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In-vitro antiviral activity of Neem (*Azadirachta indica* L.) Bark extract against BCoV, BHV-1, BPIV-3, and BEVs

Hatice Pelin Aslim*, Hasan Sercan Palanci and Oya Bulut

Selcuk University, Veterinary Faculty, Department of Virology, Konya, Turkey

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ABSTRACT

Neem extracts and bioactive compounds which have been widely utilized in medicine for some time, exhibit regulatory effects in relation to various biological mechanisms, and include antioxidant, anti-inflammatory, antihyperglycemic, as well as antifungal, antibacterial, and antiviral properties. Thus, this clearly underscores the pivotal role of Neem Bark in modulation of various biological pathways. The present study evaluated the antiviral activity of *Azadirachta indica* (Neem Bark) extract on Bovine Corona Virus (BCoV), Bovine Herpes Virus-1 (BHV-1), Bovine Parainfluenza Virus-3 (BPIV-3) and Bovine Entero Virus (BEV) *in vitro*. The aim of the present study was to determine whether its antiviral activities were effective in the cell entry or replication phase of each virus. As a result of the WST-1 test, performed to determine the non-cytotoxic dose of NBE, it was determined that concentrations lower than 0.87 mg/mL were not cytotoxic in an MDBK cell line. Although Neem Bark extract (NBE) did not have any significant effect on the attachment of Bovine Parainfluenza Virus-3 and Bovine Entero Virus to the host cell, a 100-fold decrease in TCID50 (50% tissue culture infectious dose) values of Bovine Corona Virus treated with Neem extract was detected, and virus replication was completely blocked in Bovine Herpes Virus-1. In conclusion, we suggest that it would be valuable to evaluate its antiviral activity on Bovine Corona Virus and Bovine Herpes Virus-1 *in vivo*. Additionally, the detailed determination of the effectiveness of NBE against other viruses would contribute to future antiviral drug trials.

Key words: Neem Bark; Bovine Herpes Virus-1; Bovine Corona Virus; Bovine Parainfluenza Virus-3; Bovine Entero Virus

Introduction

Various plants are reported to possess a multidimensional range of therapeutic potential that are effective against diabetes (ABDULLAH et al., 2023), have anthelmintic (DAG et al., 2023), and antibacterial activity (GHAZWANİ et al., 2023), are effective against cypermethrin resistant parasites (NASEER et al., 2022) and have an immunomodulatory effect (HUSSAİN et al., 2023). This fact highlights the importance of various plant extracts in medical interventions. Neem, also known as Azadirachta indica or Indian lilac, is a native plant obtained from the Neem tree (ASCHER, 1993). Neem leaves and their components have been reported to exhibit antimutagenic and anticarcinogenic effects (SINGH et al., 2005), anti-inflammatory activity (BISWAS et al., 2002), antihyperglycemic effects (PATIL et al., 2013), immunomodulatory properties (KUMAR

* Corresponding author:
Hatice Pelin Aslım, Selcuk University, Veterinary Faculty, Department of Virology, Konya, Turkey, e-mail: hpelinucan@gmail.com

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et al., 2013), antibacterial activity (FRANCINE et al., 2015), antiviral properties (AMER et al., 2010), antioxidant activity (KIRANMAI et al., 2011), antiulcer effects (CHATTOPADHYAY et al., 2004) and antifungal activity (EZEONU et al., 2018).

Coronaviruses cause various clinical syndromes, including enteritis, liver and respiratory tract infections, neurological disorders, and nephritis in various species, such as pigs, poultry, humans, cows, cats, dogs, horses, and mice (HAN et al., 2006). Coronaviruses in the family *Coronaviridae* are positive-stranded viruses with the largest genome (27 to 32 kb) among all the RNA viruses (LAI and CAVANAGH, 1997; HAMMADI and ALMOUSAWI, 2021). Coronaviruses are classified into three antigenic and genetic groups (VIJGEN et al., 2005; KAUR et al., 2021). Human coronaviruses are categorized into group 1 (HCoV-NL63 and HCoV-229E) and group 2 (HCoV-HKU1) (ICTV, 2009). Bovine coronaviruses belong to group 2, and are known as pneumoenteric viruses, causing winter dysentery, neonatal calf diarrhea, and respiratory disease in cattle (ATHANASSIOUS et al., 1994; CHO et al., 2000; DECARO et al., 2008).

Bovine Herpesvirus-1 (BHV-1) is one of the eight herpesviruses isolated from naturally infected cattle. BHV-1 is categorized in the subfamily *Alphaherpesvirinae* of *Herpesviridae.* Its genome shows a double-stranded DNA structure, with a molecular weight of 135.3 kpb. BHV-1 can be divided into subtypes, as follows: 1.1, 1.2a, 1.2b, and 1.3 (MUYLKENS et al., 2007; AYTOĞLU et al., 2022). BHV-1.3 is a neuropathogenic agent and is also referred to as BHV-5. BHV-1 is primarily associated with three clinical manifestations: IBR (Infectious Bovine Rhinotracheitis), Infectious Pustular Balanoposthitis (IPB), and Infectious Pustular Vulvovaginitis (IPV). Moreover, the virus can also cause various syndromes, including infertility, abortion, encephalitis, and conjunctivitis. A mixed infection (Shipping Fever) in calves with BHV-1 and others may also occur (NANDI et al., 2009).

Bovine Parainfluenza virus type 3 (BPIV-3) is a non-segmented, enveloped, negative-stranded RNA virus in the *Respirovirus* genus (GUO et al., 2021). Other viruses in this group include human Parainfluenza 1 and 3 (HPIV-1 and HPIV-3) and Sendai virus strains. The clinical symptoms of bovine BPIV-3 infections can vary significantly, from asymptomatic to a severe respiratory disease. Disease-related cases of BPIV-3 are usually characterized by mild clinical manifestations, such as cough, fever, and a runny nose.

Bovine enteroviruses (BEVs) are small, non-enveloped, single-stranded, positive-stranded RNA viruses, in the family *Picornaviridae* (BEATO et al., 2018). BEVs isolated from cattle worldwide have various clinical manifestations, including abortion, stillbirth, infertility, neonatal deaths, diarrhea and fever, dehydration, and weight loss (ZELL et al., 2006).

Previous studies have reported that Neem extract significantly inhibits poliovirus (FACCIN-GALHARDI et al., 2012), HIV, Coxsackie group B virus, and Dengue virus in the early stages of viral genome replication. Neem extract is also virucidal against coxsackievirus B-4 by interfering in the early stages of replication, finally causing virus inactivation (TIWARI et al., 2010). Similarly, the inhibitory effect of Neem extract in the replication stage of Dengue virus type 2 was confirmed through virus inhibition and RT-PCR tests (PARIDA et al., 2002).

Our aim was to determine *in vitro* whether the antiviral activity of Azadirachta indica (Neem Bark) extract on BCoV, BHV-1, BPIV-3 and BEV is effective on the cell entry or replication phases of each virus, by this study.

Materials and methods

Cell cultures and viruses. The viruses used for the study were obtained from the Virus Collection Bank of Selcuk University Faculty of Veterinary Medicine, Department of Virology.

In propagation of the viruses and in trials for determination of concentration-dose, infectious potency and antiviral activity, MDBK (Madin-Darby Bovine Kidney Epithelial Cells ATCC® CCL 22) continuous cell cultures were used. Growth of MDBK cells and virus replication processes were carried out using Modified Eagle's medium (DMEM, Gibco) at 37°C in a 5% CO_2 incubator. The medium

was supplemented with 10% FBS, 1% antibiotic 5,000 U/mL (Gibco), and 1% 200 mM L-glutamine (Gibco) in cell generation processes. During virus replication, the FBS rate was reduced to 2%.

Neem Bark extract (NBE). The plant extract used in the study was commercially available (Sigma cat no: 73279-100M).

Cytotoxicity test. A cell proliferation reagent WST-1 (Roche, Cat No: 11644807001) kit, commonly used as one of the cell viability tests, was used to determine the non-cytotoxic dose of NBE, in accordance with the manufacturer's instructions. The study was repeated three times, and the test was terminated at the end of the 48th hour. After calculating the arithmetic average of the optical density values obtained, the inhibitor concentration (IC) values of the substance were then calculated. The value at which the inhibitory concentration was less than half the dose (IC50) was used for the antiviral study.

In addition, the toxic concentration ranges of the substance quantity on the cells were evaluated using daily microscopic examinations, and the number of toxic concentration ranges was calculated according to the formula below:

$$\text{Od Sample} = \text{Od Sample}_{\text{Raw}}(450\text{nm}) - \text{Od Sample}_{\text{Raw}}(650\text{nm})$$

$$\text{Od Control} = \text{Od Control}_{\text{Raw}}(450\text{nm}) - \text{Od Control}_{\text{Raw}}(650\text{nm})$$

Inhibitor concentration % = [(Od Control - Od Control Blank) - (Od Sample- Od Sample Blank)] / (Od Control- Od Control Blank)

Antiviral activity studies. The antiviral activity of NBE was evaluated, along with the determination of TCID50 (50% Tissue culture infectious dose) of virus-infected cells, and assessment of changes in TCID50 values when infected cells were treated with NBE. In this test, individual 96-well plates were used for each virus. Antiviral activity was studied separately for each virus species, to determine the hostile activities against replication and adsorption (Fig. 1).

The antiviral activity of NBE used on the virus was calculated according to the Spearman (1908) and KARBER (1931) method, and the 50% endpoint titer of the infective doses in the group without Neem Bark of the same virus species was calculated and compared. The study plan shown above was used to determine the effect of Neem Bark on both replication and adsorption.

Effect of NBE on virus replication. A cell suspension of $8.0x10^5$ cells/ml was prepared from previously subcultured MDBK cell cultures. 100µl of this suspension, prepared for 96-well plates, was placed and incubated for one night at 37°C in a 5% CO_2 incubator, to allow the cells to enter the exponential growth phase. The cell growth medium was removed from the wells when the cells covered approximately 90% of the healthy surface as a monolayer. The wells in the plate were numbered up to 10 from top to bottom and were divided into two wells, with and without antiviral agents. The control group was located in the plate surface's last two rows of cells and viruses. 100 µl of growth medium, containing a maximum non-toxic concentration of Neem solution, was placed in the sections containing Neem extract, in four columns and four rows, up to the number 10. Likewise, 100 µl of cell growth medium that did not contain any antiviral agent was added to the wells in the other half of the plate. The plates were incubated for 2 hours in a 5% CO_2 incubator at 37˚C, for the cells to metabolize the Neem extract. Just prior to the end of the incubation period, the virus solutions were diluted serially, between 10^{-1} and 10^{-10} according to the Log_{10} base. At the end of the incubation, the substances in the plate wells and the media content were removed, and the wells were washed with Phosphate-buffered saline (PBS). Virus suspensions, diluted to 100 µl in each well, were transferred to the wells treated with NBE and no Neem was added until the 10^{th} dilution step. The virus dilutions were left to incubate with cells in an incubator with 5% CO_2 at 37˚C for one hour, then were removed from the wells at the end of the period, and washed with PBS. A growth medium containing the highest concentration of non-toxic NBE was added to the wells that were previously incubated with Neem, with 100 µl of the growth medium and 100 µl of Neem-free growth medium in the wells in the other half of the plate that was not treated with Neem extract The plates were then

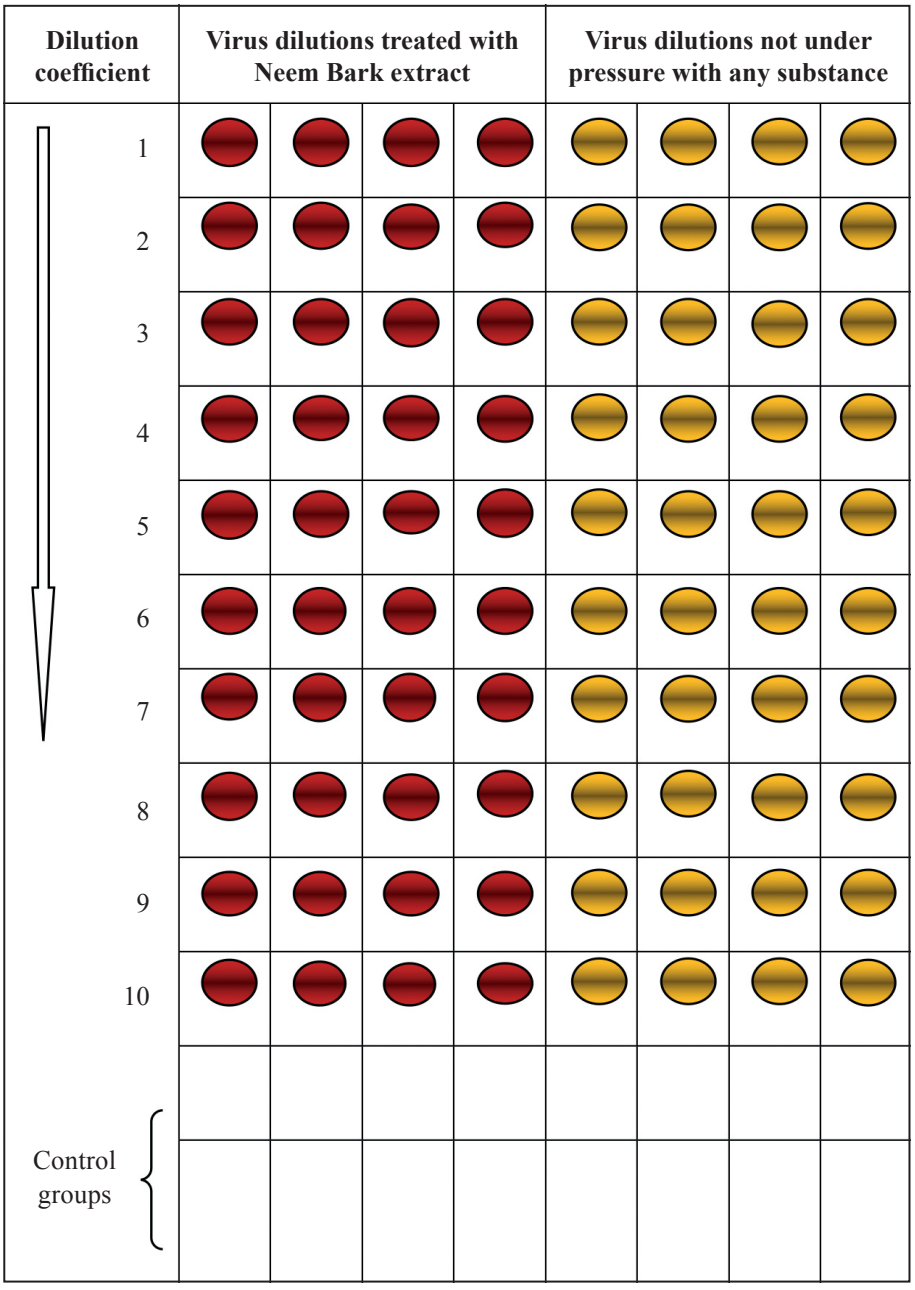

Fig. 1. 96-well cell culture plate study plan

The antiviral activity of NBE used on the virus was calculated according to the Spearman (1908) and KARBER (1931) method, and the 50% endpoint titer of the infective doses in the group without Neem Bark of the same virus species was calculated and compared. The study plan shown above was used to determine the effect of Neem Bark on both replication and adsorption.

transferred to the incubator at 37˚C and 5% CO_2. The cells inoculated with the virus were checked daily with a tissue culture microscope for the presence of any cytopathic effect (CPE) of the virus on the cell. The test was terminated at the end of 72 hours. To determine the infective dose of viruses in the wells with and without Neem, a 50% endpoint titer of the virus was calculated according to the Spearman (SPEARMAN, 1908) and Karber (KARBER, 1931) methods.

Effect of NBE on virus attachment. The plate layout was designed as in the replication study. The cell suspension was transferred into 96 well plates constituting a 90% monolayer, and incubated for 24 hours at 37˚C in a 5% CO_2 incubator. At the end of the time period, the virus groups in which antiviral activity was to be studied, were serially diluted with a non-toxic maximum concentration of Neem solution in a range from 10^{-1} to 10^{-10} according to the Log_{10} base. Simultaneously, the reconstitution of the virus with a cell generation medium containing no substance was performed separately in a similar manner. At the end of the procedure, both groups (virus dilutions and virus dilutions treated with Neem) were kept at room temperature for 90 minutes. At the end of the incubation period, if the Neem extract had affected the external structure of the virus and its receptors, the estimated time required for this effect was recorded. At this point, the cell growth medium was removed from the plates and the wells were washed with PBS. Virus suspensions with and without Neem treatment were transferred to each dilution step on microplates, in 4 copies, 100 µl volume according to their dilution coefficients. At the end of the process, the plates were transferred to an incubator with 5% CO_2 at 37˚C. The cells inoculated with the virus were checked by microscope daily for the presence or absence of the cytopathic effect (CPE) of the virus on the cell. The study was terminated after 3 days. To determine the infective dose of the viruses in wells with and without Neem, the 50% endpoint titer of the virus was calculated according to the Spearman (SPEARMAN, 1908) and Karber (KARBER, 1931) methods.

Statistical analysis. All the experiments were repeated three times. The independent sample T-test was applied to the data to compare the mean viral titer (TCID50/1 ml) values of the NBE-treated test groups with the virus control titer values. The statistical significance level was defined as $P<0.05$. Analysis of the results was carried out using the GraphPad prism t test calculator program.

Results

Cytotoxicity tests. The cytotoxicity of IVM was evaluated by determining the effect of various NBE concentrations on the IC rate of the cultured MDBK cells. The cytotoxicity of some NBE dilutions (1/4, 1/8, 1/16, 1/32, 1/64, 1/128) on MDBK cells are shown in Table 1. NBE diluted to 1/32 and more were all determined to be toxic (inhibitory concentration; %48 = 1/32). Therefore, a 1/32 dilution of NBE was considered to demonstrate the antiviral activity of NBE.

Antiviral studies: Effect of NBE on BCoV, BHV-1, BPIV-3, and BEV replication. The lowest non-cytotoxic dilution value of 1/32 of Neem Bark extraction was used on BPIV-3, BHV-1, BCoV, and BEV. Virus replication activities in the infected MDBK cells at a 1/32 concentration value were investigated by the method given above, and the

Table 1. Effects of NBE on cell proliferation

Dilution factors - mg/ 0.1 mL	NBE cytotoxic rate
1/128 (0,78125 mg)	22 %
1/64 (1,5625 mg)	35 %
1/32 (3,125 mg)	48 %
1/16 (6,25 mg)	88 %
1/8(12,5 mg)	100 %
1/4 (25 mg)	100 %
Cell Control	0 %

The experiment was carried out in three replicates and IC rates were calculated by taking the arithmetic mean of the results

Table 2. NBE and virus replication

Infectious strength (average scores) (TCID50/1 ml)							
BHV-1 Control	**BHV-1+Neem 1/32**	**BEV Control**	**BEV+ Neem 1/32**	**BPIV-3 Control**	**BPIV-3+ Neem 1/32**	**BCoV Control**	**BCoV+ Neem 1/32**
$1x10^{5,75}$	$1x10^{5,25}$	$1x10^{7}$	$1x10^{6,75}$	$1x10^{6,75}$	$1x10^{6,75}$	$1x10^{4,5}$	$1x10^{3,0}$

The results of the effect of NBE on virus attachment in terms of TCID50/1 ml. All experimental stages were repeated three times during the study period and the mean viral titer (TCID50/1 ml) values of the NBE-treated test groups are given with the virus control titer values.

Table 3. NBE and virus replication

Infectious strength (average scores) (TCID50/1 ml)							
BHV-1 Control	**BHV-1+Neem 1/32**	**BEV Control**	**BEV+ Neem 1/32**	**BPIV-3 Control**	**BPIV-3+ Neem 1/32**	**BCoV Control**	**BCoV+ Neem 1/32**
$1x10^{7}$	0	$1x10^{7}$	$1x10^{6,75}$	$1x10^{6,5}$	$1x10^{6,25}$	$1x10^{5}$	$1x10^{3}$

The results of the effect of NBE on virus attachment in terms of TCID50/1 ml. All experimental stages were repeated three times during the study period, and the mean viral titer (TCID50/1 ml) values of the NBE-treated test groups are given with the virus control titer values.

TCID50 results obtained at the end of the 72 hours including controls are presented in Table 2.

According to our data, NBEs, diluted at a ratio of 1/32, do not play any suppressive role in any virus replication on BPIV-3, and not cause any significant change in BHV-1 and BEV ($P>0.05$). However, it was seen that the virus infectivity strength was reduced by about 30 fold in MDBK cells that metabolized Neem extract and were infected with BCoV ($P<0.05$). In the light of the results obtained from this research, it was found that Neem extract may play a limiting role in the virus replication in BCoV.

The effect of NBE on cell surface attachment of BHV-1, BEV, BPIV-3, and BcoV. The 1/32 NBE concentration values used in the replication study were also used in this study using the same methodology given above. Recorded at the end of the 72-hour study, the TCID50 results of the viruses with the controls are expressed in Table 3.

Our data showed that NBE diluted to 1/32 produced no statistically significant differences ($P>0.05$) and did not significantly affect the attachment of BPIV-3 and BEV to the host cells. However, it was observed that there was a 100-fold decrease in TCID50 values of the BCoV virus treated with Neem extract, and virus attachment was completely blocked in BHV-1 ($P<0.05$).

Virus titration images obtained in the replication and adsorption studies are shown in Fig. 2., Fig. 3. and Fig. 4.

Discussion

Our understanding of the molecular mechanisms of antivirals provides scientists with great opportunities for the discovery of new therapeutic approaches, as well as new methods to prevent virus-cell interactions (DIMITROV, 2004). This study investigated the ability of NBE extract from Azadirachta indica (*A. indica*) to enter the cell and to inhibit the replication of BCoV, BHV-1, PI-3,

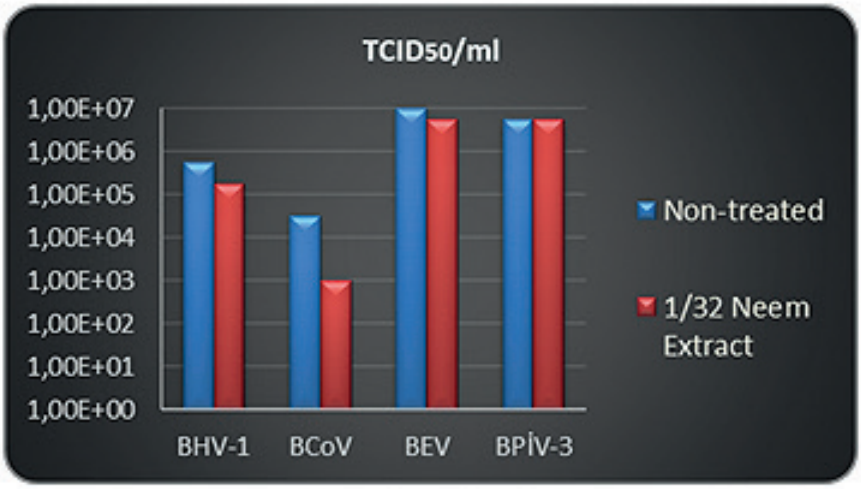

Fig. 2. Bar chart of the effect of NBE on virus replication. The chart scale was designed according to the logarithm base 10.

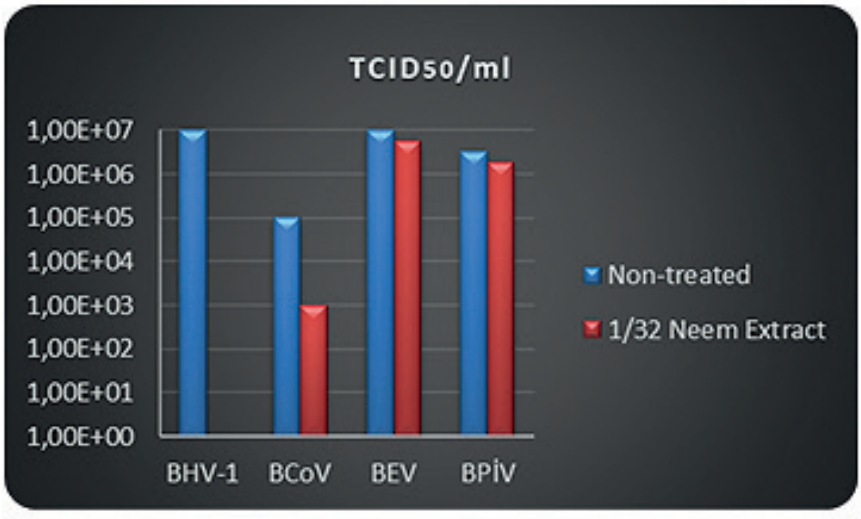

Fig. 3. Evaluation of the effect of NBE on virus attachment in the form of a bar chart. The chart scale was designed according to the logarithm base 10. No statistically significant difference was seen (P>0.05)

and BEVs, following entry. In recent years, the antiviral effects of many small molecules, such as terpenes, phenolics, polyphenols, sugar-containing compounds, and flavonoids obtained from plants, have been reported.

TIWARI et al. (2010) researched the potential of Neem extract to inhibit virus entry into target cells in a Herpes Simplex-1 (HSV-1) infection from the *Herpesviridae* family. In parallel with determination of the antiviral activity of the extract on BHV-1 (Fig. 1, Fig. 3), TIWARI et al., (2010) stated that 50-100 µg/ml concentrations of Neem extract significantly blocked the entry of HSV-1 into the cell, and that this blocking activity occurred during the pre-incubation of the virus with the extract without cells, demonstrating its direct effectiveness against the virus. They also stated that Neem extract, at concentrations of 0.1 mg/ml-100 µg/m or less, inhibits the cell entry stage and the viral fusion step through glycoprotein-mediated cell to cell fusion.

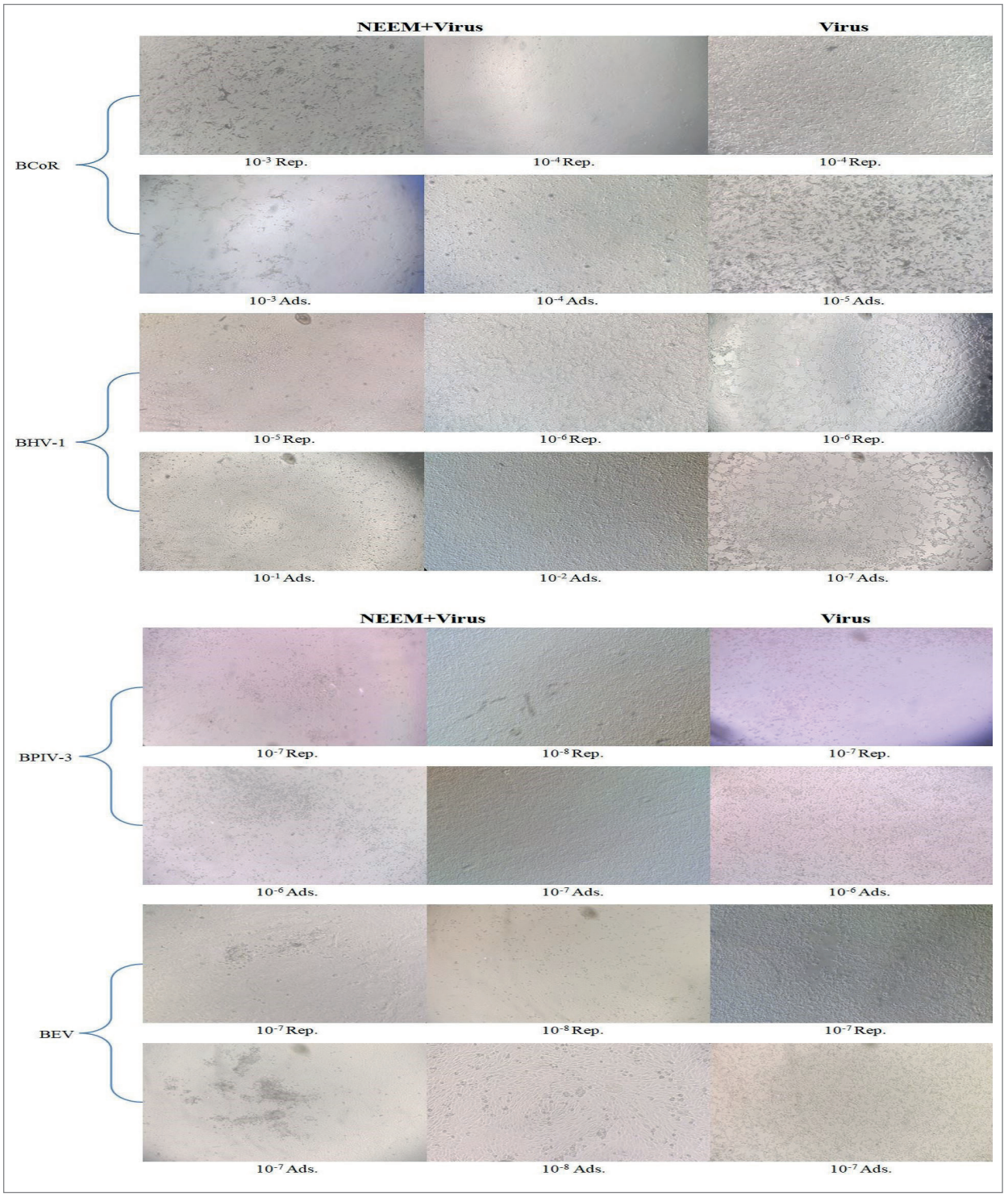

Fig. 4. Microscopic view of the final dilution step with CPE in wells containing Neem+Virus and the dilution coefficient where CPE formation ended. Any of the 4 wells of each dilution were photographed. With the same method, TCID50 values of only virus-containing groups were calculated and photographed (objectives with 4x and 10x magnifications were used in microscope images).

These findings seem to open up a potential avenue for developing Neem extract as a new antiherpetic.

MAHMOOD et al. (2018) evaluated the antiviral activity of NBE against Newcastle Disease virus (NDV), which belongs to the genus *Paramyxovirus*, using a spot test and a micro haemagglutination test *in-vitro,* and by administering the virus into an 11-day-old embryo egg *in-ovo*. Regarding *in vitro* evaluation, when the results of the non-diluted and 1:2 diluted batches of Neem Bark extract on PI-3 virus were evaluated, antiviral activities were exhibited similar to this study. However, these concentrations also showed cytotoxic activities, but no significant antiviral activities at a higher dilution. Likewise, in evaluations *in-ovo*, it was stated that high dilutions showed insignificant antiviral effects. Our study also determined no antiviral activity against PI-3, which is also a member of the *Paramyxovirus* genus (Fig. 2 and 3). Therefore, it was emphasized that a certain amount of exposure time was necessary for cytotoxicity although the researchers did not detect any statistically significant differences in the antiviral effect of different Neem Bark concentrations. ELBASUNI et al. (2023) reported that the use of NBE at a high dose (1000 µg/kg) was more effective and significant in eliminating clinical and pathological abnormalities caused by infection, compared to low dose (500 µg/kg) in experimental NDV infection in chickens. They also emphasized that the infectivity titer of the virus isolated from the trachea, spleen and cecal tonsils decreased after 3, 5 and 7 days after infection. Although no antiviral activity against PI-3 was determined in this study, it is thought that PI-3 may exhibit antiviral activity in in-vivo trials, similar to that conducted by ELBASUNI et al. (2023).

BORKOTOKY and BANERJEE (2020) determined that some compounds obtained from Neem [Nimbolin A, Nimosin and Cycloartranols (24-Methylenecycloartanol and 24-Methylenecycloartan-3-one)] exert inhibitory activity on the M and E proteins of SARS-CoV-2. Our study determined that Neem does have antiviral activity against bovine coronavirus, which is in accord with the findings by other researchers (Table 2). In another recent study, SARKAR et al. (2020) stated that some compounds derived from Neem have potential therapeutic effects on the spread and pathophysiology of Mouse Hepatitis Virus (MHV), a member of the coronavirus family.

FACCIN-GALHARDI et al. (2012) determined the activity of P1 and P2 polysaccharides isolated from the leaves of Neem Bark and their chemical sulfated derivatives (P1S and P2S) against poliovirus type 1 (PV-1). For this purpose, they analyzed their cytotoxicity by MTT and determined the antiviral effects using the "plaque reduction" test in different protocols. These authors (2012) found that polysaccharides did not exhibit any cytotoxic effect on HEp-2 cells, even at the highest concentration tested (200mg/ml), but that inhibitory concentrations of 80mg/ml, 37.5mg/ml, 77.5mg/ml, and 12.1 (IC50) showed significant antiviral activities against poliovirus type 1 (PV-1). They stated that the polysaccharides they obtained from Azadirachta Indica acted against PV-1 by inhibiting the first stage of viral replication, and that the original polysaccharides showed more effective virucidal effects than their sulfated derivatives at all concentrations tested. Similarly, in the present study, it was observed that Neem extract inhibited coronavirus replication. Neem is thought to produce an antiviral effect by acting on the RNA-dependent RNA polymerase (RdRp).

SHAILAJA et al. (2017) reported that aqueous leaf extract had antiviral activity against the Vaccinia, Chikungunya, and Measles viruses *in vitro*. The antiviral activity of aqueous Neem leaf extract assessed in C6/36 cells (cloned cells of Aedes albopictus larvae) using virus inhibition assay, showed inhibition in a dose dependent manner. On the other hand, pure Neem Azadirachtin was reported to provide no inhibition against the replication of Dengue virus type-2 *in vivo* and *in vitro*. The methanolic extract of Neem leaves (NCL-11) has been shown to have virucidal and antiviral effects against group B Coxsackie viruses. Particularly, a concentration of 1 mg/ml over 96 hours *in vitro* inhibited plaque formation of different antigenic types belonging to Coxsackievirus B (MAITHANI et al., 2011).

Punicalagin, found abundantly in P. granatum extract and juice, needs to be analyzed by bioactivity-guided fractionation studies. This punicagalin

caused a loss of infectivity apparently due to its binding to the antigenic determinants of BCoV and BHV-1. Compounds other than punicalagin may have insufficient antiviral potential to inactivate all the infectious virus particles (EL-AGUEL et al., 2022). After incubation of target cells with Neem extract in this study, the infectious potency of BCoV appeared to decrease by about 30 times (Fig. 2). Neem compounds may have inhibited the assembly of BCoV particles, resulting in reduced viral replication. Interestingly, the complete blocking activity of Neem was seen when only BHV-1 virions were pre-incubated with the extract in comparison with the pre-incubated target cells. Taken together, these observations lead us to point out two critical points: first, the tested concentrations of Neem were not cytotoxic for the cells as the viral entry was quantified when Neem was pre-incubated with the target cells; and second, the inhibitory effect of Neem targets the BHV-1 virions as the virucidal effect was seen when the extract was incubated with the virus (Fig. 3, Fig. 4).

Conclusions

In this study, the antiviral activity of Neem Bark extract was demonstrated. Further studies with phytochemical analysis of the active components in the plant will play a critical role in the development of new and effective antiviral agents. A new study in the near future could be focused on whether Neem is active against all types of herpes virus infections or if it is specific only to BHV-1 Additionally, Neem's anti-herpes activity may be further investigated against the individual BHV-1 glycoprotein itself. Obviously, further studies on the toxicity of Neem and its detailed characterization would support the development of therapeutic agents against BHV-1. It is also recommended that *in-vivo* antiviral studies of Neem Bark should be carried out in future studies.

Ethics approval

This study was approved by the local Ethics Committee of SÜVETFAK dated 20.10.2021 and numbered 2021/110.

Authorship contribution statement

HPA and OB conceived and planned the experiments. HPA and HSP carried out the experiments. OB contributed to the interpretation of the results. HPA took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript**.**

Declaration of competing interest

The authors declare that there is no conflict of interest

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At the Second International Congress on Biological and Health Sciences this paper was presented as an oral presentation (24-27 February 2022).

Data availability statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

ORCIDs

H. P. ASLIM: https://orcid.org/0000-0001-9160-1255

H. S. PALANCI: https://orcid.org/0000-0001-5408-9176

O. BULUT: https://orcid.org/0000-0002-2407-7390

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ASLIM, H. P., H. S. PALANCI, O. BULUT: Antivirusni *in vitro* učinak kore nima (*Azadirachta indica* L.) na viruse BCoV, BHV-1, BPIV-3 i BEV. Vet. arhiv 95, 75-88, 2025.

SAŽETAK

Ekstrakt kore nima (NBE) i bioaktivni spojevi koji se već neko vrijeme široko primjenjuju u medicini pokazuju regulacijske učinke kod različitih bioloških mehanizama, što uključuje antioksidacijska, protuupalna, antihiperglikemijska, antifugalna, antibakterijska i antivirusna svojstva. Time se jasno pokazuje važna uloga kore nima u modulaciji različitih bioloških procesa. U radu je istraživano *in vitro* antivirusno djelovanje ekstrakta kore nima (*Azadirachta indica*) na goveđi korona virus (BCoV), goveđi herpes virus 1 (BHV-1), virus parainfluence 3 goveda (BPIV-3) i goveđi enterovirus (BEV). Cilj istraživanja bio je ustanoviti je li antivirusno djelovanje kore nima bilo učinkovito u stadiju ulaska virusa u organizam ili u stadiju njihova razmnožavanja. Kao rezultat testa WST-1, koji je proveden kako bi se odredila doza NBE-a koja nije citotoksična, ustanovljeno je da koncentracije manje od 0,87 mg/mL nisu citotoksične u MDBK staničnoj liniji. Iako NBE nije pokazao znatniji učinak na vezanje BPIV-3 i BEV za stanice domaćina, zabilježeno je stostruko smanjenje vrijednosti TCID50% (infektivna doza kulture tkiva pri 50 % ciljanog učinka) kod virusa BCoV pri tretmanu ekstraktom kore nima, a potpuno je zaustavljena replikacija virusa BHV-1. Zaključeno je da bi bilo korisno procijeniti antivirusno djelovanje NBE-a na viruse BCoV i BHV-1 *in vivo*. Osim toga, detaljno određivanje djelotvornosti NBE-a protiv drugih virusa pridonijelo bi budućim istraživanjima antivirusnih lijekova.

Ključne riječi: ekstrakt kore nima; goveđi korona virus; goveđi herpes virus 1; virus parainfluence 3 goveda; goveđi enterovirus