Research Article

In Vitro Antiviral Effect of Interferon Produced by Bovine Ephemeral Fever Virus against Rift Valley Fever Virus

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Abstract | This work as an interesting one is a trial to prepare a natural economic antiviral agent against Rift Valley Fever virus which may be able to minimize the virus infection. This study was designed as a preliminary in vitro investigation of the effect of interferon-induced by bovine ephemeral fever virus (BEFV) in Madin-Darby Bovine Kidney (MDBK) and African green monkey kidney (Vero) cell cultures against RVFV. Such interferon was successfully obtained with high concentrations by both cell cultures up to a dilution of 10^{-5} yielding positive antiviral effect against RVFV, especially when inoculated one hour post virus infection or simultaneously with the virus. So, we could conclude that the prepared interferon has an antiviral activity against RVFV and further studies are needed to provide more understanding of the virus-host interaction and cellular antiviral response and to evaluate the probable side effects of interferon on treated animals.

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Introduction

Rift Valley Fever (RVF) is an acute viral hemorrhagic disease which affects domesticated animals including (cattle, buffalo, sheep, goats, and camels) and can also affect human. The disease is caused by (RVF) virus, which is a member of the genus Phlebovirus in the order Bunyavirales (CDC, 2020). RVFV is a negative-sense, tri-segmented RNA virus, only one serotype is recognized but strains exist of variable virulence (OIE, 2019). The disease is one of the OIE-listed diseases and must be reported to the OIE, because of its long inter-epizootic intervals it is also regarded as a re-emerging disease (OIE, 2021). Outbreaks of RVF are closely connected with climatic abnormalities as heavy rains and prolonged flooding, which make habitat is more suitable for carrier populations, thus increasing the risk of disease

emergence, transmission and spread (FAO, 2019). The epidemics of RVF may expand all over the world due to demographic changes in the climate and environment in areas with close interaction between livestock, wildlife, vectors and human population Linthicum *et al*. (2016). Intensive animal production activities close to human populations and increased Bedouin's movement of livestock through areas with potential vector breeding sites during a search for pasture and water pose a threat of outbreaks emerged by RVFV to many countries. The virus was first identified in the Rift Valley of Kenya in 1931 during spread of a disease among sheep farms. Then, outbreaks have been reported in African. Sahara. In 1977 an eruptive outbreak was reported in Egypt. The RVF virus was transmitted to Egypt through infected livestock trade along with the Nile River. In 1997–1998, a large outbreak detected in Kenya, Somalia and Tanzania after El Niño event and extensive flooding. During infected livestock transmission from the horn of Africa, RVF spread in September 2000 to Saudi Arabia and Yemen, marking the first reported disease occurrence outside Africa and raising concerns about extending to other parts of Asia and Europe (WHO, 2018).

Systems for early warning are required to develop the suitable mitigation measures Anyamba *et al*. (2019); in the last outbreaks, late warnings, especially after rains and flooding resulted in massive economic losses Murithi *et al*. (2011).

Bovine ephemeral fever virus (BEFV) a member of the genus Ephemerovirus, family Rhabdoviridae (He *et al*., 2016). The disease is characterized by different symptoms including depression, fever, difficult swallowing, serous ocular and nasal discharges, dyspnea, stiffness and lameness (Nandi and Negi, 1999).

Generalized inflammatory signs and toxicity associated with Bovine ephemeral fever (BEF) disease were attributed to the huge amounts of interferon (IFN) produced (George, 1992). Interferons (IFNs) are cytokines divided into three types according to their cell receptor, type I, type II, and type III possess a remarkable ability to evoke both innate and acquired immune responses against viral infections (Negishi *et al*., 2018). IFNs are glycoproteins in nature having strong antiviral activities which represent one of the first defense lines of the host against invading pathogens (Lin and Young, 2014). Bovine ephemeral fever disease has a complicated nature that probably reflects pathophysiologic and immunologic effects mediated by the release and activity of different inflammatory mediators (so-called cytokine storm) (Dubovi *et al*., 2011). Extracellular biomolecules can induce secretion of INFs through stimulation of Toll-like receptors (TLRs) (Cheon *et al*., 2014). Desai (2016) found that the natural INFs seems to be less toxic than synthetic ones when used in treatment. Type I interferons are a group of cytokines that have an antiviral activity and are induced during viral infection by viral replication products, such as double stranded (ds) RNA. IFNs exert their biological functions by binding to specific cell surface receptors. Interferons work in a few different ways. They: warning the immune system so it can follow the virus or cancer cells, help the immune system detect the virus or cancer cells, tell immune cells to attack, stop virus dividing and cancer cells growing besides helping healthy cells fight the infection (Stephanie, 2020).

Because of the importance of obtaining an antiviral agent in an economical way that could be used to minimize the tremendous economic losses caused by several viral diseases such as (RFV), this study was designed as a preliminary step to investigate the effect of (BEFV) interferon induced in cell cultures against (RVFV).

Materials and Methods

Viruses

Bovine ephemeral fever virus (Albehwar *et al*., 2017) with a titer 10^6 $TCID_{50}$/ml was propagated on African green monkey kidney cell culture (Vero) and Madin-Darby Bovine Kidney cell culture (MDBK) to induce interferon production. Rift Valley Fever virus (RVFV ZH 501) strain was initially passaged intracerebrally into suckling mice then propagated and titrated on Vero cells with a titer of $10^{-8.5}$ $TCID_{50}$/ml (El-Nimr, 1980). It was used to investigate the in vitro antiviral effect of BEFV induced interferon. Both viruses were supplied by Department of Pet Animal and Vaccine Research and Department of Rift Valley Fever Research, Veterinary Serum and Vaccine Research Institute (VSVRI) Abbasia, Cairo, Egypt.

Cell cultures

African green monkey kidney cell culture (Vero) and

Madin-Darby Bovine Kidney cell culture (MDBK) were used to prepare for induction of interferon production by (BEFV). Vero cell culture was used also to screen the antiviral effect of the produced interferon against RVFV. Both cell cultures were supplied by VSVRI and propagated and maintained using Minimum Essential Media (MEM) obtained from Gibco Company containing 10% Newborn Calf Serum (NCS) obtained from the U.K by GIBCO.

Induction of interferon by BEFV

Confluent sheets of MDBK and Vero cell cultures were prepared in 25ml tissue culture flasks were inoculated with 10^6 $TCID_{50}$ BEFV at multiplicity of infection (M.O.I.) 2:1 according to Tzipori (1975) and allowed to virus adsorption at 37°C for one hour.

Inactivation of (BEFV)

The infected media were collected on 12, 24 and 48 hours post cell infection and centrifuged at 800 rpm for five minutes at cold centrifuge then subjected to UV radiation at wavelength 254nm for 20 minutes, according to Tsunetsugu-Yokota (2008) so; we obtained six samples of prepared interferon.

Cytotoxicity assay of the prepared BEFV interferon

According to Jureka *et al*. (2020), cytotoxicity of BEFV interferon was tested by inoculation of tenfold dilutions (up to 10^{-7}) of each time preparation on confluent sheets of Vero cell culture prepared in 96 microtiter plates. Each dilution was inoculated in each of 6 wells leaving non-inoculated wells as standard cell control. The plates were incubated at 37°c and subjected to daily microscopic examination.

Investigation of the antiviral effect of BEFV induced interferon against RVFV

Three groups of Vero cell culture plates were used to investigate the effect of BEFV induced interferon against RVFV according to Usharani *et al*. (2017). First group of Vero cell culture plates were infected with 25 µl/ well of RVFV 100 $TCID_{50}$. After one hour allowed for virus adsorption, the cells were washed twice with PBS, then each one of the prepared interferon concentrations (from undiluted up to 10^{-7}) was added to each of 5 tissue culture wells with 150 µl of maintenance medium. The second group of plates were inoculated firstly with the interferon followed by RVFV one hour later while the third group of plates was inoculated simultaneously with RVFV and BEFV interferon. The test included normal cells and untreated virus controls and all plates were incubated at 37°C and subjected to daily microscopic examination until virus control showed 100% cytopathic effect (CPE).

Results and Discussion

The cytotoxic screening showed that all interferon preparations induced by BEFV in Vero cell cultures have normal appearance without any abnormal cellular changes (Figures 1 and 2) indicating their safety. These findings agree with what was reported by Wang *et al*. (2004) and Eickmann *et al*. (2020). Stewart (2012) stated that a number of viruses cause cell damage that can be visualized by light microscope and any of these cytopathic effects can be used to quantify the protection of cells by interferon. This method has been used to assay nearly every type of interferon against many viruses having the advantages of simplicity, speed, and economy of samples and supplies. The effect of the interferon induced by BEFV against RVFV as assayed in Vero cell culture revealed that inoculation of interferon one hour before RVFV inoculation induced antiviral effect up to dilutions of 10^{-2} for interferon produced in Vero cells and up to 10^{-3} for that produced in MDBK cells whether the used interferon was obtained 12 or 24 or 48 hours post cell infection with BEFV as shown in Table 1. These findings qualify interferon suggesting its ability to play a prophylactic role in animal protection that have not yet been infected during outbreaks and agree with the suggestion of Michael *et al*. (2002) stated that the action of interferons (IFNs) on virus-infected cells and surrounding tissues evoke an antiviral state that is characterized by the expression and antiviral activity of IFN-stimulated genes. On the other side, inoculation of the interferon one hour after the inoculation of RVFV showed antiviral effect up to a dilution of 10^{-2} and 10^{-4} using the interferon produced by Vero cell 12 and 24 hours post cell infection with BEFV, respectively. Vero cells produced interferon 48 hours post cell infection and produced in MDBK cells 12, 24, and 48 hours post-infection showed antiviral effect against RVFV up to a dilution of 10^{-5} as in Table 2. These findings suggest that the interferon produced in Vero cells is increased by time post cell infection by BEFV from 24-48 hours post-infection while the ability of MDBK to produce interferon was higher than that of Vero cells. In this respect and at the biological level, the antiviral effects of interferon may be viewed to be virus-type nonspecific. The treatment

Table 1: *Antiviral effect of interferon induced by BEFV in Vero and MDBK cell culture against RVF as inoculated in Vero cells one hour before RVFV infection.*

Interferon dilution	Vero produced interferon after			MDBK produced interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI
Undiluted	+**	+	+	+	+	+
10^{-1}	+	+	+	+	+	+
10^{-2}	+	+	+	Retarded CPE	+	+
10^{-3}	-***	-	-	-	+	+
10^{-4}	-	-	-	-	-	-
10^{-5}	-	-	-	-	-	-
10^{-6}	-	-	-	-	-	-

HPCI: hours post cell infection; ** +: Positive antiviral effect; * -: negative antiviral effect.*

Table 2: *Antiviral effect of interferon induced by BEFV in Vero and MDBK cell culture against RVF as inoculated in Vero cells one hour after RVFV infection.*

Interferon dilution	Vero produced interferon after			MDBK produced interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI
Undiluted	+**	+	+	+	+	+
10^{-1}	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+
10^{-3}	-***	+	+	+	+	+
10^{-4}	-	+	+	+	+	+
10^{-5}	-	-	+	+	+	+
10^{-6}	-	-	-	-	-	-

HPCI: hours post cell infection; ** +: Positive antiviral effect; * -: negative antiviral effect.*

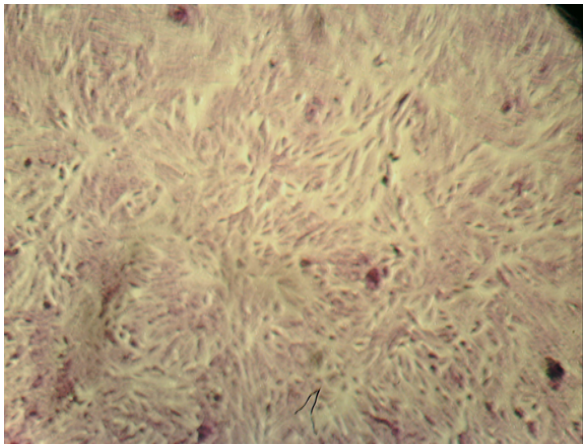

Figure 1: *Normal Vero cell culture (100xs).*

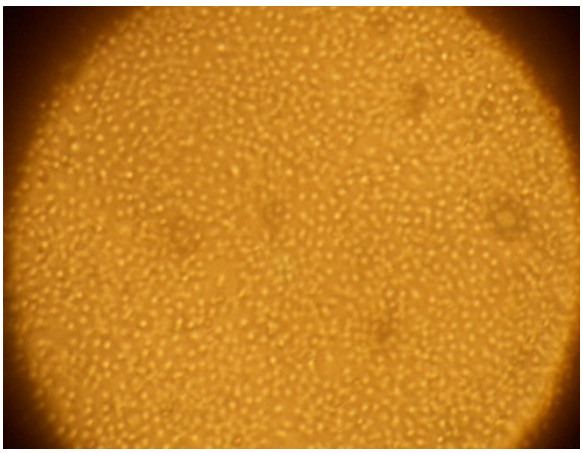

Figure 2: *Normal MDBK cell culture (100Xs).*

of cells with one type or even subspecies of interferon often leads to the generation of an antiviral state effective against a wide range of different RNA and DNA animal viruses, as explained by Charles (1991).

Regarding simultaneous inoculation of Vero cell culture with the interferon produced by BEFV, either in Vero or MDBK cell cultures, Table 3 showed that all obtained interferon products induced antiviral effect against RVFV up to dilutions 10^{-5}. These results indicated that the best antiviral effect induced by BEFV-produced interferon against RVFV is obtained by simultaneous inoculation followed by interferon inoculation after the virus inoculation. INFs can change the pathogenicity of the virus by inhibiting virus replication of a wide range of different DNA and RNA animal viruses, in both animals and cell culture at the intracellular level and by stimulating the specific and nonspecific immune responses to viral antigens in the body fluids and on cellular surfaces (Pestka *et al.*, 1987; Sarkar and Sen, 1998; Stark *et al.*, 1998).

The result of this study is consistent with Usharani *et al.* (2017) who concluded that ovine interferon tau 4 has antiviral activity against Foot and mouth disease virus *in vitro* simultaneously, before and after infection.

Table 3: *Antiviral effect of interferon induced by BEFV in Vero and MDBK cell culture against RVF as inoculated in Vero cells simultaneously with RVFV.*

Interferon dilution	Vero produced interferon after			MDBK produced interferon after		
	12HPCI*	24HPCI	48HPCI	12HPCI	24HPCI	48HPCI
Undiluted	+**	+	+	+	+	+
10^{-1}	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+
10^{-4}	+	+	+	+	+	+
10^{-5}	+	+	+	+	+	+
10^{-6}	-***	-	-	-	-	-

HPCI: hours post cell infection; ** +: Positive antiviral effect: * -: negative antiviral effect.*

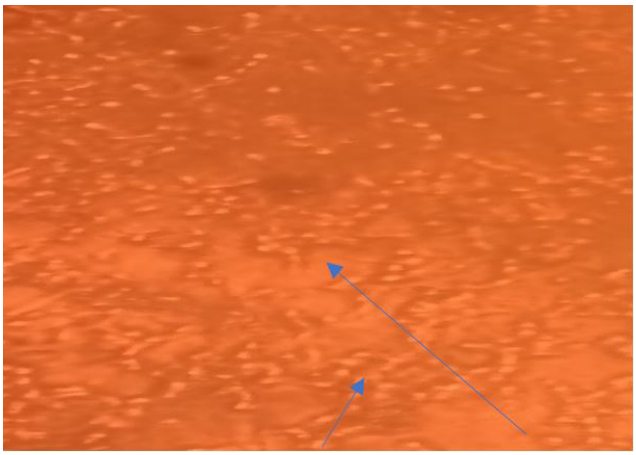

Figure 3: *Vero cell culture infected with RVF virus showing cell rounding and vacuolation (100Xs).*

The relative sensitivity of different animal viruses to interferon effects varies noticeably. Three essential components are required to measure the effectiveness of antiviral response: The species of interferon administered, the kind of cell treated and the type of the virus used to challenge the interferon-treated host cell. The viral sensitivity to the inhibitory action of IFN is attributed to the qualitative nature and quantitative amount of the individual IFN-regulated cell proteins that may participate in the inhibition of virus replication. Moreover, virus gene products or intermediates that serve as activators of IFN-regulated protein function must be balanced with those products that inhibit IFN-regulated protein function (Charles, 1991).

Several studies reported that IFNs are strong regulators of the essential cellular processes. Viruses are able to control the metabolic functions of the host cells to replicate and produce infectious particles efficiently. So, interfering with metabolic pathways seems to represent one of the essential antiviral properties of IFNs (Jorg, 2016).

Regarding the nature and the antiviral effect of interferon, they were found to be potent pleiotropic cytokines that vastly change cellular functions in response to viral and other infections. These changes include altering in protein synthesis, proliferation, membrane structure, and the nutritional microenvironment. Jorg (2016) suggests that antiviral responses are supported by an IFN-induced rewiring of the cellular metabolism. Although it was known that interferon is secreted by cells in response to stimulation by a virus or other foreign substance; but it does not directly inhibit the virus multiplication. Rather, it stimulates the infected cells and those adjacent ones to produce proteins that prevent the virus replication inside them. All interferons were found to have common effects: They are antiviral agents, and they modulate the functions of the immune system (Anon, 2021).

Depending on the present obtained results, we could conclude that it was a success to prepare interferon by infecting Vero and MDBK cell cultures with BEFV in a high concentration that was able to act as antiviral against RVFV. We support that the use of IFNs, both in cell culture and in animals, should be subjected to further research to provide significant contributions to our understanding of the virus-host interaction and cellular antiviral response. Also, further *in vivo* studies are required to investigate the probable side effects of interferon on treated animals to determine to what extent it could be used as a post-infection treatment.

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Novelty Statement

This work includes exploiting the cytokine Storm property that characterizes BEFV to produce an effective and economically abundant amount of interferon to be used.

Author's Contribution

Sahar M. Saber: Research idea and working protocol.
Ahmed M. A. Albehwar: Preparation of interferon in tissue cultures.
Amani M. Abas: Tissue culture preparation.
Omaima A.A. Alshamandy: Three-day fever virus injected into tissue cultures.
Noha A. El said and Marwa Y. Hamad: Study of the effect of interferon on Rift Valley Fever virus in tissue cultures.

Conflict of interest
The authors have declared no conflict of interest.

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