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In Vitro and In Vivo Antiviral Effect of Bee Venom on Rift Valley Fever Virus

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ABSTRACT

This work aimed to document the in vitro and in vivo antiviral activity of Bee Venom (BV) against Rift Valley Fever virus (RVFV) in Baby Hamster Kidney (BHK) cell culture and in adult mice respectively. The toxicity inspection of Bee Venom on BHK cells and baby mice was studied in order to define the non-toxic dose clarified that various concentrations of BV (started from the premier concentration "1mg / ml up to 0.1 µg / ml) didn't show any up normal cellular changes and no clinical signs or deaths in mice. The effective antiviral effect of BV against RVFV in BHK cells; as in vitro assay; was detected depending on the inhibition of the cytopathic effect of RVF virus using the undiluted venom and that diluted to100µg/ml that was used at a dilution of

In vivo investigation showed that mice received BV (100µg/ml/mouse) on the o, 1st, 2nd, 3rd and 4th day post infection were able to resist the contagion showing protection rate of 100 - 70%, while those received the treating on the next subsequent days were unable to beat the infection and died by the 2-3 days next to infection. It could be consummated that bee venom could be used to treat or even to stop the progress of Rift Valley Fever infection when administrated on the appropiate time post exposure.

Introduction:

Rift Valley fever (RVF) is an acute, arthropod (Mosquito) born viral zoonotic disease **(**Ndeye *et al.,* 2017**)**. It is caused by Rift Valley Fever virus which is a member of Phlebovirus of the bunyaviridae, single stranded RNA virus **(**Samia *et al.,* 2011**)**. The disease fundamentally affects sheep, goats, cattle, camels, buffaloes and humans causing fever, weakness, fetid diarrhea, reduction in milk production with storm of abortions in pregnant animals **(**FAO. 2003**)**. In human, it causes conjunctivitis with ocular elaboration and headache **(**CDC, 2007**)**. RVF outbreaks were related to mosquito spreading (Nguku**,** 2006**)**. The disease was first reported through livestock in Kenya around 1915, but it

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was first identified in 1931 during an investigation into an epidemic among sheep on a farm in the Rift Valley of Kenya (WHO, 2018**)**. It was introduced to Egypt meanwhile importation of infected ruminants and camels from Sudan (Sellers *et al.*, 1982) and reappeared in 1993 in Egypt **(**El-Gabry *et al.*, 1994**)**. It was detected in Egypt at Belbes and Zagazig Governorate and the virus has been isolated and designated **(**Imam et al., 1979**)**. There for together control and preventive measures are necessary. Traditionally, the preferable tool for protection of animal inhabitance and indirectly humane being is the vector monitoring and vaccination (Abdel -Gaffar et al., 1979). Rapid and effective control of RVF can be accomplished by the use of antiviral compounds. Current days there is tendency toward the use of natural antiviral materials to overcome or decrease the serious of viral diseases **(Eiaka** *et al*., 2016**)**.

Bee venom is a compound mix of proteins, peptides and low molecular component. BV has been detected to have antiviral and anti-inflammatory effects as it has a diversity of compounds present in it, that involve peptides such as Melittin (MLT), Adolapin, Apamin and enzymes such as phospholipase (Son *et al,* 2007). The main ingradient are proteins and peptides. BV has abundant polypeptides, the main one is melittin (Shkenderov and Ivanov, 1983) in addition contains many biochemical or pharmacologically active substances, including antibacterial, antiparasitic and antiviral properties (Guillaume *et al.,* 2006). The viricidal action of bee venom on Herpes simplex virus type -1(HSV1) and Adenovirus type -7(adeno-7) as a DNA virus models studies reported that there was a significant depletion rate of virus infectivity titer 6 and 24 hrs. post treatment recording 3.7 log10/0.1 ml and 2.75 log10/0.1 ml respectively. Also, the evaluation of viricidal activity of bee venom against RNA model virus of West Nile virus (WNV) revealed that there was a critical decrease in WNV infectivity titer post thermal treatment incubation at 37^{o}C for 3, 6, and 24 and 48hours post treatment with venom recording 5.1 log10/0.1 ml, 4.5 log10/0.1 ml, 2.1 log10/0.1 ml and 0.55 log10/0.1 ml respectively (Rawhia *et al*., 2009).

The co-incubation of noncytotoxic concentrations of bee venom or melittin, the main component of bee venom, significantly inhibited the replication of the Influenza A virus (PR8) and the respiratory syncytial virus (RSV) (Uddin *et al*., 2016). Also, bee venom significantly enhanced the discrimination of FOXP3-expressing cells in CD4 T cells and mature CD4 thymocytes. It was suggested that bee venom stimulates the differentiation of human regulatory T cells, that plays very important role in the immune response induced against SARS-COV infection (Caramalho *et al*., 2015).

The current study steers to spot the light on the antiviral activity of bee venom against RVFV infection as neoteric directions toward the use of antiviral components for security or treatment of viral diseases.

MATERIALS and METHODS

*Ethical approval:

This study was confirmed by the Animal Ethics Committee of the Veterinary Serum and Vaccine Research Institute (VSVRI). All experiments agreed with the VSVRI guidelines for animal research.

1. Bee venom: it was obtained from Sera plant, The Holding Company for Biological Products and Vaccines (VACSERA). The original concentration is 1mg/ml saline

2- Rift Valley Fever virus (RVF): Local Rift Valley Fever (RVF) virus isolated from a human patient in Zagazig, Sharkia Province **(**Imam *et al*.,1979**)**, Egypt and supplied by NUMRU -3 with a titer of 10^{8} $TCID_{50}$ /ml was obtained from the Department of Rift Valley Fever Research (DRVFR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia Cairo.

Tissue culture: Baby Hamster Kidney cells (BHK21), were supplied by the tissue culture unit in RVF department, VSVRI. They were propagated using minimum essential medium with Earl's salts supplemented with 10% sterile newborn calf serum (Macpherson, and Stocher, 1962).

4-Mice:

4.1-Baby mice: Forty, 3-4 days old Swiss Albino baby mice were supplied by the unite of laboratory animals (ULA); VSVRI and used for detection of BV toxicity test.

4.2-Adult mice: One hundred and forty, 21-28 days Swiss albino mice supplied by the same source were used to detect the in vivo antiviral action of BV against RVFV

5- Determination of honey bee venom toxicity:

5.1- In vitro cytotoxicity assay:

The bee venom safety was tested on cultivation of monolayer of Baby hamster kidney cell culture (BHK) according to Eiaka *et al*., (2016), by fixing its cytotoxic effect by applying serial tenfold dilutions of the Bee venom in Hank's balance salt solution. Then from each dilution, 25 µl was inoculated in each of 5 wells of tissue culture 96 wells plate. Wells with normal cell culture were considered as control. Then incubate the plate at 37° C with daily microscopic examination for detection of any cell abnormalities up to 7 days.

5.2-In vivo toxicity assay: Was applied according to Eiaka *et al*., (2016), by preparing tenfold dilutions of bee venom in normal saline 7.2 pH started from the original matter up to 10^4 (1/10000) then each dilution was inoculated in ten mice intraperitoneally (I/P) using a dose of 0.2 ml / mouse. Control ten mice were kept without inoculation. Under hyegenic conditions, all mice were kept with daily observation up to 15 days post inoculation for detecting any abnormal signs.

6-Investigation of the antiviral effect of bee venom against RVF virus:

6.1- In vitro antiviral assay:

BHK cultured plates were infected with RVF virus, according to Eiaka *et al*., (2016) using 25 µl/ well of 10^2 $TCID_{50}$ of the used virus. After one hour allowed for virus adsorption, the cells were washed twice with phosphate buffer saline then 25 µl of each of the prepared bee venom concentrations (undiluted up to 10^4 i.e., from 1mg to 10µg/ml) was added to each of 5 tissue culture wells with 150 µl of maintenance medium. The test included normal cells and untreated virus controls. All plates were subjected to daily microscopic examination at 37°C.

6.2-In vivo antiviral assay: Ten groups of adult mice (10 mice/group) were experimentally infected with 0.2 ml RVF virus 10^3 $TCID_{50}$ / ml intraperitoneally. All mice groups were treated with BV inoculated I/P using a dose of 50 ug/ mice (0.5 ml) on the 0,1,2,3,4,5,6,7,8,9 and 10 days post infection.

In addition, other 3 adult mice groups were included as positive control RVFV infected untreated group; negative uninfected and untreated group and BV inoculated group.

All mice were kept under hygienic measures receiving balanced ration and adequate water and subjected to daily observation for 15 days for detecting any abnormal signs or deaths.

Results:

Toxicity testing of Bee venom:

1.1-In vitro:

Inoculation of different concentrations of BV in tissue culture (starting from the master concentricity 1mg / ml up to 0.1 ug / ml) didn't show any abnormal cell growth or cellular changes with a regular growth rate.

1.2-In vivo:

Mice inoculated with different concentrations of BV (starting from the master concentration 1mg / ml up to 0.1 ug / ml) didn't show any abnormal clinical signs and no deaths. These data are tabulated in table (1).

Table 1. In vitro and in vivo toxicity assay of Bee venom mice :

Tested dilution of bee venom	Tested concentration of bee venom	In vitro assay Cytotoxicity % in BHK cells	In vivo assay No. of dead mice
Original concentration	1 mg/ml	0	0/6
1:10	100 ug/ ml	0	0/6
1:100	10 ug/ml	0	0/6
1:1000	1 ug / ml	0	0/6
1:10000	0.1 ug / ml	0	0/6

2-Antiviral effect of Bee venom against RVFV:

2.1- In vitro antiviral assay:

Positive BV anti-RVFV effect was obtained using the undiluted (1mg/ml) and a dilution of 1:10 (100µg/ml) as determined by complete inhibition of the CPE of 100$TCID_{50}$ of the virus while higher dilutions had no antiviral effect as shown in table (2).

Table 2. In vitro BV-anti-RVFV assay :

Tested Concentration of bee venom	Used BHK cell culture
Undiluted (1mg/ ml)*	Positive anti-RVFV (No CPE)
1:10 (100 ug/ ml)*	
1:100 (10 ug/ ml)	Negative anti-RVFV (positive CPE)
1:1000 (1 ug/ ml)	
1:10000 (0.1 ug/ml)	

*Effective concentration of Bee venom.

2.2. In vivo BV antiviral assay:

The in vivo assay of Bee Venom anti-RVFV assay showed that mice treated with 100µg/ml/ mouse on the o, 1st, 2nd, 3rd and 4th day post infection were able to withstand the infection showing protection rate of 100 - 70%. Delayed treatment of infected mice did not enable them to overcome the infection and died by the 2-3 days post infection as shown in table (3).

Table 3. In vivo Bee venom anti-RVFV assay :

TD*	No. of mice	Number of mice during the experimental period (1-15 days)															**Survival %**	**Mortality %**
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0 time	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	0
1DPI**	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	0
2DPI	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	0
3DPI	10	10	10	10	10	10	10	10	10	9	9	9	9	9	9	9	90	10
4DPI	10	10	10	10	10	9	9	8	8	8	7	7	7	7	7	7	70	30
5DPI	10	10	10	10	9	8	8	7	5	4	3	3	3	3	3	3	30	70
6DPI	10	10	10	9	7	5	5	4	2	1	1	1	1	1	1	1	10	90
In 7DPI and positive control untreated infected mice typical RVF signs began to appear and death within 2-3 days after that showing 100% mortality																		
Control -VE	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	0
Treated With BV	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100	0

*TD- treatment day **DPI= day post infection

DISCUSSION:

The evolution of antiviral drugs is yet in its infancy with rapid changes and progress almost daily. The latest two decades have been the most efficacious in the history of viral infections and their handling. Antiviral drugs proved to be efficient for only a few groups of viruses until now. Majority of antiviral drugs do not yield a cure, but allow holding the infection. However, the limitations of antiviral therapy due to the high costs of drugs, making the requirement for prohibition even more demanded (Trying , 2005)**.**

The virucidal drugs have been advised for minimizing the outbreaks, and to decrease the number of infected animals through outbreaks. This paper studied the antiviral activity of bee venom against RVFV.

lately, the power of bee venom as a therapeutic or prophylactic operator has got the awareness of researchers around the world. Researches have shown the efficiency of bee venom versus different types of cancer as well as anti-viral action including human immune deficiency virus (HIV) and the made of action on the virus particles (Wehbe et al., 2019).

The in vitro and in vivo toxicity assays of the Bee venom on the susceptible cells (BHK cells) and baby mice were studied. The venom has to be active against the virus without induction of any presumed toxicity on the cells and baby mice. subsequently, the maximum concentration of the BV at which there is no marked toxicity to the cell and baby mice was detected. Regarding this aspect, the results demonstrated in table-1 showed clearly that the different tested concentrations of Bee venom didn't show any retarded cell growth or cellular changes. Parallel to these results of in vitro studies, the in vivo investigation of BV cytotoxic effect revealed that mice inoculated with different concentrations of BV showed no deaths and no abnormal clinical signs. These results agreed with that obtained by Eiaka et al. (2016), who reported that various concentrations of BV (started from the master concentrations 1mg/ ml up to 1:1000) didn't show any cell expansion inhibition or cellular changes only normal growth and normal cell shape appeared.

On the other side, it was found that 1mg/ml and 100 ug/ml of bee venom were able to inhibit the replication of 10^2 $TCID_{50}$ of Rift Valley Fever virus in BHK cells while the concentrations 10, 1 and 0.1 µg/ ml were unable to do this showing clear specific CPE of RVFV as shown in table -2, in agreement with (15), who reported that the viricidal action of bee venom against RNA virus of West Nile virus (WNV) revealed that there was a marked decrease in the virus infectivity titer post incubation at 37^{o}C for 3, 6, 24 and 48 hours post treatment with bee venom.

Parallel to these findings of in vitro studies, the investigation of in vivo BV antiviral effect showed that mice inoculated with 0.5 ml (50 µg/ mice) on the time of infection and on the first and second day post infection showing protection percent 100%, and this percent started to be decreased starting from 90% in group treated in the 3rd day post infection, then 70%, 30%, 10% in groups treated with BV in 4th, 5th, 6th days post infection respectively, till reach 0% in 7th and 8th days post infection as shown in table -3. These results confirmed what reported by Eiaka et al. (2016), who found that post exposure treatment against rabies should be as fast as possible and the late administration leads to fast disease rise. Also, our results agree with Filotti *et al*., (1967**)** who regarded the virucidal activity of bee (Joshua *et al*., 2015) venom at 24 hrs. post infection In addition, stated that the toxin found in bee venom Melittin destroys some viruses which can poke holes in the protective viral envelope that surrounds the viruses and Noura *et al*. (2020) suggested that this action is possible where Melittin attacks the membranes of the virus.

Also, Noura *et al*. (2020) reported that bee venom inhibited FMDV in two ways. first, it induced antiviral state in cells as indicated by increased IFN gamma level. Secondly, Melittin in BV inactivated FMDV by directly binding with the virus particles i.e., virucidal activity.

Depending on these results, the administra-

tion of BV in the field for handling of RVFV infection could aid to prevent or even minimize RVFV infection as a new control measure of the disease.

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