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TREATMENT TRAILS FOR EQUINE VIRAL ABORTION BY USING NATURAL COMPOUND IN VITRO

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ABSTRACT

Equine herpesvirus 1 (EHV-1), infections is found in horse populaces worldwide and cause a febrile respiratory ailment every year among foals in zones with concentrated steed populaces, the result of infection is dictated by viral strain, resistant, pregnancy status and potentially age, disease of pregnant mares with EHV-1, may abort after weeks to months by clinical or subclinical infection. Treatment of viral infection in animals it appears to be exceptionally troublesome and require a considerable measure of cost, in the present investigation, we used a steroidal saponin compound from dry roots of shallot, the infection rate of EHV-1 in vitro was detected by neutralization test and flowcytometric analysis, the result of infection rate was decreased after 24 hour by 55.7 - 59.2 % when added saponin with concentration of 50µg-100µg, this natural compound may be used as effective materials for treatment animals against viral infection.

Key word: *EHV-1, antivirus*

INTRODUCTION

Horses can get EHV-1 infection by inhalation of infectious aerosols or direct contact with infectious secretions (Allen and Bryans, 1986). The virus attaches and replicates in the epithelium of the upper respiratory tract, including the nose, turbinate, pharynx, soft palate and trachea (Patel *et al.,* 1982; Kydd *et al.,* 1994). Subsequently, the virus penetrates the basement membrane and disseminates into the stroma, thereby infecting mononuclear cells and endothelial cells of local blood vessels (Edington *et al.,* 1986; Kydd *et al.,* 1994). Replication in the respiratory tract can be extensive and is accompanied by erosions with vesiculation of the mucosa and thrombosis (Kydd *et al.,* 1994). Infected steeds shed a lot of infection into the environment, nasal shedding is seen from day one up till two weeks after infection (Gibson *et al.,* 1992; Heldens *et al.,* 2001; van der Meulen *et al.,* 2006). EHV-1 and 4 were considered as two subtypes of the same virus namely EHV-1, the separation amongst EHV-1 and 4 came after their genomic DNA were analyzed (Turtinen *et al.,* 1981; Sabine *et al.,* 1981; Studdert *et al.,* 1981).

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Today in many cases numerous viruses stay without powerful vaccination and just of few antiviral medications is authorized for clinical practice, consequently there is a critical need to find an antiviral that are profoundly effectual and financially savvy for the administration and control of viral contaminations when antibodies and standard treatments are deficient (Liang *et al.,* 2014).

Herbs have been utilized as a part of society pharmaceutical since numerous years and the utilization of home grown inferred common items as a remedial device has been expanding extensively (Slader *et al.,* 2006; Eisenberg *et al.,* 2001).

The Allium genus is one of the essential monocytic genera containing more than 850 species that expand broadly finished the northern portion of the globe from the boreal zone to the dry subtropics (Kamenetsky and Rabinowitch, 2006; Fritsch *et al.,* 2010). Various sorts of this genus, for instance, *A. cepa* (onion), *A. cepa Aggregatum gathering* (shallot), *A. sativum* (garlic), *A. fistulosum* (Japanese grouping onion) and *A. ampeloprasum* (leek), have been used as a piece of food planning and society pharmaceutical for long time (Mostafa *et al.,* 2013). Onion use as a food settling or ethnomedicine is generally credited to its dietary and helpful properties, including antiasthmatic, anticholesterolemic and antimicrobial properties

(Caruso *et al.,* 2014; Abdelrahman *et al.,* 2014, 2016). Until now there is no immunization can be complete protect the horse against EHV-1, although primary and recurrent infections are very difficult to control in horse worldwide.

MATERIALS AND METHODS

1. Cells

FHK-Tcl3.1 (Maeda *et al.,* 2007; Andoh *et al.,* 2009) cells were kept up in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (FCS) and 100 unit of penicillin and 100 µg of streptomycin (Gibco, Invitrogen) per ml and incubation at 37ºC with 5% CO_2.

2. Viruses

EHV-1 strain 89c25 was isolated from a racehorse with respiratory diseases in the epizootic of EHV-1 respiratory infection (Matsumura *et al.,* 1992), EHV-1 strain 89c25 was plaque-purified three times in primary fetal horse kidney (FHK) cells and termed as 89c25p. Virus was released by three cycles of solidifying and defrosting. The harvested culture fluids were clarified by centrifugation at 3500 rpm for 15 min at 4 ºC to remove cell debris and stored at -80ºC. Viral infectivity was measured by plaque assay.

3. Saponin compound

The extraction and segregation of the unadulterated saponin compound named Cepa2, from shallot dry roots (40 g) was performed by the method of (Mostafa *et al.,* 2013). The characterizing of this compound with it is ability as anticancer activity in vitor was done by (Mostafa *et al.,* 2017).

4. Virus infection

Saponin compound with concentration (50-100 µg) or medium alone (control) were mixed with equal volume of virus solution containing 50 PFU/100$\mu\ell$, incubated at 37 ºC for 1 hr and then the mixtures were added to FHK-Tcl3.1 cells, after incubation for 60 min at 37 ºC in 5% CO_2, the cells were washed twice with DMEM and overlaid with 0.8% agarose (Sea Plaque GTG agarose, Lonza) in DMEM containing 10% FCS. The plates were then incubated at 37 ºC in 5% CO_2 for one day. The cells were settled with 5% buffered formaldehyde for 1 hr and the agarose layers were expelled and staining with crystal violet and plaques were checked.

5. Flowcytometric analysis

The infected and control cells were harvested in phosphate buffer solution on ice then the cells were washed three times and resuspended in flowcytometric analysis buffer (1x PBS + 2% fetal calf serum + 0.1% sodium azide in PBS) discard the supernatant, vortex and centrifuge at 2000 rpm for 2 minutes at 4°C and then incubated with monoclonal antibody specific to EHV-1(Mahmoud *et al.,* 2013) diluted to (1:500) for 20 min at 4°C, after three times washed cells with flowcytometric analysis buffer, cells were incubated with Alexa Fluor® 488 goat anti-mouse IgG (H+L) (Invitrogen) for 20 min at 4°C and then washed three times with flowcytometric analysis buffer (McSharry, J.J., 1994; Huang *et al.,* 2005). Cells were suspended in flowcytometric analysis buffer and then analyzed by the flow cytometer software (BD Accuri™ C6, Biosciences).

RESULT

1. Microscopic Examination

The cytopathic effect (CPE) appear very clear in the FHK-Tcl3.1 cells infected by 50 PFU /100$\mu\ell$ after 24 hr post infection (Fig. 1 A), in addition to there is decreased in the number of CPE in case of added saponin by concentration of 50 µg (Fig. 1B) compared to CPE that appeared in case of EHV-1 alone, from another side there is more decreased in the number of CPE in case of added saponin with concentration 100 µg (Fig. 1 C and D).

2. Staining cells

After overlaid the cells with 0.8% agarose the cells were settled with 5% buffered formaldehyde for 60 min and the agarose layers were expelled then the cells were staining with crystal violet and plaques were checked, it is clear that there is decreased in the number of CPE in case of added saponin compared to CPE in case of EHV-1 alone (Fig. 2 A, B and C).

3. Flowcytometric analysis

EHV-1 infected to FHK-Tcl3.1 used as antigen, and FHK-Tcl3.1 cells alone were used as control for cytometric analysis. By using monoclonal antibody specific to EHV-1(Mahmoud *et al.,* 2013) as first antibody with dilution (1:500) and using Alexa Fluor® 488 goat anti-mouse IgG (H+L) as a secondary antibody diluted to (1:500), the result was reduction in infection rate to FHK-Tcl3.1 by 55.7% and 59.2% in case of added saponin by concentration of 50 µg and100µg compared to EHV-1 alone (Fig.3).

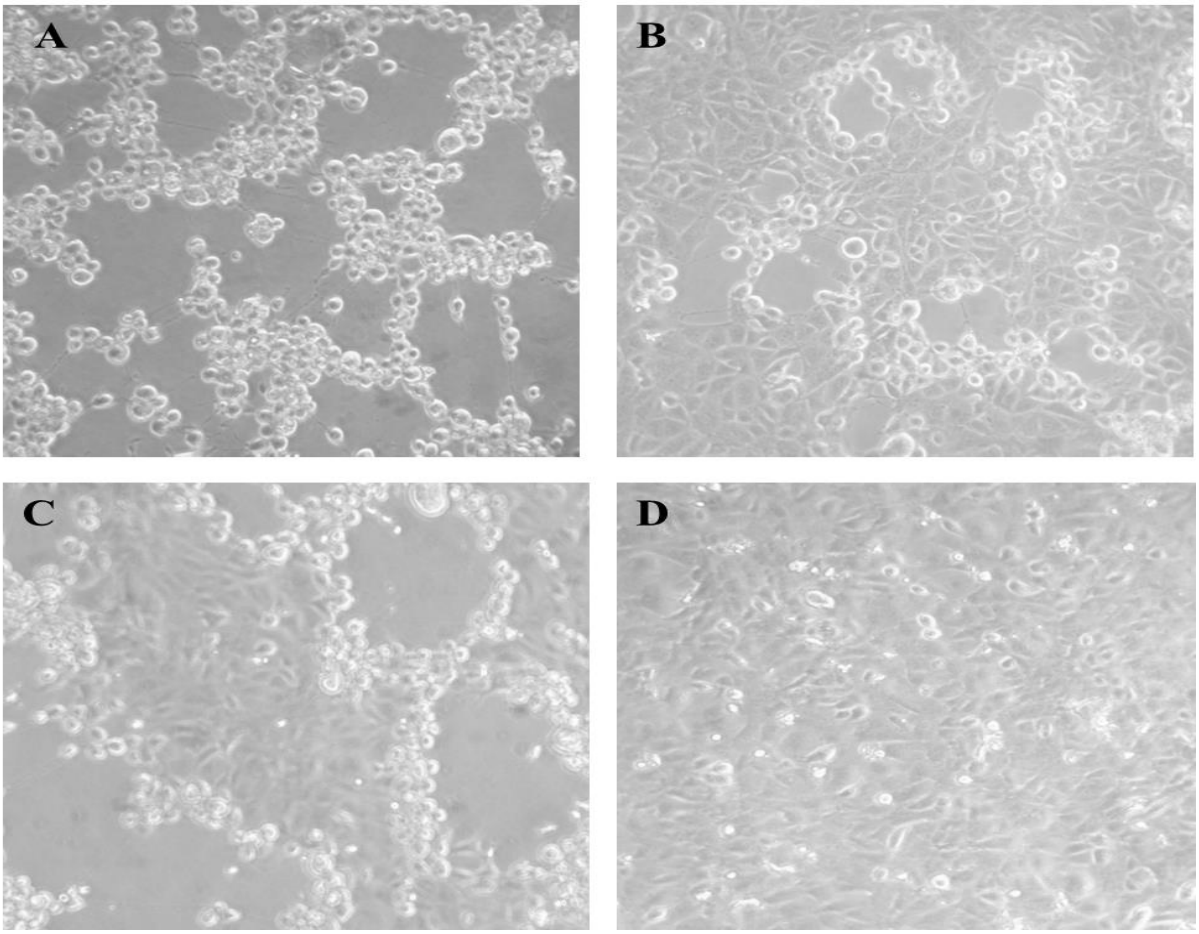

Fig. 1. A and C are microscopic examination of FHK-Tcl.3.1 cells after 24 hours from infection with EHV-1. B and D are cells that treated with saponin by concentration of (50µg and 100µg).

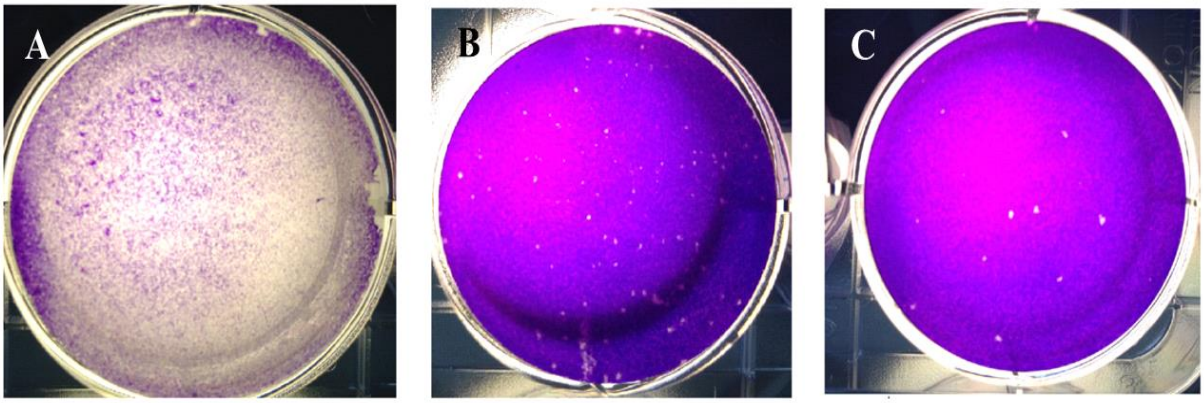

Fig. 2. A is a microscopic examination of stained cells with crystal violet after 24 hours from infection. B and C are microscopic examination of cells infected with EHV-1 which treated with saponin by concentration (50µg and 100 µg).

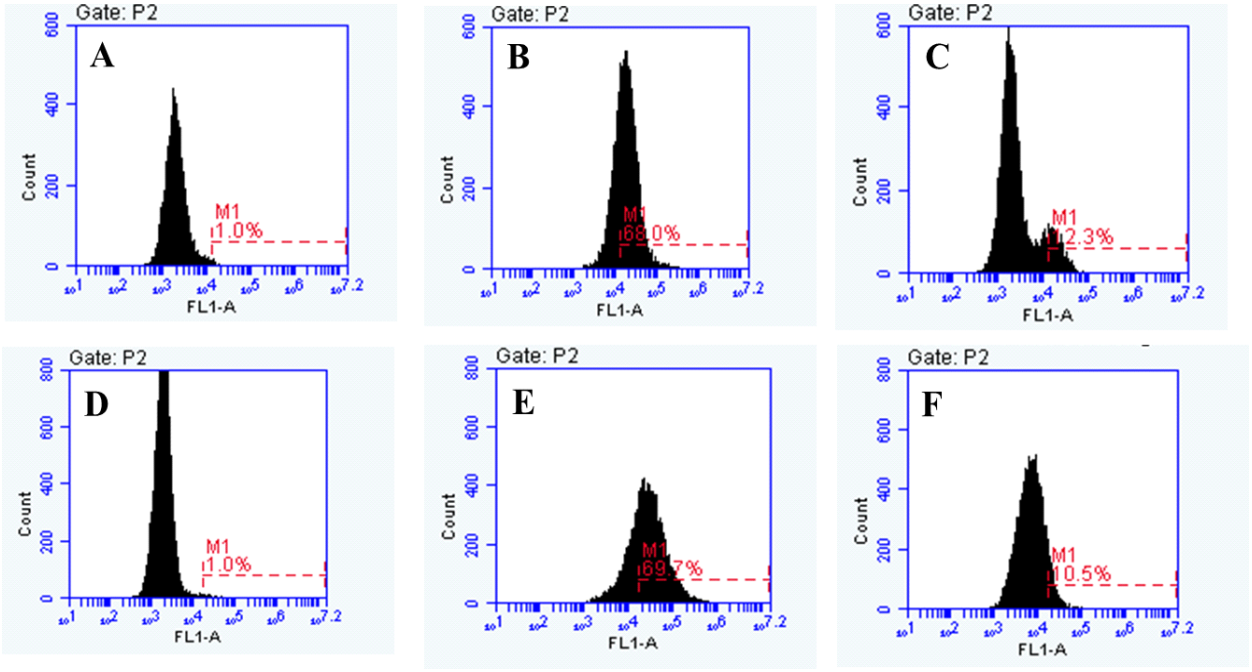

Fig.4. A and D are flowcytometric analysis using FHK-Tcl3.1cells as control without infection. B and E flowcytometric analysis using EHV-1 infected to FHK-Tcl3.1. C and F flowcytometric analysis using EHV-1 and saponin with concentration (50 µg and 100 µg).

DISCUSSION

EHV-1 is a relentless infection disease settles after a few weeks, yet EHV-1 caused an industrious infection characterized by latency with chronic reactivations. The treatment of EHV-1 is testing and the result is specifically identified with the seriousness of the neurological deficits in the influenced horse, as no treatment is available, the management of affected animals is directed towards supportive nursing and wholesome care to decreasing CNS inflammation (Pusterla *et al.*, 2009).

Numerous steeds are latently infected with EHV-1 and reactivation of the infection can happen under stress, where upon latently infected carriers begin to shed infection virus that may spread to in contact stallions (Pusterla *et al.*, 2009-2010).

Herbal pharmaceuticals and purged characteristic items give a rich asset to novel antiviral medication improvement (Liang-Tzung, 2014). In this study, we try to use the antiviral compound from natural products and it is action against EHV-1 by using flowcytometric analysis and virus neutralization test which is considered as the standard test for a laboratory diagnosis of EHV-1 infection, we utilized it to determine the effective of saponin in the reduction of EHV-1 infection to cells in vitro, it is appear that more than fifty percent was reduction in infection rate of virus to cells after 24hr with concentration between 50-100 µg. The mechanism of this compound may be due to the interact with the life cycle of virus inside the cells or maybe due to interact with the methods that used by viruses to enter cells, in addition to this compound was chartered by anticancer activity (Mostafa et al., 2017). More study well be needed to clarify the role of the compound as antiviral in the future for treatment the viral infection, it can be utilized as a part without bounds to treatment the viral infection, more investigation it will be expected to decide the method for it is activity in viral pathogenesis in vivo.

CONCLUSION

This saponin compound was active in vitro as antiviral, it was reduction in the infection rate of EHV-1 to cells, so it may be used as antiviral drugs in the future for treatment the viral infection, more study will be needed to determine the effective of this compound in vivo, this to be complete the clear the action of this compound in both cells and virus.

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علاج فيروس الإجهاض الخيلي باستخدام مركب طبيعي في المختبر

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مرض هربس الخيول من الامراض التى تصيب الحهاز التنفسى فى الخيول بمختلف انحاء العالم بالاضافه الى قدرته على احدث اجهاض بعد عده أسابيع إلى أشهر من العدوى الظاهريه أو دون الظاهريه. ان علاج العدوى الفيروسية من الامور الهامه ولذلك فقد استخدمنا فى هذه الدراسة مركب طبيعى وهو السابونين فقد وجد ان معدل العدوى قد انخفاض بنسبة 55.7 - 59.2٪ باستخدام هذ المركب بتركيز يتراوح بين 50 الى100 ميكرو جرام وهذه النتيجه تدل على ان هذا المركب له قدره للحد من العدوى الفيروسية فى الاختبارات المعملية ويحتاج الى المزيد من الدراسة لمعرفة قدره هذا المركب للحد من العدوى فى الحيوانات الحيه.