beside oral erosions and ulcers, lameness, serous to bloody nasal discharge, fever and muscle necrosis were observed by MacLachlan (17). Especially cattle's also have been developed protective immune response to the disease (16).

Nerium oleander (NO) is a member of the Apocynaceae family. NO has anticancer and cardio tonic effects and it is used in the treatment of skin diseases (6). NO is a toxic plant after digestion. NO grows tropic and subtropics regions. NO includes cardiac glycosides (3). NO water extraction contains polysaccharides, some cardenolids and triterpenoids (11). *Nerium oleander* distillate (NOD) and chemical extract were found beneficial to cancer, diabetes and cholesterol (5, 20). Furthermore, NO chemical extract has antimicrobial and antifungal activity (12, 13). But antiviral effect of NOD on the BTV has not been investigated. Chemical extract of NO has no antiviral effect against *Autographa californica nuclear polyhedrosis virus* (AcNPV) (9) while NOD has antiviral effect to PI-3 virus (1).

It is hypothesized that NOD may show antiviral affect against to BTV like its antiviral effects to PI-3. The aim of this study was to determine the effect of NOD against BTV in Vero cell lines in vitro.

MATERIALS and METHODS

Fresh twigs and leaves of NO plant were collected from Mediterranean region of Turkey. The identification of these specimens was done and confirmed at the Department of Biology, University of Selcuk. Gathered NO was washed and chopped. Chopped plants included in distillatory and enough distilled water added. The mixture was boiled in the distillatory. When liquid started to vaporize, vapor was accumulated to sterile glass containers by using a surface cooled with cold water. The accumulated NOD was lyophilized within 20 mL glass bottle in lyophilizator (5). It has been reported that higher concentrations than 10 mg/mL of NO distillate had cytotoxic effects (2). As a result lyophilized NOD was distributed at concentrations of 10 mg/mL in sterile distilled water and used in the antiviral assays.

Vero (African green monkey kidney cells) monolayers cell lines and BTV were used in this study. All cell line and BTV (virus control) were obtained from Department of Virology, Faculty of Veterinary Medicine, University of Selcuk. The cells were grown in sterile Dulbecco's Modified Eagle's Medium (DMEM) and 10% Fetal Calf Sera (FCS). The cells were protected as monolayers in 25 cm^2 culture flasks at 37°C in a moisturized atmosphere containing 5% CO_2 in air. Concentrate antibiotics and antifungals were utilized to eliminate the growth of bacteria and fungi. 50 µL NOD was treated with 50 µL 100 $TCID_{50}$ diluted BTV in 96-well plates. Other wells were

evaluated to cell control (CC), NOD control, and virus control (VC). After treatments, Vero cells ($3.5x10^5$/mL) in DMEM supplemented with 10% FCS were seeded into well of 96-well plates at 4th, 8th, 12th, 24th and 36th h, respectively and cultured for 72 h at 37°C. All wells were observed for cytopathogenic effect (CPE) under inverted microscope on a daily basis.

RESULTS

No CPE was observed in NOD and CC in Vero cell lines after 24th, 48th and 72th hours (Figure 1), however, CPE was identified in all of BTV treated with NOD and VC (Figure 2).

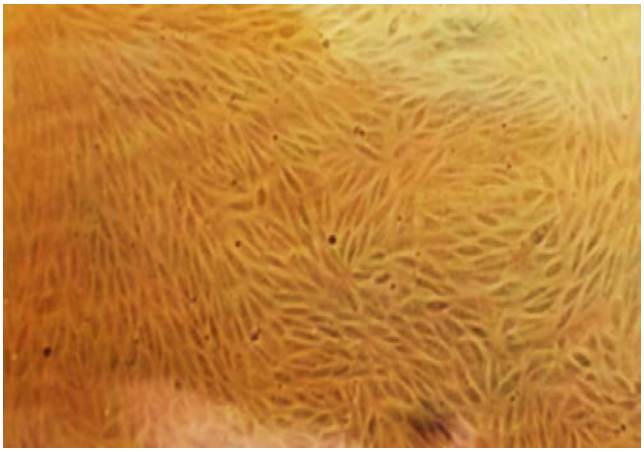

Figure 1. Cell control (x200).

DISCUSSION

Nerium oleander L. is used in medical treatment in Chinese folk medicine (10, 14). Previous studies showed the pronounced toxicity, antimicrobial, anticancer and antifungal activities of NO chemical extract (12, 13, 15, 19). Also, studies have suggested that the NOD has beneficial effect to cancer, diabetes and cholesterol in vivo studies (5, 20).

NO parts and chemical extracts were investigated to determine their antimicrobial and antifungal activities (6, 8, 12). NO is same family that

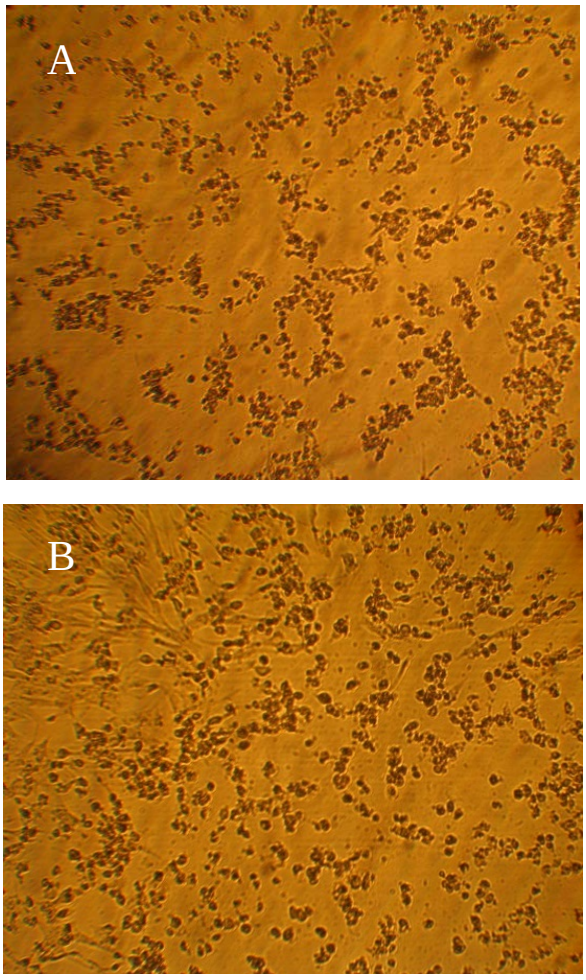

Figure 2. Cytopathogenic effect of BTV on 24th h on Vero cell. A: Virus control B: BTV treated with NOD (x200)

is *Nerium indicum* has antiviral activity against herpes simplex virus (22). Whereas, NO chemical extract was no discovered antiviral activity (9). There has not enough literature information about the antiviral effects of NO and NOD. To our best knowledge, no any research exists about the effect of NOD against to BTV. In the current study, it was determined that NOD had no antiviral activity against to BTV in vitro (Figure 2). Although NOD may not to be effective against BTV in vitro design with this method; the data can be stored as a guide to help future research about antiviral activity of NOD. Research with different DNA viruses or methods can be planned for determine antiviral effect of NOD in future.

In conclusion, NOD may be useless against to BTV, but further studies of the activity of NOD associated with the different virus types are necessary. In addition, NOD should be tested at different methods and may provide useful comparative information in the future.

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