ANTIVIRAL POTENTIAL OF IODINE COMPLEX AGAINST FOOT AND MOUTH DISEASE VIRUS

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Abstract- Foot and Mouth is an infectious disease caused by FMD Virus. The disease is major problem for beef and dairy industries due to high spreading capacity and genetic diversity of virus. Immunization is the only way to limit and eradicate the disease where the disease is endemic. However, vaccination is constrained by lack of or incomplete protection. Therefore, an alternative application of antiviral agents for inhibition of the FMD virus (FMDV) is needed. This study was conducted to investigate the antiviral potential of Renessans (Iodine complex) against FMDV type O, A and Asia-1 serotypes. The MTT assay was carried on BHK-21 cell line to assess the Cytotoxicity of Renessans. Viral inhibition assays using the ten non-cytotoxic concentrations (serial dilution)(0.133, 0.0668, 0.0334, 0.0167, 0.0083, 0.0041, 0.0020, 0.001, 0.0005, and 0.00025mg/ml) of Renessans (Iodine complex) were performed to check the antiviral potential of Renessans on different stages of viral infection. At these concentrations (0.133, 0.0668, 0.0334, 0.0167, 0.0083, 0.0041, 0.0020, 0.001, 0.0005, and 0.00025mg/ml) of Renessans the virus titer was reduced significantly ($p < 0.001$) by three log in all three strains of viruses at non-toxic concentrations. The virus titer in strain O control was $10^{6.3}$ Tissue Culture Infective Dose 50 (TCID50)/mL and was reduced to $10^{1.89}$ TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. In the case of strain Asia-1, the virus titer was reduced from (control) $10^{5.8}$ TCID50/mL to $10^{1.42}$ TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. The titer of strain A was reduced from $10^{5.5}$ TCID50/mL to $10^{1.51}$ TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. Moreover, the virus titer was reduced more at the late replication & assembly stages as compared to attachment and entry stages. This study showed the in vitro anti-FMDV potential of Renessans (Iodine Complex) for the first time and predicted its potential use against FMDV infections.

Index Terms- FMD, FMDV, TCID50, Renessans (Iodine complex), BHK-21 cell line

I. INTRODUCTION

Foot and Mouth is an infectious disease caused by FMD Virus. It infects all domesticated cloven hoofed animals and many wild species. The clinical symptoms of the disease include lameness, fever, lymphopenia and blister-like sores on mouth as well as in between the hooves. The disease is in the priority list among veterinary infectious diseases in the world. The annual economic loss due to this disease is estimated almost US$6.5 and 21 billion. It is endemic in various regions including Pakistan [1]. Virus is excreted into milk, semen, saliva, nasal discharge, feces, urine and fluid from ruptured vesicles [2]. It can survive for approximately two months at 4°C (39.2° F) on wool and in bovine feces or slurry for two to three months [3]. The FMDV belongs to picornaviridae family and aphtovirus genus.FMDV is the single stranded, +sense RNA as genetic material, approximately 8500 nucleotide, with in a protein capsid of icosahedral symmetry made up of sixty copies of each structural protein. There are 7 important types (A, O, C, Asia 1, SAT 1, SAT 2 and SAT 3). It is endemic in different countries but the familiar serotype is O then Asia 1 and A [4]. There is no cross immunity against other types and subtypes. Immunization is the only way to control and limit the disease. It requires at least seven days to generate protective immune response in animals [5]. The livestock population in Pakistan consists of 27.1 million sheep, 56.7 million goats, 31.8 million cattle and 29 million water buffalo In Punjab, major portion of livestock such as water buffalo (61%) and cattle (43%) [6]. Foot and Mouth disease is more present in cattle (62%) and less present in buffalo (40%). Furthermore in young calf fatality is greater (0.62%) as compared to adult cattle in which 0.08% is present. In the spread of disease carrier bulls, milk man, veterinary workers, watering points, vehicles used in transport play an important role [7]. In Pakistan Foot and Mouth disease causes greater economic loss such as buffaloes, up to 307.8 liters per animal in 45 days milk production loss was recorded and prevalence of disease animal population due to exotic breed importation and cross breeding of local breeds (Farooq et al. 2017). [8]. Previous studies reported that worldwide due to FMDV vaccine failure 6.5 and 12 billion US dollar loss is recorded and decrease milk production [9]. The high mutating nature of FMDV and quick evolution of virus develops seven main serotypes. Many variants and subtypes have been evolved from these serotypes [10]. There is no remedy available for FMDV at present, the limitation and drawbacks in the use of vaccine change the focus of researcher towards other resources antiviral drugs like iodine complex [11]. However, the antiviral activity of Renessans (Iodine Complex) against FMDV has not yet been reported. In this study, we examined the antiviral efficacy of Renessans (Iodine Complex) in vitro. We

show that Renessans (Iodine Complex) severely inhibited the production of infectious FMDV and acted its action at the extracellular level and post entry stages of viral replication. This is the first time the antiviral effect of Renessans (Iodine Complex) against FMDV in vitro has been demonstrated.

II. IDENTIFY, RESEARCH AND COLLECT IDEA

Antiviral Agent

The drug Renessans was used for antiviral potential against FMDV. The drug was provided by MTI Pvt. Ltd. (patent no: 141316, IPO, Pakistan) and registered by MTI, 84 Pakistan (DRAP registration # 505620098). By Composition: each 10 ml contains: polyiodides as iodine/iodide equivalent to 50 mg iodine/iodide; Excipients: glycerine 2 ml and ascorbic acid 2 mg, sorbitol 1 ml and water.

Cytotoxic Assay

The cytotoxic concentration of Renessans was determined by using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Roy et al. 2015). The cytotoxicity assay was carried out on BHK-21 cell line in 96 well plates. Two fold (serial) dilutions of iodine complex (Renessans) were prepared in media. The respective dilutions were poured on BHK-21 cell line monolayer and incubated at 37°C with 5% CO2. Optical density (OD) values of each well were measured by multi well ELISA reader at 570 nm [12]. These OD values were statistically converted in to cell survival percentage [13].

III. WRITE DOWN YOUR STUDIES AND FINDINGS

Cell Culture & Media

Preserved baby hamster kidney cells (BHK-21) were obtained from Quality Operation Laboratory, University of Veterinary and Animal Sciences Lahore. To establish a monolayer the cells were revived The BHK-21 Cell line was revived and propagated by using Glasgow Minimal Essential Media (GMEM) (Caisson laboratories) with 10% Fetal Bovine Serum (Capricorn Scientific). Growth media was added to the flask, incubated at 37ºC with 5% CO2 for 18-24 h. After incubation cells were observed under inverted microscope.

Virus identification by ELISA

The viral strains of FMDV were identified and confirmed by ELISA. The antigen detection kit (Pirbright institute, UK) was used for the test. The confirmed A, O and Asia 1 strains was used in research [14].

Biological Titration of FMD Virus

The biological titer of FMDV strains A, O and Asia 1 was estimated by TCID50 [15]. The test was performed in 96 well plates. A plate with 80% monolayer was taken. Media was removed. Monolayer was washed 2-3 times with PBS. Then, 100 µl of 10 fold diluted viral suspension was added that was obtained from Quality Operation Laboratory (QOL), University of Veterinary and Animal Sciences Lahore. Cell and virus controls were run alongside the experiment. Plates were examined for the presence of CPEs. The titer was calculated by Reed and Muench Method (1938) [16]. The biological titer of FMDV O was 106.3, FMDV A was 105.5, and FMDV ASIA-1 was 105.8

Anti-Viral Activity

The antiviral activity of the iodine complex (Renessans) was assessed with in the safety range as described previously. The anti-viral activity was assessed both extra- and intra-cellular against ten concentrations (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) of Renessans (Iodine Complex). The extra-cellular antiviral activity was assessed by incubating the mixture of (Viral strain +iodine complex (45 min). The mixture was poured on to confluent monolayer of BHK-21 cell line propagated in flat bottom 96 well plates and incubated for 16 h at 37oC in CO2 incubator. As a control, the cells were infected with 106.3 TCID50 of "O" Strain of FMDV, 105.8 TCID50 of "Asia-1″ and 105.5 TCID50 of "A" strain of FMDV in the absence of Renessans. The effect of iodine complex on cell receptors was evaluated by incubating the mixture of (Iodine complex+ BHK-21 cell 45 min at 37oC in CO2 incubator). The plates were washed with PBS to remove unabsorbed drug after incubation. After washing, The BHK-21 cells were infected with 106.3 TCID50 of "O" Strain of FMDV, 105.8 TCID50 of "Asia-1″ and 105.5 TCID50 of "A" along with fresh maintenance media separately for 16 h at 37oC in CO2 incubator. The cell and virus control was run along with. Optical density (OD) values of each well were measured by multi well ELISA reader at 570 nm to check Cell Survival Percentages (CSP). The samples were collected for each experiment to check the virus titer via TCID50 procedure.

Antiviral activity during Post entry stages

Confluent BHK-21 cells propagated in flat bottom 96 well plates were incubated with viral strains with 106.3 TCID50 of "O" Strain of FMDV, 105.8 TCID50 of "Asia-1″ and 105.5 TCID50 of "A" for 2, and 12 hours at 37oC in CO2 incubator[17]. The plates were washed with PBS to remove unabsorbed virus after incubations. After washing, fresh maintenance media was added along with two fold concentrations (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) of Iodine complex up to 10th well and incubate it at 37oC in CO2 incubator. The samples were collected from each well after 16 h for virus titration.

Statistical Analysis

All the experiments were performed three times individually, and the data were presented as means ± standard deviation (SD). The results were analyzed by Graph pad prism software (version 5.01). Student t-test was applied to the results to compare the means of the TCID50 value of the test group with the control. Statistical significance represented by asterisks is marked correspondingly in the figures (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Results

Non-toxic concentration of Renessans (Iodine Complex)

The cytotoxicity assay carried out on BHK-21 cell line propagated in 96 well plate. Ten dilutions (2 fold dilution up to 10th well) of iodine complex (Renessans, complex of iodine commercial .product) will prepared in media. The respective dilutions poured on BHK-21 cell line monolayer to select the safety range of iodine complex. These concentrations (1.07mg, 0.535mg, 0.267mg, 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg/ml) were analyzed for their cytotoxic evaluation. The results revealed that at concentrations of 1.07mg, 0.535mg, 0.267mg/ml the cell survival percentage (CSPs) were 38%, 41%, 48% respectively. These concentrations were considered cytotoxic because CSPs were below 50%. The rest of the concentrations showed CSPs greater than 50% as shown in the table (3.1).

Table 1 Cytotoxicity Assay of Renessans (Iodine Complex)

Drug dilution	Mean±S.D	CSP	Remarks
1.07	0.183±0.021	38%	Cytotoxic
0.535	0.213±0.023	41%	Cytotoxic
0.267	0.251±0.031	48%	Cytotoxic
0.133	0.336±0.022	64%	SAFE
0.0668	0.418±0.025	87%	SAFE
0.0334	0.463±0.027	99%	SAFE
0.0167	0.430±0.032	90%	SAFE
0.0083	0.462±0.034	99%	SAFE
0.0041	0.458±0.036	97%	SAFE
0.0020	0.443±0.038	94%	SAFE

Extracellular Antiviral Activity (effect of drug on virus viability)

The extracellular antiviral activity was performed by incubating viral strains (O, A & Asia-1) with ten concentrations (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) of drug separately for 45 min at room temperature. The respective mixtures were then pour on 96 well plate having BHK-21 mono-layer in a triplicate manner and plates were incubated at 37oC for 16 hrs. in CO2 incubator. The virus control was infected with the same titer viral strains in the absence of Renessans. For FMD type O virus, the results revealed that at safe concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 1.89, 10 2.37, 10 3.39, 10 3.64, 10 3.91, 10 4.35, 10 4.95, 10 5.34, 10 5.94, 10 6.24) as compared to Control (i.e. 10 6.3) as shown in (table. 2). For type A virus, the results revealed that at safe concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 1.51, 10 1.80, 10 1.99, 103.01, 10 3.71, 10 4.29, 10 4.46, 10 4.94, 10 5.09, 10 5.34) as compared to Control (i.e. 105.5) shown in (table. 2). For type Asia 1 virus, the results revealed that at safe concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 1.42, 10 1.62, 10 1.83, 102.21, 10 2.71, 10 3.45, 10 4.55, 10 4.92, 10 5.24, 10 5.45) as compared to Control (i.e. 105.8) shown in (table. 2). The results of TCID50 shows that the virus titer was reduced significantly in the drug-treated virus culture, and Renessans (Iodine complex) has the ability to exhibit antiviral activity against FMDV. The fact indicates that drug successfully stop the attachment of virus in vitro cell culture.

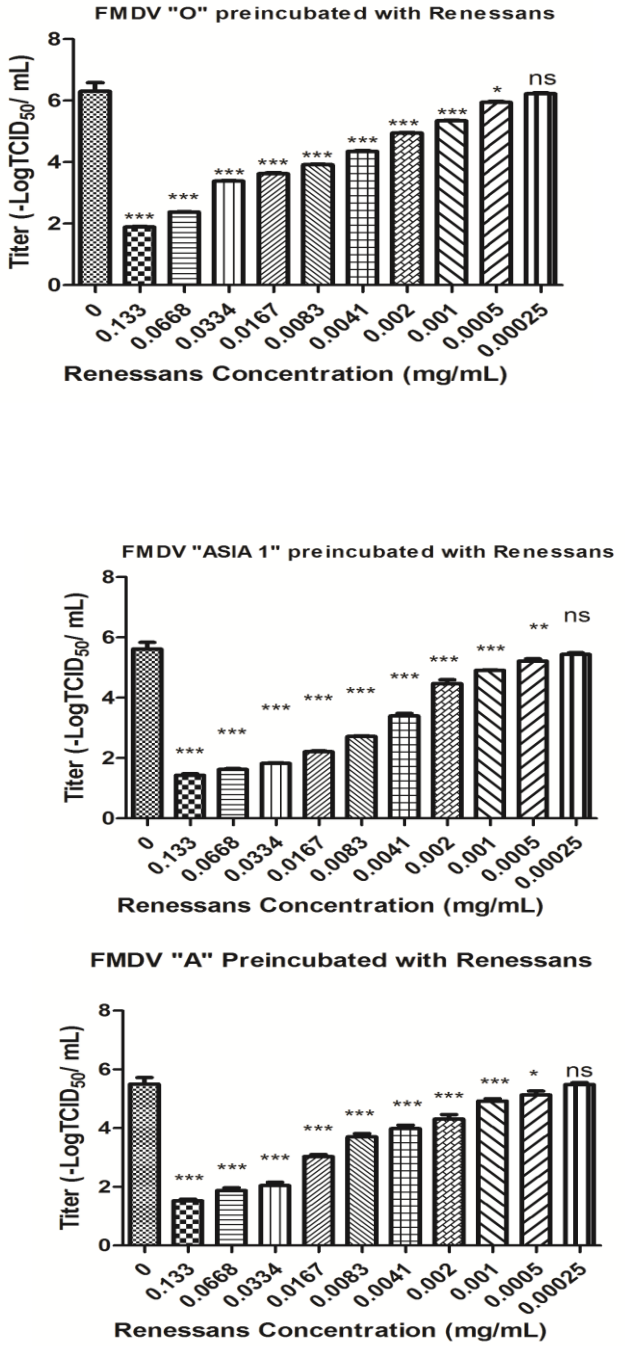
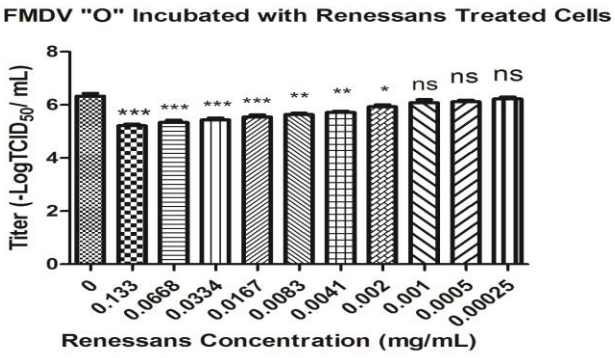


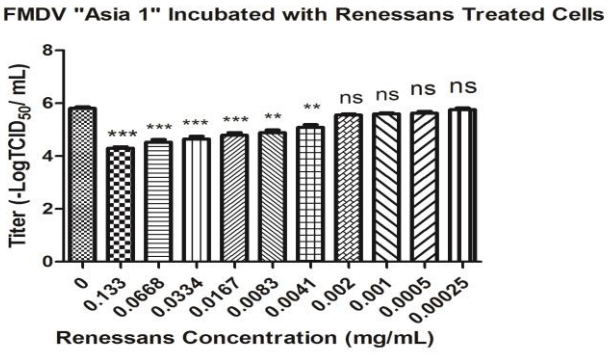
Fig.2. Antiviral Activity of Renessans against FMDV, FMDV strains were preincubated with Renessans and antiviral effect was determined using TCID50 after the drug exposure in three strains of FMDV (O, Asia-1, A) (0 =Virus control without the drug; (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg,

0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) =Renessans Concentrations (2-fold dilutions).

Extracellular Antiviral Activity (Drug treated cell line)

Ten drug concentrations (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) were incubated with BHK-21 Cells in 96 well plates having confluent monolayers separately and incubated for 1 hrs. The drug concentrations were then discarded in sterile condition. The plates were washed with PBS to remove free drug after incubations. After washing, fresh maintenance media along with viral strains FMDV O, A, Asia 1 was added separately in triplicate manner and plate were incubated at 37oC for 16 hrs. in CO2 incubator. The virus controls run along with. For FMD type O virus, the results revealed that at safe concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 5.24, 10 5.33, 10 5.41, 10 5.46, 10 5.51, 10 5.62, 10 5.84, 10 5.94, 10 6.21,10 6.25) as compared to Control (i.e.10 6.3) as shown in (table. 3). For type A virus, the results revealed that at safe concentrations concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 3.84, 10 3.99, 10 4.15, 10 4.26, 10 4.43, 10 4.68, 10 4.88, 10 5.14, 10 5.27, 10 5.36) as compared to Control (i.e. 105.5) as shown in (table. 3). For type Asia 1 virus, the results revealed that at safe concentrations concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were (10 4.22, 10 4.35, 10 4.41, 10 4.89, 10 4.99, 10 5.17, 10 5.35, 10 5.37, 10 5.64, 10 5.75) as compared to Control (i.e. 105.8) as shown in (table. 3). These results indicate that drug (Renessans) at these concentrations showed little effect or couldn't block cell receptors and viral particles successfully enter inside and started replication so it could infect the cells.





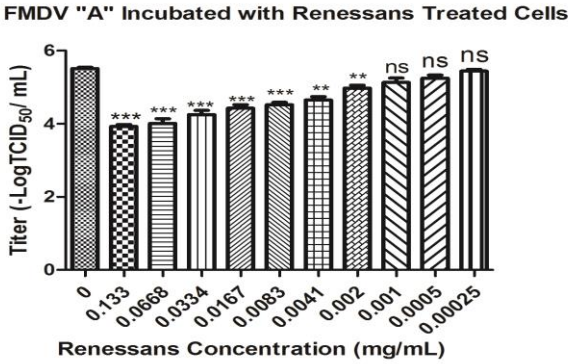


Fig.3 Effect of Renessans on viral attachment, the figure shows viral attachment no significant difference when FMDV strains (O, Asia-1, A) was introduced on Renessans treated BHK-21 Cells (0= Virus control without the drug; 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) =Renessans Concentrations (2-fold dilutions).

Intracellular Antiviral Activity (Post entry stages)

The BHK-21 cell line propagated in 96 well plates was incubated with viral strains (O, A, Asia 1) separately for 2, 12 hours (binding and entry stage, late protein synthesis and Assembly stage respectively). After incubation the viral suspensions were replaced with ten concentrations of Renessans (iodine complex). The virus replication/inhibition was evaluated by TCID 50.

Effect of Renessans on FMDV binding and entry stage

The intracellular antiviral activity was performed by incubating viral strains (O, A, Asia-1) in 96 well plates separately having confluent mono-layer of BHK-21 cell line for 2 hrs. The controls were also infected with the same titer of the virus in the absence of Renessans. The viral suspensions were removed after 2 hrs incubation. The plates were washed with PBS three times to remove unabsorbed virus after incubation. After washing, fresh maintenance media was added along with ten concentrations of Renessans (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) of iodine complex on BHK-21 mono-layer and plates were incubated at 37oC for 16 hrs in CO2 incubator. For FMD type O virus, the results revealed that at safe concentrations (i.e 0.133mg/ml 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 4.24, 10 4.83,10 5.21 ,10 5.54 ,10 4.71,10 5.28,10 5.64,10 5.91 10 6.25,10 6.35 as compared to control (i.e 10 6.3) as shown in (table. 4). For type Asia 1 virus, the results revealed that at safe concentrations i.e (0.133mg, 0.0668mg,

0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 3.75,10 3.95 ,10 4.21,10 4.39, 10 4.67, 10 4.95 ,10 5.25, 10 5.36 , 10 5.54 ,10 5.61 as compared to control (i.e 105.8) as shown in (table. 4). For type A virus, the results revealed that at safe concentrations i.e e (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 4.34 ,10 4.81 , 10 4.99 10 4.35, 10 4.81,10 5.18 ,10 5.22,10 5.29, 10 5.31, 10 5.54 as compared to control (i.e 105.5) as shown in (table. 4). The results concluded that drug could not restrict the replication of virus at entry stage as CSPs was below 50% and there was no significance.

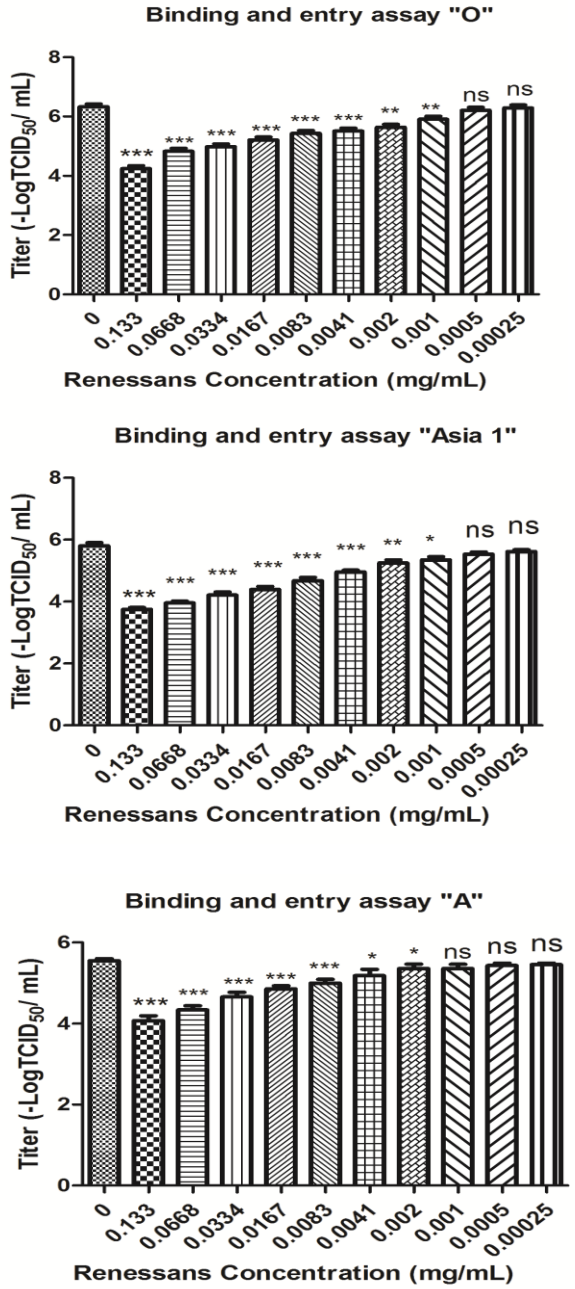
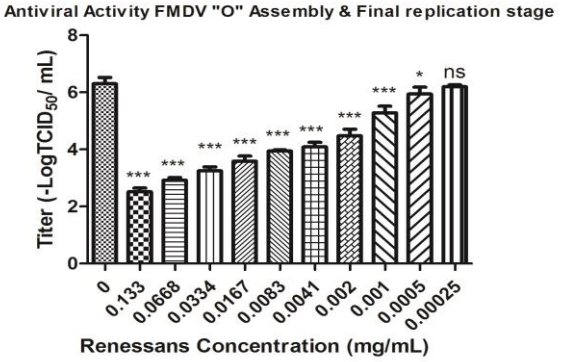


Fig.4 Effect of ivermectin on FMDV binding and entry stage, the figure shows the results calculated in binding and entry stages of viral infection. A significant difference can be observed at higher concentrations in the graph as compared to the controls. (0= Virus control without the drug; 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) =Renessans Concentrations (2-fold dilutions).

Renessans inhibits FMDV during post-entry stages

The intracellular antiviral activity was performed by incubating viral strains (O, A, Asia-1) in 96 well plates separately having confluent mono-layer of BHK-21 cell line for 12 hrs. The control well was infected with the virus without adding drug. The viral suspensions were removed after 12 hrs incubation. The plates were washed with PBS three times to remove unabsorbed virus after incubations. After washing, fresh maintenance media was added along with ten concentrations of Renessans (0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) of iodine complex on BHK-21 mono-layer and plates were incubated at 37oC for 16 hrs in CO2 incubator. For FMD type O virus, the results revealed that at safe concentrations (i.e 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 2.31 ,10 2.48 ,10 2.83 ,10 2.99 ,10 3.21 ,10 3.79, 10 4.08 ,10 4.88 ,10 5.75 ,10 6.21 as compared to control (i.e 10 6.3) as shown in (table. 7). For type Asia 1 virus, the results revealed that at safe concentration (i.e 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 2.25 10 2.69 10 2.97 10 3.66 10 3.97 10 4.46 10 4.51 10 5.11 10 5.19 10 5.35 as compared to standard virus (i.e 105.5) as shown in (table. 7). For type A virus, the results revealed that at safe concentration (i.e 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) the TCID50 were 10 1.96,10 2.42 ,10 2.83 , 10 3.10, 10 3.45, 10 3.91, 10 4.26, 10 4.73, 10 5.02, 10 5.28 as compared to control (i.e 105.8) as shown in (table. 7). The data exhibited that the Renessans successfully hinders the assembly of viral particle at this stage. These results showed that Renessans largely inhibited the replication steps of FMDV.



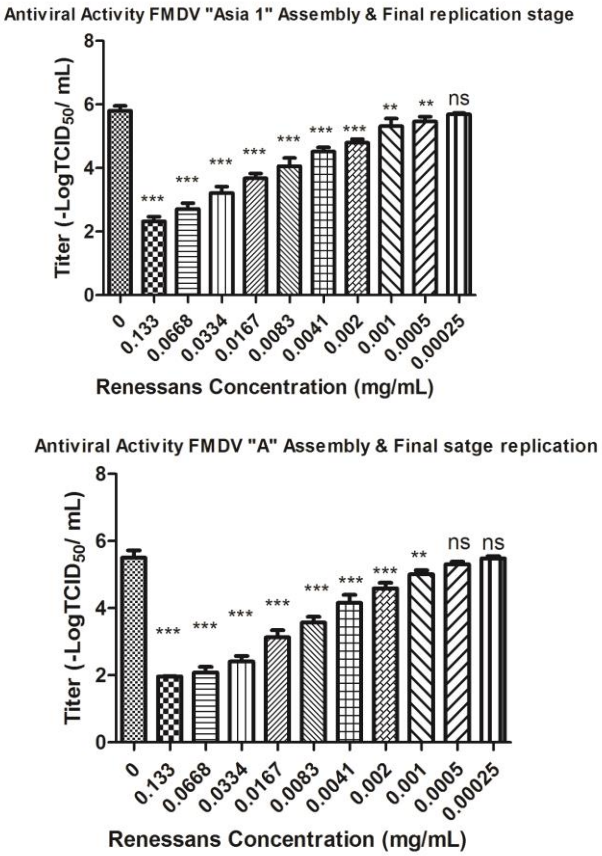


Fig: 5. Effect of Renessans on viral Assembly & final replication stage: The figure shows the results of viral assembly & final replication stages of viral infection (O, Asia-1, A). A significant reduction in viral replication can be observed in the graph as compared to the controls. (0= Virus control without the drug; 0.133mg, 0.0668mg, 0.0334mg, 0.0167mg, 0.0083mg, 0.0041mg, 0.0020mg, 0.001mg, 0.0005mg, and 0.00025mg/ml) =Renessans Concentrations (2-fold dilutions).

DISCUSSION

Foot and Mouth is an infectious disease caused by FMD Virus. There are several vaccines available against disease including inactivated whole virus vaccines, new inactivated whole virus marker vaccines and subunit vaccine. The FMD vaccine has problems including low immunogenicity, instability of the antigen, insufficient maintenance of the antibody, and less or no cross-protection across serotypes [18]. These vaccines do not provide clinical protection until seven days post-vaccination. Therefore, there is a need for developing effective and safe alternative antiviral strategies against FMDV.

The antiviral potential of micronutrients is still an area wide open for research. Among the micronutrients, Iodine has known for antimicrobial properties, and therefore used in topical applications. The role for inactivation of enveloped and non-enveloped viruses [19] its use in physical inactivation of SARS-CoV-1, MERS has already been demonstrated [19]. In a study, efficacy of povidine-Iodine was tested against MERS-CoV. The reduction in the virus titer indicated that it restricted the viral growth [20]. The use of iodine as a systemic therapy is yet debatable for its toxicity. There is need of cytotoxic analysis to check the systemic antimicrobial potential of Iodine [21]. In a recent study, it was demonstrated that highly complexed iodine could rapidly and efficiently inactivate African swine fever virus ASFV, representing an effective disinfectant for ASF control. [22].

In our study, the antiviral activity of Iodine complex (Renessans) against FMDV (O, ASIA-1 & A) was evaluated. The Iodine complex (Renessans) has been approved from Drug Regulatory Authority of Pakistan (DRAP) for clinical use. The cytotoxicity assay was assessed on BHK-21 cell line by MTT Assay. Ten dilutions of iodine complex (Renessans, complex of iodine commercial product) were checked in BHK-21 cell line [23]. The ten non-toxic concentrations (2-fold dilution) (0.133, 0.0668, 0.0334, 0.0167, 0.0083, 0.0041, 0.0020, 0.001,0 .0005, 0.00025mg/ml) were evaluated against FMDV (O, ASIA-1 & A). There was no change in cell morphology observed at these concentrations. The Renessans revealed great antiviral potential against FMDV (O, ASIA-1 & A) at higher non-toxic concentrations.

We find out Renessans's antiviral activity by viral inhibition assays and TCID50 by using the ten non-toxic drug concentrations. Different treatments were given to the BHK-21 cells along with infection to know at which point of the replication cycle Renessans employ its effect, which indicated that it significantly decreased the titer of virus in case of all three strains of FMDV. The titer of each strain used in the experiments was calculated by TCID50 independently. The virus titer in strain O control was 106.3 TCID50/mL and was reduced to 101.89 TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. In the case of strain Asia-1, the virus titer was reduced from (control) 105.8 TCID50/mL to 101.42 TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. The titer of strain A was reduced from 105.5 TCID50/mL to 101.51 TCID50/mL at highest non-toxic concentration (i.e 0.133mg/ml) and gradually titer increases as drug serially diluted. In line with the TCID50 data, cell morphological analysis revealed that there is no/few CPE were observed in the presence of Renessans as compare to control cells.

Our research results supported the study conducted by Sriwilaijaroen and his team studied the inhibition of avian and human influenza A virus infection by PVP-I and explain that PVP-I restrict both viral HA binding activity and viral NA catalytic hydrolysis, mediating virus entry into host cells, and virion release and spread to a new host cell, respectively [17]. Similar results were observed in case of CupriDyne, a disinfectant solution containing Iodine was assessed against COVID-19. The iodine solution effectively inactivate virus in time dependent manner, reducing the virus titers by 99% and reducing the virus titers below detection limit after 60 min [24]. Similarly, iodine complex had exhibited a virucidal activity against MERS virus, the virus inactivation of $\geq$ 99.99% within 15 s of application. Moreover, iodine product had reduced the SARS-CoV infectivity to undetectable levels in 2 min of exposure in Vero infected Cells [25]. The iodine formulations have shown antiviral activity up to 99.99% in an experiment against modified vaccinia virus [26] and it also prove inhibitory

effect against adenoviral conjunctivitis. In a recent study Povidone-iodine (P-I) is being tested as a topical antiviral treatment for eye infections caused by adenovirus. P-I produced greater than 3-Log10 reductions of titers at 1–5 min for most of the ocular types tested [27].

The overall previous studies confirm our results of iodine complexes which have showed great antiviral potential against FMDV (O, ASIA-1 & A). Our study also indicated that the titer of viral strains was reduced more when FMDV preincubated with Renessans and post-entry stages of the replication cycle. This predicts the function of Renessans during replication of FMDV. This study concludes that Renessans has the potential as an anti-FMDV drug and may help control this disease.

The previous research work on antiviral potential of Iodine suggest the mechanism involved in the activity of iodine against viruses, it is more likely that iodine makes some changes on the viral receptors. The current study revealed that the drug (Renessans) also restricted the replication inside cell. It seems to be a general mechanism underlying the inhibitory effect of iodine on other viruses, Limitation of the study is that we only used TCID50 to measure viral concentrations but there is need to design further experiments to expose the mechanism that how iodine restrict the viral replication inside the cell.

IV. Conflict of interest

The author declare that they have no known competing financial interest or personal relationship that could have appear to influence the work reported in this paper.

V. CONCLUSION

This study indicates that Renessans (iodine containing formulation), has potential to inhibit FMDV (O, A, Asia 1) which needs to be further investigated through in vivo clinical trials. The FMDV strains (O, A and Asia 1) were inhibited in the presence of Renessans.

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