Determination of Antiviral Activity of Isoprinosine on FMDV Serotypes O, A, and SAT2

Ehab El-Sayed Ibrahim[1], Heba Atia[1], Mahdy S. E.[1] and Eman R. Abdo[2*]
[1]Foot and Mouth Disease Vaccine Research Department; Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo; Agricultural Research Center (ARC)
[2]Quality Control laboratory (QCL); Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo; Agricultural Research Center (ARC)
Corresponding author: Eman R. Abdo, emanredav05@gmail.com

Abstract

Background: One of the most contagious viral infections that affects animals with cloven hooves is Foot and Mouth Disease (FMD), which causes significant financial losses for the livestock sector. Due to the limitations of current vaccines and treatments, there is a growing need for effective antiviral agents. Isoprinosine, an immunomodulatory compound, was evaluated in this study for its virucidal activity against FMDV.

Aim: This study assessed the *in vitro* and *in vivo* antiviral efficacy of Isoprinosine against FMDV serotypes (O, A, and SAT2).

Material and Methods: FMDV serotypes O, A, and SAT2 were subjected to the present work. The experimental design included cytotoxicity assays of Isoprinosine in the BHK21 cell line, followed by investigation of its in vitro virucidal effect at various concentrations (1000, 500, 250, and 125 µg/ml). Isoprinosine *in vivo* assays were conducted in mice and guinea pigs against viral challenge.

Results: Toxicity assays revealed that all tested concentrations of Isoprinosine did not show any abnormal changes in the BHK21 cell line, as they were safe in mice and guinea pigs. It induced strong in vitro virucidal activity, achieving 100% virus inactivation at 1000, 500, and 250 µg/ml concentrations. At 125 µg/ml, partial inhibition was still observed, with a virus titer reduction of approximately 75–78%. *In vivo*, Isoprinosine provided 100% protection against FMDV at 1000, 500, and 250 µg/ml concentrations in both animal models. Even at 125 µg/ml, high protection rates (75–86.6%) were recorded, while control groups showed 100% infection and mortality.

Conclusion: Isoprinosine could be an effective antiviral agent for withstanding and controlling FMD infections. Further studies are warranted to explore its mechanism of action and application in field conditions.

Keywords: FMD - Isoprinosine - BHK - Mice - G. pig

Introduction

Animals with cloven hooves are susceptible to Foot and Mouth disease (FMD), a highly contagious virus that causes significant financial losses for the cattle industry globally. The causative agent, the Foot and Mouth disease virus (FMDV), is a member of the family Picornaviridae and is classified into seven serotypes: Asia1, SAT1, SAT2, SAT3, O, A, and C. Among these, serotypes O, A, and SAT2 are the most prevalent in many endemic regions, including parts of Africa and the Middle East (***Grubman and Baxt, 2004; Jamal and Belsham, 2013***). Current disease control strategies rely primarily on vaccination and biosecurity measures. However, vaccines require frequent updates due to antigenic variability, and there is a critical need for effective antiviral agents that can be used as complementary tools, particularly during outbreaks, aiding in minimizing the virus spread (***Mahapatra and Parida, 2018***).

Isoprinosine (inosine pranobex) is a synthetic immunomodulatory and antiviral agent that has been shown to stimulate host immune responses and inhibit the replication of several RNA viruses. It enhances lymphocyte proliferation, cytokine production, and interferon responses, making it a potential candidate for controlling animal viral infections (***Janković et al., 2001; Růžek et al., 2014***). Nevertheless, its antiviral activity against FMDV has not been extensively studied.

For culture, isoprinosine at doses ranging from 50 to 800 µg/ml showed no harm. It has been demonstrated that IFNα dramatically reduces these viruses' ability to multiply. The decrease in infectious titers was proportional to the IFN-α concentration ***Al-sherees et al., (2019)***.

Mice and tissue cultures were used to investigate isoprinosine's antiviral properties. The infectivity of influenza and her pes hominis viruses decreased in tissue cultures at concentrations ran ging from 25 to 1000 µg/ml. However, this was not the case for parainfluenza, rhinovirus, or adenovirus.

There was significant variation in t he inhibitory concentration of isopri nosine among influenza A strains ***Muldoon et al., (1972)***.

Influenza virus, PR-8 and A2 strains; herpes virus, LU strain; polio virus 3; and adenovirus 10 were all susceptible to the antiviral actions of isoprinosine. Isoprinosine has been shown to have therapeutic antiviral properties against the A2 strain of influenza and against a herpes infection in neonatal mice in in vivo mortality tests. Studies showed a connection between the decrease in the virus titer in the lungs of infected mice

and the in vivo anti-influenza (PR-8) actions of Isoprinosine, ***Paul and Eric (1972)***.

It has been demonstrated that isoprinosine can alter viral RNA levels, preventing the proliferation of several viruses. Since 1971, it has been used extensively to treat viral infections and diseases, including subacute sclerosis panencephalitis, herpes simplex virus, human papilloma virus, human immunodeficiency virus, influenza, acute respiratory infections, cytomegalovirus, and Epstein-Barr virus infections, because of its immunomodulatory and antiviral qualities as well as its safety *profile* ***Sliva et al., (2019)***.

This study aims to evaluate the ***invitro*** and ***invivo*** antiviral activity of Isoprinosine against FMDV serotypes O, A, and SAT2 in cell culture and lab animal models. The results may contribute to developing supportive therapeutic strategies for FMD control.

Material and Methods

Ethical approval: The experiment was conducted according to the Institutional Animal Ethics Committee protocol and Veterinary and Serum Vaccine Research Institute regulations.

1-Virus Strains and Infectivity Titration

Serotypes O Pan-Asia/2012, A Iran 05, and SAT2/EGY/2012 of the FMDV were acquired from the FMD Vaccine Research Department (FMDVRD) repository at the Veterinary Serum and Vaccine Research Institute (VSVRI), Abasia, Cairo. The infectivity titer of each serotype was determined using the tissue culture infectious dose 50 ($TCID_{50}$) method on the BHK21 cell line and expressed as log_{10} $TCID_{50}$/ml as described by ***(Reed and Muench, 1938)***.

2-Cell culture:

The baby hamster kidney (BHK21) cell line was propagated and maintained in FMDVRD using minimum essential Eagle Medium (MEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 µg/ml) at 37°C in a 5% CO_2 incubator. This cell line was used for virus propagation, titration, in vitro cytotoxicity, and antiviral assays.

3-Isoprinosine:

A commercial pharmaceutical supplier (Medical Union Pharmaceutical) provided 5.0 mg of isoprinosine (inosine pranobex), which was then suspended in phosphate-buffered saline (PBS, PH=6.9) and filtered using a syringe filter (Filter Unit, 0.2 µm). Their antiviral efficacy and toxicity were tested *invitro* and *invivo* using serial dilutions (125, 250, 500, and 1000 µg/mL) produced in culture medium.

4-Cytotoxicity and safety assays of Isoprinosine

Different dilutions of Isoprinosine were inoculated in the BHK21 cell

line for an in vitro cytotoxicity assay and in mice and Guinea pigs as an animal model for in vivo safety assays.

4.1-Cytotoxicity in vitro assay:

Isoprinosine's safety was evaluated in a monolayer culture of Baby hamster kidney (BHK) cells by assessing its cytotoxic effect. Five wells of 96-well tissue culture plates were injected with 25 µl of each serial 2-fold dilutions made in PBS. Regular cell culture wells were used as test controls. For 48 hours, the cell culture plates were incubated at 37°C and examined under a microscope daily to look for any abnormalities in the cells.

4.2- In vivo safety assay:

4.2.1-In G. Pigs

Twenty-five G. pigs (supplied by the Lab Animal Farm; VSVRI) were divided into five groups (5 G. pigs/group), where each animal was inoculated intraperitoneally (I/P) with 0.4 ml of a 2-fold dilution of Isoprinosine, leaving an animal group inoculated with normal saline as a test control. All animals received a balanced ration and adequate water and were kept under hygienic measures and daily clinical observation to detect any abnormal post-inoculation reactions. The safe concentration was considered the one that did not cause any post inoculation reaction, as stated by (***Saad and Fawzy, 2004)***

4.2.2- In Baby Mice:

According to (***Richards et al., 1978)*** and ***(Saad and Fawzy, 2004)***, twenty-five, 2–3-day-old baby Swiss mice (supplied by the Lab Animal Farm; VSVRI) were each inoculated with 0.2 ml I/P with different dilutions of Isoprinosine (five mice for each dilution). Besides that, a control group of baby mice was inoculated with saline. After 48h. post inoculation, all the baby mice were examined to evaluate the action of Isoprinosine. The death of mice means that this concentration of Isoprinosine is toxic.

5-Antiviral assay:

5.1-*Invitro* assay:

It was done according to (***Boseila and Hatab, 2011)*** and ***(El-Baz et al., 2013).*** A mixture of 25 µl containing 100 $TCID_{50}$ of the used virus with 25 µl of each 2-fold dilution of Isoprinosine was kept at 37 °C for 30 minutes, then inoculated in each of 6 wells of cultured BHK plates. The test included standard and virus-infected cell controls, and the plates were examined microscopically to detect viral cytopathic effect (CPE). The presence of complete CPE in virus-infected control means an adverse antiviral effect.

5.2-*Invivo* antiviral assay

5.2.1-In baby mice

According to (***Richards et al., 1978) and (Saad and Fawzy, 2004);*** sixty-five and five of 2-3-day old baby Swiss mice were used for this assay where each mouse was inoculated with 0.2 ml I/P from the mixture of each of the three serotypes of FMDV having virus

titer 10^7 ID_{50}/ml with different dilution of Isoprinosine (fifteen mice were used for each dilution). A group of baby mice was inoculated with FMDV only, and another group was kept without inoculation as a control. After 48h. post inoculation, all the baby mice were examined to evaluate the antiviral action of Isoprinosine. Paralysis of the hind limbs or death of infected mice means that these mice, infected with FMDV, died after 24h and were considered non-specific.

5.2.2-In G. Pigs

As described by ***(Mansour et al., 2016),*** sixty-five G. pigs were divided into five groups (15 animals/group), and each mouse in a mouse group was inoculated in the foot pad with 0.4 ml of FMDV (10^7 ID_{50}/ml) mixture with Isoprinosine in different dilutions. Non-inoculated and virus-inoculated groups were included as test controls. All animals were kept under daily clinical observation to detect any signs of FMD virus infection.

Results

1. Infectivity Titer of FMDV Serotypes

As shown in Table 1, the infectivity titers of the FMDV serotypes, O Pan-Asia/2012, A Iran 05, and SAT2/EGY/2012 were recorded as 8.5, 8.0, and 7.0 log_{10} $TCID_{50}$/ml respectively.

2. *Invitro* Cytotoxicity of Isoprinosine

The *invitro* cytotoxicity test on BHK cells revealed 0% cytotoxicity for all tested concentrations of Isoprinosine (Table 2), showing that Isoprinosine is non-toxic to BHK cells at concentrations up to 1000 µg/ml.

3. *In vivo* safety assay in Guinea Pigs and Mice

No post-inoculation toxic reactions were observed in any inoculated animals, and a 0% toxicity rate was reported (Table 3). All animals remained healthy throughout the observation period, confirming the safety of Isoprinosine at the tested dose.

4. *In vitro* Virucidal Activity of Isoprinosine Against FMDV

The virucidal assay results (Table 4) demonstrated a 100% reduction in virus titer for all three serotypes of FMDV (O, A, and SAT2) induced by Isoprinosine at concentrations of 1000, 500, and 250 µg/ml. The mean initial virus titers ranged from 6.5 to 8.5 log_{10} $TCID_{50}$/ml, (not accepted), and all dropped to 0 after exposure to Isoprinosine, confirming vigorous virucidal activity *invitro*. While at 125 µg/ml (1/8 dilution), partial virucidal activity was detected: For serotype O, the virus titer was reduced from 8 log_{10} to 1.5, achieving a reduction of 6.5 log_{10} and a virucidal activity of 75%. For serotype O, from 7 to 1.5, with an average reduction for serotype A, and of 5.3 log_{10}, equating to 75.7% activity for SAT2.

Control samples (untreated) showed no reduction in virus titer, confirming the effectiveness of Isoprinosine and ruling out spontaneous virus inactivation.

5.*Invivo* virucidal effect of Isoprinosine against FMD viruses: The *in vivo* virucidal assay results in mice and guinea pigs are presented in Tables 5 and 6. It was found that Isoprinosine has a strong protective effect against FMDV at all tested concentrations except at 1/8 dilution (125 µg/ml), with varying levels of efficacy depending on the inoculated dilution. The undiluted concentration (1000 µg/ml) of Isoprinosine induced complete protection (100% virucidal action) against the tested FMDV serotypes (O, A, and SAT2). None of the guinea pigs in these groups showed clinical signs of infection, and all survived. Similarly, the 1/2 and 1/4 dilutions also provided complete protection against all serotypes, with 0% infection rate and 100% survival. However, at the 1/8 dilution (125 µg/ml), the protective effect of Isoprinosine decreased slightly, where some infection signs were observed, showing virucidal activity ranged between 80% and 86.6% depending on the serotype. In contrast, the untreated control groups showed a 100% infection rate and 0% survival.

Table 1: The infectivity titer of FMDV serotypes

FMDV serotype	Infectivity titer ($log_{10}TCID_{50}$/ml)
O Pan-Asia/ 2012	8.5
A Iran 05	8.0
SAT2/EGY/2012	7.0

Table 2: *Invitro* Cytotoxicity of Isoprinosine on BHK cells

Tested dilution	Isoprinosine concentration (µg/ml)	Cytotoxicity %
Un-diluted	1000	0
½	500	0
¼	250	0
1/8	125	0

Table (3): Safety % of Isoprinosine in G. pigs and Mice

Inoculated dilution	Isoprinosine concentration (µg/ml)	Number of experimental G. pigs and Mice			Toxic reaction %
		Total	Reacted	Non-reacted	
Undiluted	1000	5GP&5M	0	5	0
½	500	5GP&5M	0	5	0
¼	250	5GP&5M	0	5	0
1/8	125	5GP&5M	0	5	0
Control	Saline	5GP&5M	0	5	0

N.B: GP= Guinea pigs M= mice

Table (4): *Invitro* virucidal effect of Isoprinosine in BHK21 cell line

Tested dilutions	Isoprinosine Concentrations (µg/ml)	FMDV serotypes	Observed CPE	Virucidal Effect %
Undiluted	1000	O	Completely inhibited	100
		A		
		SAT2		
Untreated virus controls			Completely Clear	0
1/2	500	O	Completely inhibited	100
		A		
		SAT2		
Untreated virus controls			Completely Clear	0
1/4	250	O	Completely inhibited	100
		A		
		SAT2		
Untreated virus controls			Completely Clear	0
1/8	125	O	+ = (25%)	75
		A		
		SAT2		
Untreated virus controls			Completely Clear	0

CPE: Cytopathic Effect

Table 5: Virucidal assay of Isoprinosine against FMDV in mice.

Tested dilution of Isoprinosine	Isoprinosine Concentration (µg/ml)	FMDV serotype	Number of experimental mice			Virucidal action %
			Total number	Number of infected	Number of survived	
Undiluted	1000	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	0
1/2	500	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	0
1/4	250	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	0
1/8	125	O	5	1	4	80
			5	1	4	
			5	1	4	
		A	5	1	4	80
			5	1	4	
			5	1	4	
		SAT2	5	1	4	86.6
			5	1	4	
			5	0	5	
		Control	5	5	0	0

Table (6): Virucidal assay of Isoprinosine against FMDV in G. pigs

Tested dilution of Isoprinosine	HBV Concentration (µg/ml)	FMDV serotype	Number of experimental G. pigs			Virucidal action %
			Total number	No. of infected	No. of survived	
undiluted	1000	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	-
1/2	500	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	-
1/4	250	O	5	0	5	100
			5	0	5	
			5	0	5	
		A	5	0	5	100
			5	0	5	
			5	0	5	
		SAT2	5	0	5	100
			5	0	5	
			5	0	5	
		Control	5	5	0	-
1/8	125	O	5	1	4	86.6
			5	0	5	
			5	1	4	
		A	5	0	5	86.6
			5	1	4	
			5	1	4	
		SAT2	5	1	4	93.3
			5	0	5	
			5	0	5	
		Control	5	5	0	-

Discussion

The present study investigated the *in vitro* and *in vivo* virucidal effects of Isoprinosine against FMDV serotypes O, A, and Sat2 across different experimental models, including tissue culture, mice, and guinea pigs. The results consistently demonstrated that Isoprinosine potently inhibits FMDV replication in the BHK21 cell line and infection in baby mice and Guinea pigs.

Invitro cytotoxicity assay in BHK21 cell line (Table 2) indicated that all used concentrations of Isoprinosine did not show any abnormal signs of the cell shape or growth rate, revealing its safety for cell culture. It was stated that Isoprinosine is not cytotoxic to A549 cells; it also does not change the morphology or affect biological activity (in the MTT test), HEp-2 and HEL 299 cell lines ***(Majewska et al., 2016)*.** Isoprinosine-mediated cytotoxicity was confirmed before *invitro* studies, but no cytotoxicity was observed at concentrations of 0–5 mg/ml ***(Kim et al., 2024)*.**

In BHK21 cell line assays, treatment of FMDV serotypes O, A, and SAT2 with Isoprinosine (Table 4) at various concentrations (1000, 500, 250, and 125 µg/ml) resulted in complete inhibition of the virus CPE. At concentrations of 1000, 500, and 250 µg/ml (100% reduction), indicating full virucidal activity across test viruses. While, at 125 µg/ml, although a significant reduction was still observed, the virucidal effect slightly decreased, with the mean percentage of virus inactivation about 75, depending on the serotype. These findings confirm the concentration-dependent activity of Isoprinosine. It was found that the ability of Isoprinosine (50-800 µg/mL) to inhibit virus multiplication in cell cultures. The cytopathic effect of the virus was evaluated 48 h after infection of cell cultures with the virus using light, inverted microscopy under controlled conditions. Campoli has extensively reviewed the antiviral effect of IP- ***(Campoli-Richards et al., 1986)*** found it modest and inconsistent in standard tissue cultures. IP was successful in inhibiting the replication of several RNA and DNA viruses. It was noticed that higher concentrations of Isoprinosine strongly inhibited the multiplication of all viruses. HPIV-2 and HAdV-2 showed the highest sensitivity to the antiviral activity of Isoprinosine as compared with the control; however, increasing concentrations of Isoprinosine up to 800 µg /ml slightly enhanced the antiviral activity of 400 µg/ml Isoprinosine ***(Hashim et al., 2023)*.** In addition, Isoprinosine demonstrates antiviral effects against FMDV in cell lines, primarily by interfering with viral replication and potentially modulating the immune response. It achieves this by inhibiting viral RNA synthesis, promoting host cell RNA and protein synthesis, and

influencing immune cell function ***(Kim et al., 2024)***.

Due to the small number of antiviral medication ingredients that interfere with certain viral replication stages, research on the antiviral action of Isoprinosine must be conducted. The traditional "one virus, one medicine" approach maximizes the therapeutic potential. Therefore, considering the pleiotropic effect and bolstering the antiviral response, searching may be one tactic for managing viral infections. Additionally, it contains compounds that alter the immune system ***(Lin and Young, 2014)***.

In vivo safety assessments (Table 3) showed that Isoprinosine was well tolerated in mice and guinea pigs at all tested concentrations. No post-inoculation abnormal reactions were observed, even at the highest undiluted concentration (1000 µg/ml), indicating the compound's safety for animal use in these doses. Similar findings indicated that the highest survival rates were noted in animals receiving undiluted or half-diluted Isoprinosine, supporting the compound's efficacy when used at higher concentrations. No significant toxic reactions were reported in any animal models at the tested doses, indicating a high margin of safety, consistent with previous toxicity assessments in humans and animals ***(Chirigos and Fouts, 1984).*** To date, it is uniformly supported that IP is a drug that is well tolerated and free from serious side effects. Based on results from cytotoxic assays, comet and micronucleus assays, and Ames's testing, it was concluded that IP is neither cytotoxic, genotoxic nor mutagenic ***(Hadden and Wybran, 1981 and Majewska et al., 2016)***

In mice models (Table-5), Isoprinosine offered complete protection against FMDV at concentrations of 1000, 500, and 250 µg/ml, with a 100% survival rate and no clinical signs of infection. The protective effect was slightly reduced at the lowest tested dose (125 µg/ml), with survival rates dropping to 75–80%. Nevertheless, this still reflects a strong antiviral effect, especially compared to the control group, which showed complete mortality and clinical infection. These findings agree with the conclusion that isoprinosine has antiviral activity and a confirmed immunomodulatory effect ***(De Clercq, 2013).*** Regarding the *in vivo* antiviral assay in Guinea pig (Table-6), the results in alignment with those observed in mice and tissue culture, complete protection was achieved at 1000, 500, and 250 µg/ml with a 100% survival rate and 0% infection. At 125 µg/ml, while a minor drop in effectiveness was noted, virucidal action remained high (80–86.6%), and the infection rate was significantly lower compared to control groups.

Isoprinosine exhibits potent virucidal activity against the three tested FMDV serotypes *invitro* and

in vivo. The reduction in virus titer observed in tissue culture systems was significant, especially at concentrations of 1000 µg/ml and 500 µg/ml, where complete inhibition (100%) of viral activity was recorded. These results are consistent with earlier findings that Isoprinosine has broad-spectrum antiviral activity against RNA viruses, likely via interference with viral RNA replication and enhancement of host immune mechanisms ***(De Clercq, 2002; Hassan and Salem, 2019).***

Conclusion and Recommendation:

The present findings suggest that Isoprinosine exhibits virucidal activity against FMDV serotypes in a concentration-dependent manner. Its ability to prevent infection *in vitro* and *in vivo* and its low toxicity profile highlight its potential as a promising antiviral candidate for controlling FMDV outbreaks. Furthermore, the consistent efficacy across different animal models strengthens the translational value of these findings toward potential application in larger animals or field use.

Vigorous virucidal activity and increased survival rates in treated animals demonstrated that isoprenaline was a safe and efficient antiviral drug against FMDV. Its immunomodulatory properties also suggest a potential benefit in lessening the seve
rity of the illness.

Additionally, by stimulating cellular immunity and interferon production, isoprinosine may improve immune response and increase vaccine efficacy when combined with FMD vaccination. This demonstrates its potential as a helpful adjuvant in regimens aimed at controlling FMD.

Future studies should explore the mechanism of action of Isoprinosine in FMDV inhibition, evaluate its long-term safety profile, and investigate its synergistic potential with other antiviral agents or vaccines.

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"تحديد تاثير ماده الايزوبريوسين كمضاد فيروسى ضد عترات فيروس الحمى القلاعية A, O, SAT2"

ا.د ايهاب السيد ابراهيم [1], د. هبه عطية محمد بيومى[1], د. صفى الدين مهدى[1], د. ايمان رضا عبده[2]

[1] قسم بحوث الحمى القلاعية - معهد بحوث الامصال واللقاحات البيطرية - مركز البحوث الزراعية -القاهرة- مصر

[2] معمل مراقبة الجودة - معهد بحوث الامصال واللقاحات البيطرية - مركز البحوث الزراعية - القاهرة- مصر

الملخص العربى

تُعد الحمى القلاعية (FMD) من الأمراض الفيروسية شديدة العدوى التي تصيب الحيوانات ذات الظلف المشقوق، وتتسبب في خسائر اقتصادية كبيرة في قطاع الثروة الحيوانية. وبسبب القيود التي تواجه فاعلية اللقاحات والعلاجات التقليدية، ظهرت الحاجة الملحة لاكتشاف عوامل مضادة للفيروسات ذات فاعلية. في هذه الدراسة، تم تقييم مركب الإيزوبرينوسين، وهو مركب مناعي منظم، لاختبار نشاطه كمضاد للفيروسات تجاه فيروس الحمى القلاعية. هدفت الدراسة إلى تقييم فعالية مركب الإيزوبرينوسين كمضاد للفيروسات في النماذج المختبرية والحيوية ضد الأنماط المصلية(A,O,SAT2) لفيروس الحمى القلاعية.

شملت الدراسة استخدام الأنماط المصلية O، A، و SAT2 من فيروس الحمى القلاعية. وقد تم إجراء اختبارات السُمية لمركب الإيزوبرينوسين على خط خلاياBHK21 ، تلاها اختبار تأثيره الفيروسي عند تركيزات مختلفة: 1000، 500، 250، و125 ميكروغرام/مل. كما تم إجراء تجارب في الجسم الحي (in vivo) باستخدام الفئران وخنازير غينيا لاختبار تأثيره الوقائي ضد العدوى.

أظهرت اختبارات السمية أن الإيزوبرينوسين لم يسبب أي تغيرات خلوية غير طبيعية في خلايا BHK21، وكان آمنًا على الفئران وخنازير غينيا. وأظهر المركب فعالية قوية في تعطيل الفيروس بنسبة 100% عند التركيزات العالية (1000، 500، 250 ميكروغرام/مل)، بينما حقق تثبيطًا جزئيًا بنسبة 75–78% عند التركيز 125 ميكروغرام/مل. في التجارب الحيوانية، وفر المركب حماية بنسبة 100% عند التركيزات العالية، وحماية بين 75% و86.6% عند التركيز الأدنى. أما الحيوانات في المجموعات الضابطة فقد أظهرت إصابة ونفوق بنسبة 100.

تشير النتائج إلى أن مركب الإيزوبرينوسين قد يكون مرشحًا واعدًا كعامل مضاد لفيروس الحمى القلاعية. وتوصى الدراسة بإجراء مزيد من الأبحاث لفهم آلية عمله والتأكد من جدواه التطبيقية في الحقل.