



Evaluation of the antiviral effect of bee venom on rabies virus

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ABSTRACT

It is an interesting thing to know the effect of natural matter as bee venom as antiviral agent and to any extent it could be used in case of experimental rabies infection. In vitro study revealed that different concentrations of bee venom (started from the original concentration "1mg/ml" up to 1:1000) did not show any cell growth retarders or cellular changes showing normal growth rate and normal cell shape. 1mg/ml and 100µgm/ml of bee venom were able to inhibit the replication of 100 TCID₅₀ of rabies virus (ERA strain) in using baby hamster kidney BHK cells while 10 and 1µgm/ml were negative. In vivo study showed that mice received rabies immune globulin and BV were able to withstand the rabies infection when they were administrated on the 0, 1st, 2nd and 3rd day post infection showing protection rate of 90 and 80% respectively but those mice received the treatment on the following days did not survive the infection and showed paralysis of the hind limbs by the 4th day and died by the 6-7th day post infection. It could be concluded that bee venom could be used in case of rabies infection when administrated on the suitable time post exposure.

Key word: Antiviral, Bee Venom, Rabies Virus.

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1. INTRODUCTION

Rabies is an acute viral disease of the nervous system of warm-blooded animals that is caused by a rhabdo virus (species *Rabies virus* of the genus *Lyssa virus*) (National Centre for Disease Control, 2014). The disease transmitted by infected saliva usually through the bite of rabid animal as well as scratches and that is characterized typically by increased salivation, abnormal behavior, and eventual paralysis and death of untreated cases (WHO, 2014). The disease is almost fatal following the onset of clinical signs. The disease is preventable by timely administration of post exposure prophylaxis (PEP) consisting of four or five doses of rabies vaccine combined, in the most severe cases of exposures, with anti-rabies immunoglobulin (RIG). PEP consists of a multimodal approach to decrease an individual's likelihood of developing clinical rabies after a possible exposure to the virus. Every year, approximately 55000 individuals worldwide die due to rabies infection (Bourhy et al., 2009). A study of one year follow-up enrolled 193 patients; aged 16 months to 79 years; received post exposure prophylaxis (PEP) encompasses wound cleaning; infiltration of rabies immunoglobulin (RIG) and rabies vaccination following exposure to baiting of rabid animals. Where serious adverse events were

considered related to PEP recommended that PEP treatment is highly effective where 191 (99%) patients were in good health one year post exposure (Quiambao et al., 2009). Nowadays there is an attention toward the use of natural antiviral agent to overcome serious viral diseases. Bee venom is a complex mixture of proteins, peptides and low molecular component. The main components are proteins and peptides. BV has numerous polypeptides the main one being melittin (Shkenderov and Ivanov, 1983) in addition contains several biochemical or pharmacologically active substances, including antibacterial, antiparasitic and antiviral properties (Guillaume et al., 2006). The viricidal activity (direct action) of bee venom on Herpes simplex virus type-1 (HSV-1) and Adenovirus type -7 (adeno-7) as a DNA virus models studies revealed there was a significant depletion rate of virus infectivity titer 6 and 24 hrs. post treatment recording 3.7 log (10)/0.1 ml and 2.75 log (10)/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 significant decreased in WNV infectivity titer post thermal treatment incubation at 37°C for 3, 6, and 24 and 48 hours post treatment with venom

recording 5.1 log (10)/0.1 ml, 4.5 log (10)/0.1 ml, 2.1 log (10)/0.1 ml and 0.55 log (10)/0.1 ml respectively (Rawhia et al., 2009). The evaluation of veridical activity of bee venom against RNA model virus of West Nile virus (WNV) revealed that there was significant decrease in Adeno virus titer 3, 6 and 24 hours post incubation at 37°C with BV was noticed (Jason, 2013). Also, evaluation of the antiviral activity of bee venom against RNA model virus WNV revealed that there was a non-significant decrease in West Nile Virus titer after incubation period of 3, 6 and 24 hours' post treatment at 37°C. Melittin can poke holes in the protective viral envelope that surrounds the human immunodeficiency virus, as well as other viruses (Hood et al., 2013). Hassan et al. (2015) used two different virus models namely Adeno-7 as DNA model and RVFV as RNA virus models to investigate the antiviral effect of BV. The depletion of viral infectivity titer of ABW to Adeno-7 virus showed strong antiviral activity recorded a depletion of viral infectivity titer (1.66 log (10)/ml) that gave equal action with BV and more than interferon IFN (1 log (10)/ ml).

The present study aims to spot the light on bee venom as antiviral in rabies infection as recent directions toward the use of antiviral components in trials for protection or treatment of viral diseases.

2. MATERIALS AND METHODS

2.1. Bee venom:

Honey BV was supplied by Sera plant, The Holding Company for Biological Products and Vaccines (VACSERA). It was obtained at a concentration of 1mg/ml saline

2.2. Challenge virus strain (CVS):

CVS mice brain adapted rabies virus with a titer of 10^6 MLID₅₀/ml was supplied by DPAVR and used for the induction of experimental rabies infection in mice.

2.3. Rabies immune-globulin:

It was prepared by Albehwar (2009) and supplied kindly by the DPAVR and used in a comparative evaluation with bee venom as antiviral substances.

2.4. Safety assay of honey bee venom:

2.4.1. In vitro safety assay:

The safety of bee venom was tested in cultivation monolayer of Baby hamster kidney BHK cell culture through determination of its cytotoxic effect where serial tenfold dilutions of

the venom were prepared in Hankes balance salt solution HBSS and 25µl of each dilution were inoculated in each of 5 wells of 96 wells tissue culture plates. Normal cell culture wells were included as test control. The cell culture plates were incubated at 37°C and subjected for daily microscopic examination for determination of any cell abnormalities up to 7 days.

2.4.2. In vivo safety assay:

Ten fold dilutions of bee venom were prepared in normal saline 7.2 pH started from the original material up to 10^3 where each dilution was inoculated intraperitoneally in ten mice using a dose of 0.5ml/mouse. Ten mice were kept without inoculation as test control. All mice were kept under observation up to 15 days post inoculation

2.5. Investigation of the antiviral effect of bee venom:

2.5.1. Invitro antiviral assay:

BHK cultured plates were infected with rabies virus using 25 µl/ well of 100 TCID₅₀ of the used virus. After one hour allowed for virus adsorption, the cells were washed twice with phosphate buffer saline PBS then each one of the prepared bee venom concentrations (undiluted up to 1 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.

2.5.2. In vivo antiviral assay:

Four hundred albino Swiss mice were divided into 6 groups as follows: Group 1 and 2 each of 100 mice were experimentally infected with the CVS through the intra-cerebral route using 0.03 ml/mouse according to Seligmann (1973). Group-1 was subdivided into 10 subgroups which were treated with BV inoculated I/P using a dose of 50µgm/mouse on the 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days' post infection. Group-2 was subdivided into 10 subgroups (10 mice/subgroup) treated with rabies immunoglobulin (342mg/mouse) according to Albehwar (2009) inoculated I/P on the same periods post infection. Groups 3-6 (each contains 50 mice) were treated as follow: Group-3 was kept infected with the CVS without treatment. Group-4 was inoculated I/P with rabies immunoglobulin alone. Group-5 was inoculated I/P with BV alone. Group-6 was kept without infection and without treatment as normal control. All mice were kept under hygienic measures receiving balanced ration and adequate water and subjected to daily observation.

3. RESULTS

3.1. In vitro testing the safety and antiviral effect of bee venom:

Inoculation of different concentrations of BV (started from the original concentration “1mg/ml” up to 1:1000) did not show any cell growth retarders or cellular changes where normal growth rate and normal cell shape. It was found that 1mg/ml and 100µg/ml of bee venom were able to inhibit the replication of 100 TCID₅₀ of rabies virus (ERA strain) in BHK cells while 10 and 1µg/ml were unable to prevent or reduce the virus

replication showing clear specific CPE as demonstrated in table (1)

3.2. In vivo safety and antiviral effect of BV:

It was found that mice received rabies immune globulin and BV were able to withstand the infection when they were administered on the 0, 1st, 2nd and 3rd day post infection showing protection rate of 90 and 80% respectively but those mice received the treatment on the following days did not survive the infection and showed paralysis of the hind limbs by the 4th day and died by the 6-7th day post infection as demonstrated in table (2).

Table (1): In vitro safety and antiviral activity of bee venom in BHK cell culture

Tested concentration of bee venom	Determined changes in BHK Cell culture	Antiviral activity of bee venom against 100TCID ₅₀ of rabies virus
Undiluted (1mg/ml)		Inhibited virus replication showing no CPE
1:10 (100µg/ml)	There were neither cell changes nor growth inhibition	Did not affect virus replication showing clear specific CPE
1:100 (10µg/ml)		
1:1000 (1µg/ml)		

Table (2): In vivo safety and antiviral effect of bee venom

Inoculated Material	IgG	Number of survived mice/number of treated infected mice = protection%							
		1DPI*	2DPI	3DPI	4DPI	5DPI	6DPI	7DPI	8DPI
Rabies (342mg/mouse)		100	100	90	40	10	0	0	0
BV (50µg/mouse)		100	100	80	30	3	0	0	0
Infected control		Began to show typical rabies symptoms by the 4 th day post infection and death by the 6 to 7 days later							
Normal control									
Immune globulin control		Remain healthy all over the experimental period showing neither local or general abnormalities							
BV control									

*DPI= days post infection

4. DISCUSSION

The development of antiviral drugs is still in its infancy with rapid changes and progressive milestones encountered almost daily. The last two decades have been the most dynamic in the history of viral infections and their management. Unfortunately, antiviral drugs have been effective for only a few groups of viruses until now. Most antiviral drugs do not produce a cure, but rather allow control of the infection. However, the limitations of antiviral therapy, including the high costs of drugs, make the need for prevention even more urgent (Trying, 2005).

In vitro investigation of the effect of different concentrations of BV did not show any cell retarded growth or cellular changes. On the other side, it was found that 1mg/ml and 100µg/ml of bee venom were able to inhibit the replication of 100 TCID₅₀ of rabies virus (ERA strain) in BHK cells while 10 and 1µg/ml were unable to do this showing clear specific CPE as demonstrated in table (1). In this respect Rawhia et al. (2009) reported that evaluation of viricidal activity of bee venom against RNA model virus of West Nile virus (WNV) revealed that there was a significant decreased in WNV infectivity titer post incubation at 37°C for 3, 6, and 24 and 48hours post treatment with venom recording 5.1

log (10)/0.1 ml, 4.5 log (10)/0.1 ml, 2.1 log (10)/0.1 ml and 0.55 log (10)/0.1 ml respectively. The RNA model virus; WNV was well influenced by bee venom than IFN where the virus infectivity titer recorded 1 log (10) /0.1 ml post BV treatment compared with 2.5 log (10)/0.1 ml virus infectivity titer post IFN treatment. Also Hassan et al. (2015) used two different virus models Adeno-7 model and RVFV as RNA virus models to determine the antiviral effect of BV. They found that the depletion of viral infectivity showed strong antiviral activity recorded a depletion of viral infectivity titer (1.66 log (10)/ ml) that gave equal action with bee venom and more than interferon IFN (1 log (10)/ ml).

Parallel to the results of in vitro studies, the in vivo investigation of BV anti-viral effect revealed that mice received rabies immune globulin and BV were able to withstand the infection when they were administered on the 0, 1st, 2nd and 3rd day post infection showing protection rate of 90 and 80% respectively while treatment on the following days did not overcome the infection showing paralysis of the hind limbs by the 4th day and death by the 6-7th day post infection as demonstrated in table (2) and photo (3). These observations indicate that post exposure treatment against rabies should be as soon as possible and the late administration leads to fast disease arise. There were no available data that discuss emergency immunization of animals against rabies but Barnas (2001) stated that rabies immune globulin could be applied for animals. In addition Khodeir and Daoud (2008) found that passive immunization of experimentally infected mice showed that these mice were able to withstand the infection when treated with the antiserum on the 1st, 2nd and 3rd day post infection but cannot survive after that. Also, Albehwar (2009) advised that post exposure immunization of animals should be carried out as soon as possible on time post exposure and recommended the same periods. either homologous or heterologous species anti-rabies sera could be used safely in emergency cases. In addition, Hood et al. (2013) 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. Joshua and Hood (2015) suggested that this action is possible where melittin attacks the membranes of the virus.

This work indicates the inoculation of mice with BV as anti-rabies virus was sufficient to possibility to use rabies immune globulin or bee venom in emergency cases of rabies exposure administered on the suitable time post exposure in other species.

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