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INVITRO AND INVIVO ANTIVIRAL EFFECT OF HOMEOPATHY MEDICINES AGAINST TILAPIA LAKE VIRUS

Paramanandan Rakesh 1 , * Panchavarnam Sivasankar 1 , Bagthasingh Chrisolite 1 , Paulraj Magesh Kumar 1 , M. Mohamed Mansoor 1 , Muthumariappan Selvamagheswaran 1 , Mulloorpeedikayil Rosalind George 2 , Palaniyappan Subash 1

1 Department of Fish Pathology and Health Management , Fisheries College and Research Institute, Thoothukudi - 628 008, Tamil Nadu, India.

2 Department of Aquatic Animal health Management, Kerala university of Fisheries and Ocean Studies, Cochin, India.

Abstract:

Tilapia lake virus (TiLV) is a novel virus that causes large scale mortalities in Tilapia farming and is considered a threat to the global tilapia industry. In the present study, the antiviral effects of homeopathy and herbal remedies against TiLV were investigated both *in vitro* and *in vivo*. A total of ten homeopathy medicines including *Belladonna* 200C, *Pyrogenium* 200C, *Influenzinum* 200C, *Crotalus horridus* 200C, *Arsenicum album* 200C, *Hepar sulphur* 200C, *Aconitum napellus* 200C, *Tuberculinum* 200C, *Bothrops lanceolatus* 200C and *Nux vomica* 200C were investigated at different concentrations *viz*, 100 µl /ml, 250 µl /ml and 500µl /ml of medium for screening the antiviral effects against TiLV using cell culture. Antiviral effect exhibited by homeopathy remedies including *Pyrogenium* 200C, *Influenzinum* 200C, *Crotalus horridus* 200C at different concentrations like 20ml, 40ml and 60ml/kg of feed were further investigated for their *in vivo* potential in Nile Tilapia against TiLV infection. homeopathy medicines exhibited no effect against TiLV in the experimental study.

Key Words **:** Cell culture, Homeopathy medicines, TiLV.

1. INTRODUCTION

Tilapia is the second most important fish species for aquaculture next to carps. During 2020, global tilapia production increased by 3.3 percent, reaching 6 million tonnes. China, Ecuador, Egypt, Indonesia and Thailand are the largest tilapia producing countries while the largest importing countries are United States. Niletilapia (*Oreochromis niloticus*) is the most important candidate species cultured globally among the 100 species of tilapiines. Tilapia is considered as an important candidate species for culture aspect due to their nutritional value, omnivorous diet, tolerance for high-density aquaculture, and relative disease resistance. Initially, tilapias were considered as more resistant to bacterial, parasitic, fungal and viral diseases compared to other cultured species. But Tilapia is susceptible to both bacterial and parasitic diseases including *Streptococcus sp, Flavobacterium columnare, Aeromonas hydrophila, Edwarsiella tarda, Ichthyophitirius multifillis, Tricodhina sp,* and *Gyrodactylus niloticus* (Klesius et al., 2008). There was no report viral disease in Tilapia until 2009. During the summer of 2009 in Israel, there was enormous amount of mortality observed in both wild and farmed hybrid tilapia (*O. niloticus* × *O. aureus*) and the etiological agent was subsequently identified in 2013

as Tilapia Lake Virus (TiLV) (Eyngor et al., 2014). 3 After that, the virus has been reported in various countries including Israel, Thailand, Ecuador, Colombia, Egypt, Kerala, West Bengal and Tamilnadu in India (Behera et al., 2018; Dong et al., 2017; Eyngor et al., 2014; Fathi et al., 2017b; Ferguson et al., 2014; Saranya et al., 2020; Tsofack et al., 2017). Several fish species including (*Oreochromis niloticus* × *O. aureus*), Nile tilapia (*O. niloticus*), Red tilapia (*Oreochromis sp*.), Mozambique tilapia (*O. mossambicus*) (Amal et al., 2018; Eyngor et al., 2014; Fathi et al., 2017b; Ferguson et al., 2014; Mugimba et al., 2018; Surachetpong et al., 2017; Waiyamitra et al., 2021), giant gourami (*Osphronemus goramy*) (Chiamkunakorn et al., 2019; Jaemwimol et al., 2018), and ornamental African cichlids (*Aulonocara spp*) (Yamkasem et al., 2021) have been found to be infected by TiLV. TiLV disease is an emerging and transboundary disease of tilapia culture, causing mortality up to 90% globally in farmed tilapia over the last 4–5 years (Aich et al., 2022; Eyngor et al., 2014). The virus is a negative-sense, single-stranded RNA virus (-ssRNA) made of an icosahedron envelope with a genome length of 10,323 kb and 55–100 nm diameter (Eyngor et al., 2014). It is an orthomyxo-like virus and the only member of the genus Tilapinevirus in the family Amnoonviridae (Eyngor et al., 2014; Bacharach et al., 2016). This virus infected fish could show symptoms that include anorexia, abnormal swimming, severe anemia, exophthalmia, skin erosion and congestion, scale protrusion, and abdominal swelling (Tattiyapong et al., 2017). The occurrence and spreading of disease can be avoided by following the proper health management approaches. Some experimental vaccines have been developed for TiLV that are heat-killed and formalin-killed vaccine (Mai et al., 2022), DNA vaccine (Yu et al., 2021), VP20-based vaccine (Zeng et al., 2021), inactivated vaccine containing montanide adjuvant (Zeng et al., 2021) but these are not yet commercialized for aquaculture use. Antibiotics and other chemotherapeutics have been used to control fish and shellfish diseases but could result in the development of drug-resistant pathogens (Le et al., 2005), environmental threats (Rico et al., 2012), and accumulation of residues in fish (Baleta et al., 2019). Homeopathy medicine can be used as an alternative option to control TiLV infection. Therefore, the present study is intended to evaluate the antiviral effect of different homeopathy medicines against TiLV in both invitro and invivo study.

2. MATERIALS AND METHODS

2.1 Virus strain and Cell line

The virus TiLV isolated from the infected tilapia fish was used for the study. The SSN1 cell line derived from snakehead fish in the Department of Fish Pathology and Health Management of FCRI, Thoothukudi was used for virus isolation and propagation. The virus titer (TCID 50 /ml) was determined by end-point dilution assay using 96 well plates and calculated according to the method of Spearman-Karber (Lei et al., 2021).

2.2 Homeopathy medicines

In the present study, ten homeopathy medicines including *Belladonna* 200C, *Pyrogenium* 200C, *Influenzinum* 200C, *Crotalus horridus* 200C, *Arsenicum album* 200C, *Hepar sulphur* 200C, *Aconitum napellus* 200C, *Tuberculinum* 200C, *Bothrops lanceolatus* 200C and *Nux vomica* 200C were tested for their antiviral effect against TiLV infection in cell culture. The homeopathy medicines used in the study were purchased from Dhivyam Medicals, Madurai, Tamil Nadu.

2.3 Determination of the cytotoxicity of the homeopathy medicines

The cytotoxicity of the homeopathy medicines was determined in SSN1 cell cultures by cell viability. For this, medicines were added to the cell cultures in concentrations of 50, 100, 250, and 500 µl/ml and incubated for 7 days. The viability of the cells was determined by staining with a trypan blue solution (0.5%).

2.4 Treatment with homeopathy medicines

2.4.1 Experiment – 1

The assay was performed in the full monolayer of SSN1 cell line developed in cell culture plastic culture flask with (25 cm^2) (Thermo, Korea). When the monolayer became sufficiently confluent, TiLV was inoculated with 500 µl of the TiLV stock ($10^{4.5}$ $TCID_{50}$/ml and $10^{8.5}$ $TCID_{50}$/ml) and incubated for 15 to 20 min at room temperature. Followed by incubation, the virus solution was removed and homeopathy and herbal remedies were added with a concentration of 500 µl/ml of medium. After the incubation at 27 ^{o}C, the result was observed (Nefedchenko *et al*., 2015).

2.4.2 Experiment – 2

A neutralisation test was carried out by titrating a constant quantity of virus with the homeopathy medicines in 96 well plate after developing the full monolayer of SSN1 cells. Maintenance medium (L-15 medium) amounting to 90 µl was added into each well except column one. TiLV preparation (100 µl) was added to the first column as per the protocol in duplicate. The virus was serially double diluted by transferring 10 µl across the plate. Back titration of the TiLV preparation was also carried out simultaneously in the same titration plate to confirm the amount of virus used in the assay. A 100 µl of homeopathy and herbal remedies were added to

each well in diluted virus bearing rows and maintenance medium was added at 10 µl into all wells having TiLV preparation. Finally, the plate was incubated at 27 ^{o}C and CPE development was recorded over 7 - 10 days.

2.4.3 Statistical analysis

All the data were analysed in triplicates using IBM SPSS 26 (SPSS Inc.). The descriptive statistics (Mean and standard deviations) were calculated for all the parameters. The effects of two factors (Independent variables) namely, the groups, the time intervals and interactions (groups × time intervals) on the dependent variable (Log $_{10}$ TCID $_{50}$) were determined by two-way ANOVA. The Tukey's post hoc test for multiple comparisons at the significance level $p < 0.05$ was used to compare the differences between the experimental groups over the dependent variable (Log $_{10}$ TCID $_{50}$) (Fig 2,3,4).

2.5 Pathogenicity experiment

Forty tilapia fingerlings (weight 8 ± 0.5 g) were separated into 2 groups (duplicate/group) with a total of 4 tanks and 10 fish of each were used for experimental infection. TiLV were injected intraperitoneally (IP) at 100 µl/ fish with a concentration of $1 \times 10^{8.5}$ TCID $_{50}$ / ml and the experimental control group was injected with 100 µl/ fish of L-15 medium. The challenged and unchallenged fish were fed twice daily with commercial pelleted feed and maintained with 20 - 30 % water exchange daily. The fish were then examined daily for any behavioural and physical changes and mortality.

2.6 Treatment using homeopathy medicines

2.6.1 Experimental Fish

A total of 160 fingerlings of Nile tilapia, *Oreochromis niloticus* (average body length 7.4cm) were collected from fish farm, FC&RI, Thoothukudi. The fish were maintained in 24 glass tanks with 100 L capacity. These fishes were acclimatized in a tank for 7 days and fed commercial pelleted feed twice a day between 9.00 am and 4.30 pm. About 30% of water was exchanged daily to maintain the water quality. These fishes were screened by PCR to confirm the fishes are TiLV free.

2.6.2 Feed preparation

The experimental diet was prepared by incorporating either homeopathy medicines or herbal extracts using binder, sodium alginate (1g /100 ml of distilled water). These remedies were incorporated at different concentrations including 20 ml, 40 ml and 60 ml per kg of feed. The feed was then air dried in a room temperature for 24 h and stored in a bottle for further use (Lewandowski *et al*., 2019).

2.6.3 Homeopathy treatment for TiLV challenged fish

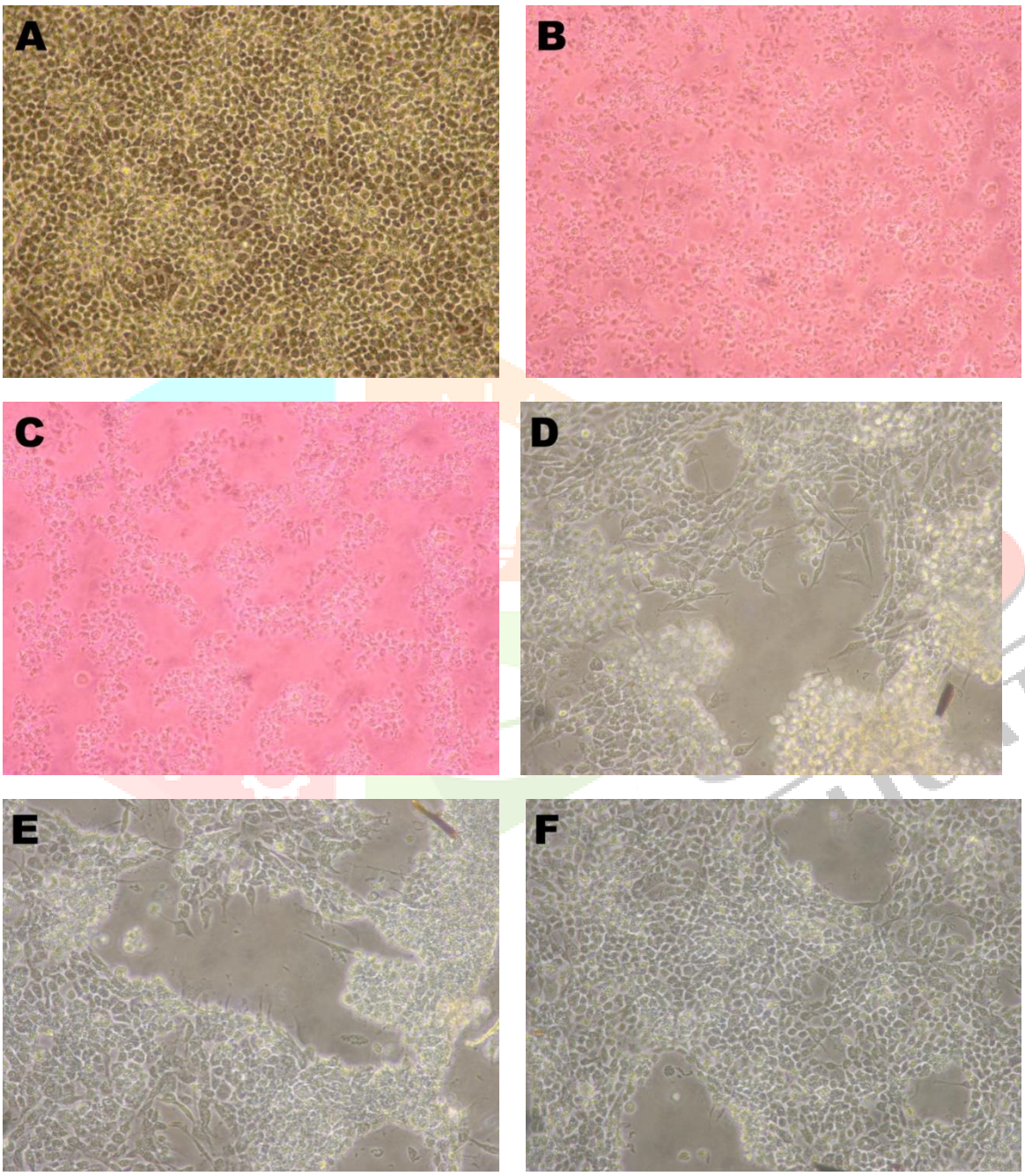
Out of 10 homeopathy medicines screened for anti-TiLV in cell culture, three medicines such as *Crotalus horridus* 200C, *Influenzinum* 200C and *Pyrogenium* 200C were found to have antiviral effects. These three medicines were further used for the treatment of tilapia following TiLV infection. Accordingly, two hundred and sixty-four tilapia fingerlings (weight 13 ± 0.5 g) were separated into eleven groups in duplicate each group with a total of 22 tanks. Each group of 12 fish was used for experimental infection followed by homeopathy treatment. The details of each group are: experimental control as group-A, Virus control as group-B, treatment with *Crotalus horridus* 200C as group-C1 (20 ml/ kg), C2 (40 ml/ kg), C3 (60 ml/ kg), treatment with *Influenzinum* 200C as group -D1 (20 ml/ kg), -D2 (40 ml/ kg), -D3 (60 ml/ kg), treatment with *Pyrogenium* 200C as group-EI (20 ml/ kg), -E2 (40 ml/ kg), -E3 (60 ml/ kg). Group-A of 12 fish in duplicate were injected by L-15 medium at 100 µl/ fish intra-peritoneally. Except for Group-A, the remaining 10 groups of 12 fish in duplicate were injected by TiLV infected cell culture supernatant ($1\times10^{8.5}$ $TCID_{50}$/ ml) at 100 µl/ fish intraperitoneally. Fish were maintained in glass tanks and were fed twice daily with medicated and non-medicated feed. The fish were examined for any behavioural and physical changes and mortality rate for 14 days.

3. RESULTS

Before testing the antiviral effect, all the homeopathy medicines were tested for their toxicity to SSN1 cell cultures (Table 5). All the medicines exhibited cell toxicity only at a concentration of 750 µl/ ml by causing morphological changes and increasing dead cells. Further, all these homeopathy medicines were tested for their antiviral effect against TiLV using SSN1 cell line. Out of ten tested, three homeopathy medicines such as *Crotalus horridus* 200C, *Influenzinum* 200C and *Pyrogenium 200C* were found to have the antiviral effect against TiLV by reducing the cytopathic effect. Among three different concentrations (100 µl/ ml, 250 µl/ ml and 500 µl/ ml) of homeopathy medicines, 500 µl/ ml of homeopathy medicines showed antiviral activity (Fig 1). Among these three medicines, *Crotalus horridus* 200C and *Influenzinum* 200C had shown highest potential against TiLV. Virus inoculated SSN1 cell monolayer exhibited a typical cytopathic effect with the appearance

of small foci with cell lysis and rounding up of cells at the edge of foci while no cytopathic effect was found in mock inoculated.

In the second experiment, the infectivity of TiLV concentrated to $10^{4.5}$TCID$_{50}$/ ml and $10^{8.5}$TCID$_{50}$/ ml were completely neutralized by *Crotalus horridus* 200C and *Influenzinum* 200C. *Pyrogenium 200C* reduced the infectivity of the virus from $10^{8.5}$ to $10^{3.5}$TCID$_{50}$/ ml and $10^{4.5}$ TCID$_{50}$/ ml to $10^{1.5}$ TCID$_{50}$/ ml during 7 days of observation (Table 1). Parallel to homeopathy medicine, the infectivity of TiLV in the SSN1 cell line was found to be $10^{8.5}$ TCID$_{50}$/ ml, while no CPE was noticed in the control cell line which is mock inoculated.



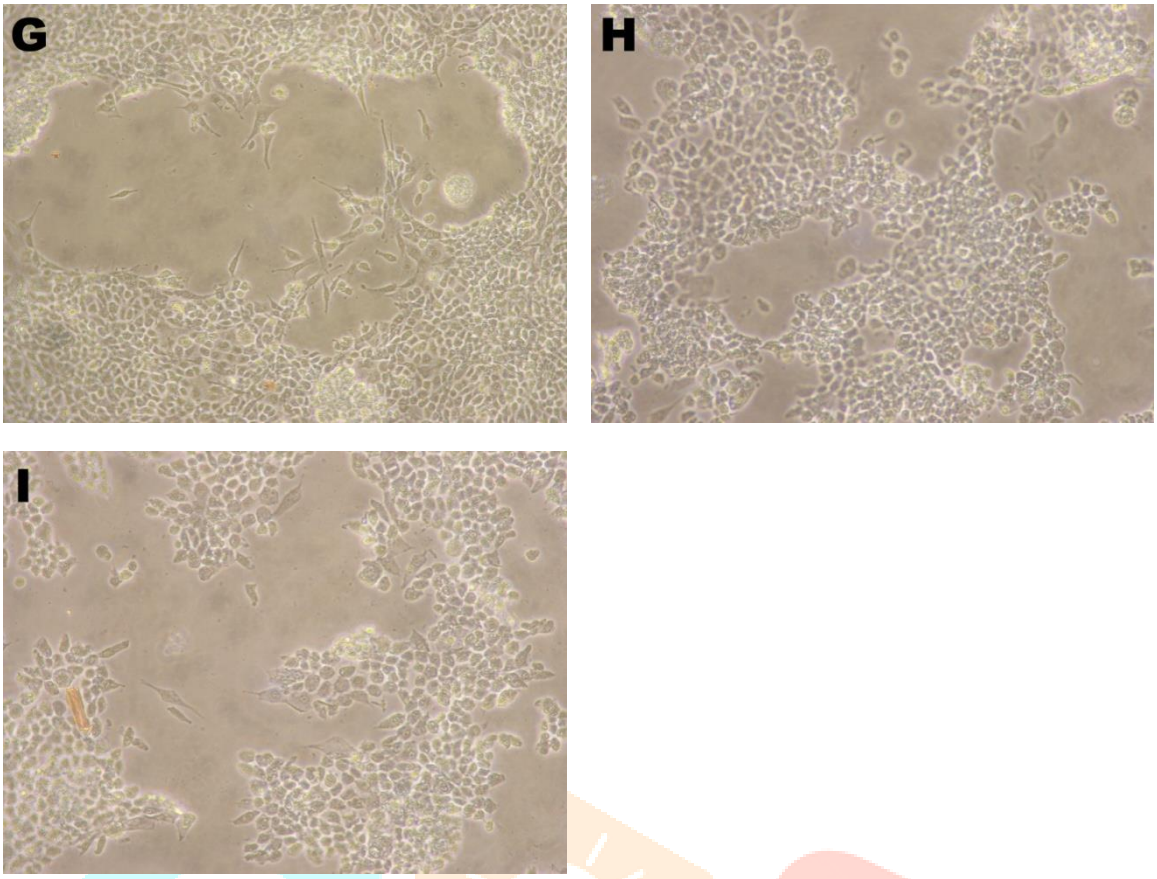


Figure 1. Cytopathic effect induced by TiLV in SSN1 cells. (A) Uninfected control SSN1 cells (B) TiLV ($10^{4.5}$ $TCID_{50}$/ml) infected cells (C) TiLV ($10^{8.5}$$TCID_{50}$/ml) infected cells (D) TiLV ($10^{4.5}$ $TCID_{50}$/ml) infected SSN1 cells treated with *Crotalus horridus* 200C (E) TiLV ($10^{8.5}$ $TCID_{50}$/ml) infected SSN1 cells treated with *Crotalus horridus* 200C (F) TiLV($10^{4.5}$ $TCID_{50}$) infected SSN1 cells treated with *Influenzinum* 200C (G) TiLV ($10^{8.5}$ $TCID_{50}$/ml) infected SSN1 cells treated with *Influenzinum* 200C (H) TiLV ($10^{4.5}$ $TCID_{50}$/ml) infected SSN1 cells treated with Pyrogenium 200C (I) TiLV ($10^{8.5}$ $TCID_{50}$/ml) infected SSN1 cells treated with Pyrogenium 200C.

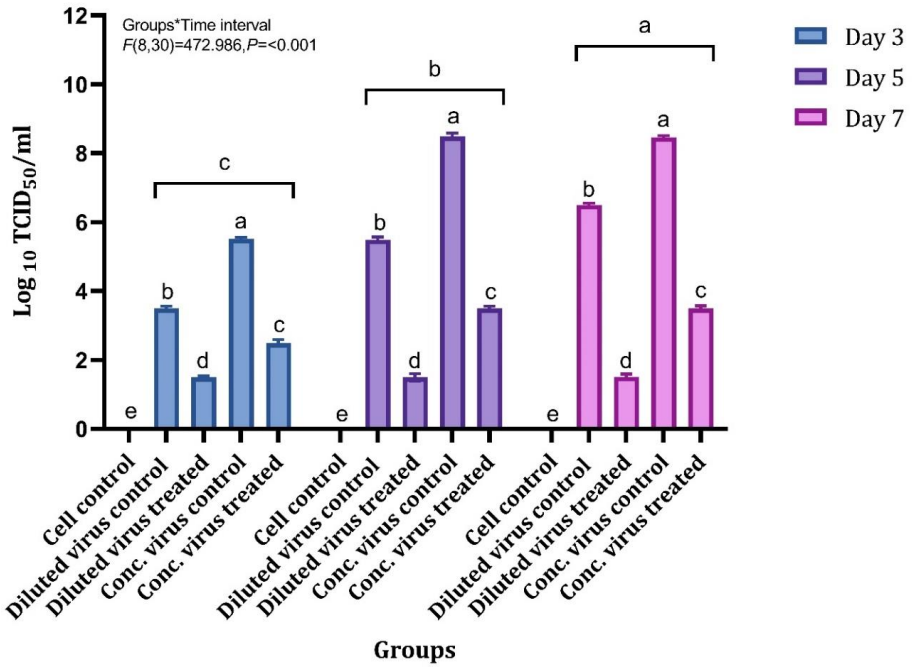


Figure 2. Antiviral potential of *Crotalus horridus* 200C. The data were provided in Mean ± SD of three replicates per group (N=3). The vertical bars with different alphabets (a,b,c…) over them are significantly different between groups and at different time intervals as determined by Two-way ANOVA followed by Tukey's post hoc test for multiple comparisons of means (P <0.05)

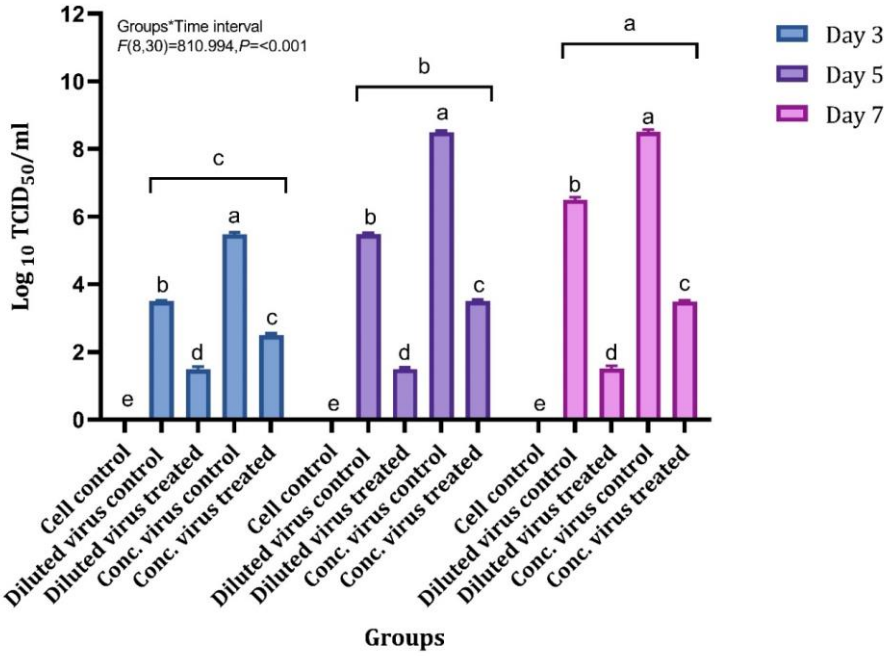


Figure 3. Antiviral potential of *Influenzinum* 200C. The data were provided in Mean ± SD of three replicates per group (N=3). The vertical bars with different alphabets (a,b,c…) over them are significantly different between groups and at different time intervals as determined by Two-way ANOVA followed by Tukey's post hoc test for multiple comparisons of means (*P* <0.05).

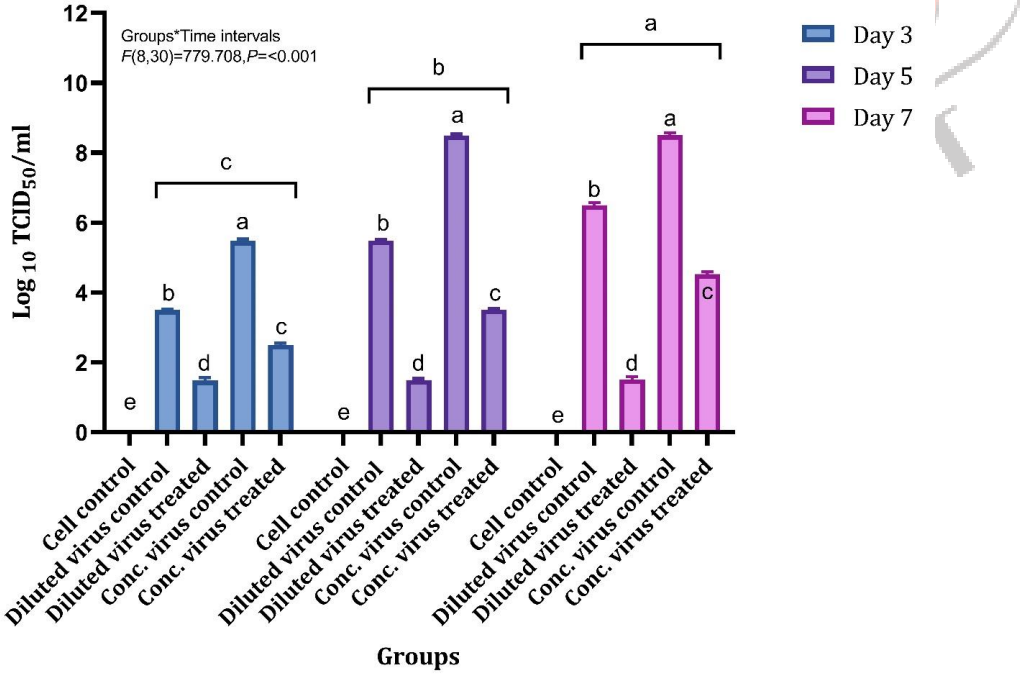


Figure 4. Antiviral potential of *pyrogenium* 200c. The data were provided in mean ± sd of three replicates per group (n=3). The vertical bars with different alphabets (a,b,c…) over them are significantly different between groups and at different time intervals as determined by two-way anova followed by tukey's post hoc test for multiple comparisons of means (*p* <0.05).

Table 1. Antiviral effect of homeopathy medicine in cell culture

Homeopathy medicines	Concentrated virus ($10^{8.5}$TCID50/1ml of medium)	Concentrated virus ($10^{8.5}$TCID50/1ml of medium) + treatment	Diluted virus (10^{2} TCID50/1ml of medium)	Diluted virus (10^{2} TCID50/1ml of medium) + treatment
Crotalus horridus 200C	$10^{8.5}$ $TCID_{50}$/1ml	$10^{3.5}$ $TCID_{50}$/1ml	$10^{4.5}$ $TCID_{50}$/1ml	$10^{1.5}$ $TCID_{50}$/1ml
Influenzinum 200C	$10^{8.5}$ $TCID_{50}$/1ml	$10^{3.5}$ $TCID_{50}$/1ml	$10^{4.5}$ $TCID_{50}$/1ml	$10^{1.5}$ $TCID_{50}$/1ml
Pyrogenium 200C	$10^{8.5}$ $TCID_{50}$/1ml	$10^{4.5}$ $TCID_{50}$/1ml	$10^{4.5}$ $TCID_{50}$/1ml	$10^{1.5}$ $TCID_{50}$/1ml

3.1 Pathogenicity study

In the experimental challenge study, the cumulative mortality of Tilapia following challenge with TiLV was recorded for 14 days. In this experiment, the mortality started at 3dpi in virus injected group and the cumulative mortality was 100% at 14 dpi (Fig 8). The infected animals exhibited various clinical signs including anorexia, haemorrhages, erratic swimming behaviour, lethargy, scale protrusion, discolouration, fluid accumulation and abdominal swelling

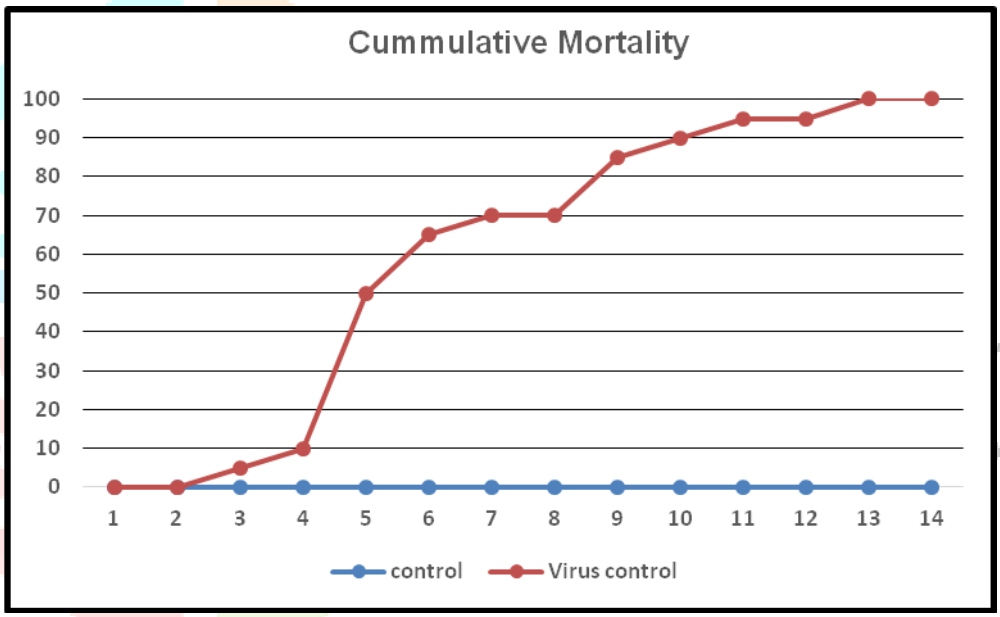


Fig 5: Cumulative mortality occurred at 14 days period of time against TiLV infected fingerlings.

3.2 Antiviral effect of homeopathy medicines in experimentally infected fishes

The antiviral effect of homeopathy medicines was evaluated following the challenge with TiLV. In this experiment, the mortality started at 3 dpi in virus control and 100% cumulative mortality was found at 10 dpi. During the experimental study, all treated and untreated fishes exhibited certain symptoms including lethargy, abdomen swelling, exophthalmia, anorexia and pale skin. Accumulation of fluid was also noticed in the internal organs of the animal. Most of the fish died of the symptoms of abdominal swelling. This study resulted that all the homeopathy medicine, *Crotalus horridus* 200C and *Influenzinum* 200C at three different concentrations of 20 ml, 40 ml and 60 ml per kg of feed had less antiviral activity against TiLV while, *Pyrogenium* 200C at three different concentrations had no antiviral activity (Fig 8).

RPS value of medicated feed of *Crotalus horridus* 200C was 16.6% while *Influenzinum* 200C 8.3% at 60 ml/ Kg of feed (Figure 6, 7). *Crotalus horridus* 200C and *Influenzinum* 200C at a concentration of 60 ml/ Kg of feed were found to have antiviral effects when compared to lower concentrations. This study resulted in high concentration of medicine having some effect against TiLV.

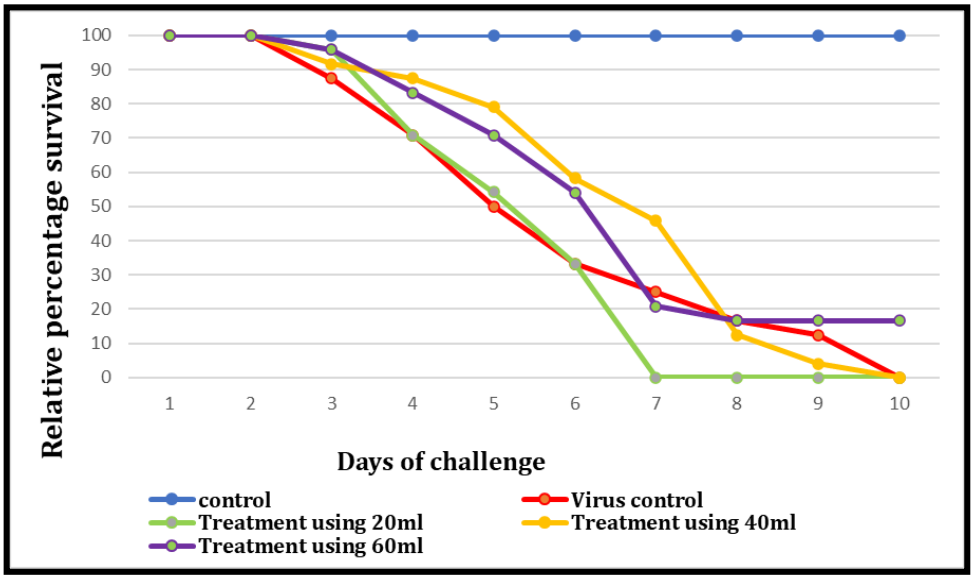


Fig 6. Antiviral effect of *Crotalus horridus* 200C treated fishes against TiLV

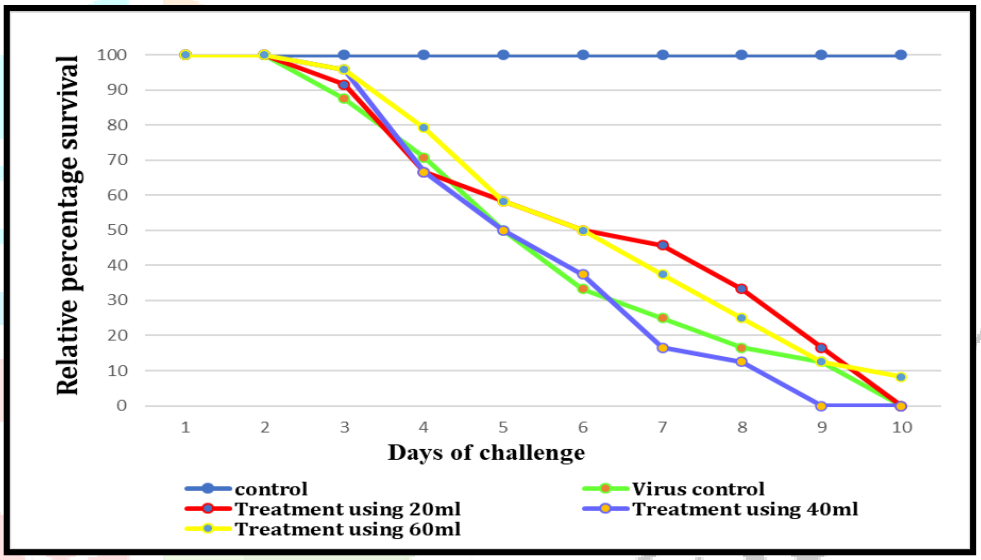


Fig 7. Antiviral effect of *Influenzinum* 200C treated fishes against TiLV

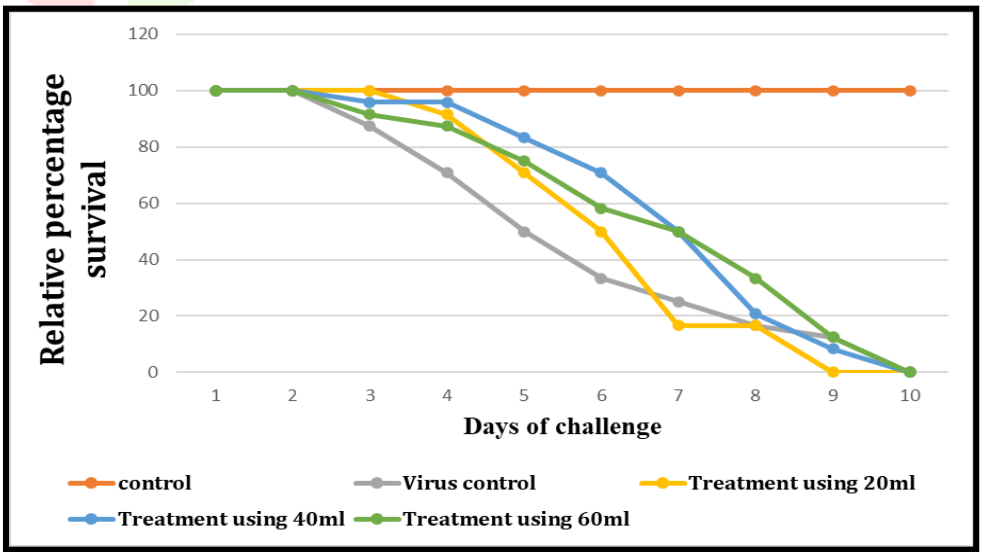


Fig: 8. Antiviral effect of *Pyrogenium* 200C treated fishes against TiLV

4. DISCUSSION

During the past few years, TiLV infecting tilapia pose a great threat to the tilapia aquaculture industries globally. TiLV outbreak has become a severe loss in tilapia farming resulting in greater risk in tilapia production (Jansen *et al*., 2019). Experimental studies are required to find out the possibility of control of TiLV infection. Treating viral diseases with chemicals or drugs is ineffective. Few vaccines are commercially available for fish viruses including ISKNV, RSIV and NNV. To date, no commercial vaccine is available to prevent TiLV as it is an emerging virus. Hence, alternative studies on natural compounds to prevent or control of TiLV outbreaks are urgently needed (Lertwanakarn *et al*., 2021).

4.1. In vitro and in vivo antiviral effects of homeopathy medicines

Cell culture is a very versatile tool that has been used as a model system to study the interaction between cells and viruses and to study the potential of different natural compounds like homeopathy medicines an their toxicity (Segeritz *et al*., 2017). TiLV could cause cytopathic effects and massive cell death within 3-7 days in various fish cell lines (Liamnimitr *et al*., 2018; Nanthini *et al*., 2019; Thangaraj *et al*., 2018; Wang *et al*., 2018, Yadav *et al*., 2021; Lertwanakarn *et al*., 2021; Haridas *et al*., 2022). As TiLV can grow well in both SSN1 and EPC cells, the SSN1 cell line was used for virus propagation and for investigating the antiviral effects of homeopathy and herbal medicines (Haridas *et al*., 2022). In the present study, TiLV infected SSN1 cell line exhibited a typical cytopathic effect with the appearance of small foci with complete cell lysis within 4 days post inoculation.

Homeopathy is a form of complementary and alternative medicine that uses very small amounts of natural substances for treating various diseases (Eldredge-Hindy *et al*., 2021). These medicines are natural substances of animal, plant or mineral origin (Ortiz-Cornejo *et al*., 2017) that could activate specific sensibility mechanisms in living organisms (Mazón-Suástegui *et al*., 2018). The medicine is useful in treating humans and veterinary diseases (Mayer *et al*., 2016). It can also be used to heal fish diseases because it works with all living organisms. This is the first attempt of using homeopathy medicines to treat TiLV infection as this medicine has been used in aquaculture with different aspects (Mazón-Suástegui *et al*., 2017; *Valladão et al*., 2015].

Homeopathy medicines such as *Engystol* and *Echinacea com* has exhibited toxicity at 500 µl/ml in MDBK and KST cell cultures (Nefedchenko *et al*., 2015). In contrast, ten homeopathy medicines including *Belladonna* 200C, *Pyrogenium* 200C, *Influenzinum* 200C, *Crotalus horridus* 200C, *Arsenicum album* 200C, *Hepar sulphur* 200C, *Aconitum napellus* 200C, *Tuberculinum* 200C, *Bothrops lanceolatus* 200C and *Nux vomica* 200C exhibited toxic effect to SSN1 cells at 700µl/ml in the present experiment. The combination of homeopathy medicines may cause low toxicity to cell culture (Nefedchenko *et al*., 2015), but the effect of toxicity could be varied based on the types and potency of the medicines. Ribavirin is a synthetic nucleoside analog that inhibited virus replication by reducing viral loads and preventing the CPE induced by ISAV, IHNV, IPNV, VHSV and TiLV (Rivas-Aravena *et al*., 2011; Marroquí *et al*., 2007; Hu *et al*., 2019; Kim *et al*., 2015, Lertwanakarn *et al*., 2021). In this study, out of ten homeopathy medicines, three medicines such as *Crotalus horridus* 200C, *Influenzinum* 200C and *Pyrogenium* 200C have significantly reduced TiLV replication and controlled the CPE in SSN1 cell line at the concentration of 500 µl/ml of medium. *Crotalus horridus* 200C and *Influenzinum* 200C remedies exhibited higher cell survival by completely reducing the CPE formation when compared to *Pyrogenium* 200C. The combination of homeopathy remedies has been found to exhibit *in vitro* and *in vivo* antiviral activity against the BHV-1 and BVDV-1 viruses in cattle disease (Nefedchenko *et al*., 2015).

In the virus neutralization test, three remedies *Crotalus horridus* 200C, *Influenzinum* 200C and *Pyrogenium* 200C were found to be neutralizing the TiLV infectivity in the SSN1 cells within 7 days. *Crotalus horridus* has previously been used as a remedy against babesiosis and canine ehrlichiosis disease in dogs (Chaudhuri *et al*., 2007; Tungnunga *et al*., 2016). It has also been used for increasing the platelet count against dengue virus in humans (Nayak *et al*., 2019). Other homeopathy drugs such as *Typhoidinum* 200, *Hydrophobinum* 1000, *Tuberculinum* 1000, *Nux vomica* 200 have been found to cause 100% inhibition of chicken embryo virus (CEV) in mice (Singh *et al*., 1985).

Homeopathy remedies are also used to stimulate digestive processes and detoxicating (*Nux vomica* and *Chelidonium*) and immune-stimulant factors (*Echinacea purpurea* and *Lycopodium*) (Sarubbi *et al*., 2012). Human virus like herpes simplex virus could be treated using homeopathy nosode medicines (Volinsky *et al*., 2021). Homeopathy drugs exhibited a positive effect in controlling influenza and acute respiratory infections

caused by the virus. Homeopathy treatment was found to be virtuous in curing foot and mouth disease in cattle when compared to allopathy treatment (Chand *et al*., 2018). Moreover, due to its safety efficacy and as it is non-toxic to living beings, there is no restriction in using this drug (Bondarenko *et al*., 2018). The homeopathic drug *Hepar sulphur* and *Arnica spray* have shown encouraging results in curing the ulcerative syndrome in fish caused by fungal infection (Mitra *et al*., 1991). The flu virus infecting humans has been treated and prevented using the homeopathy medicine *Influenzinum* (de Oliveira *et al*., 2011). Homeopathy treatment can protect against viral infections by strengthening the body's immune system. *Pyrogenium* was found to be inducing fever by mediating the release of cytokines such as TNF, interleukin IL-1, IL-6 and interferons into the blood stream of rabbits (Ahmad *et al*., 2019). However, in the present study, all three homeopathy medicines were found to have very less antiviral effect against the fish virus. In particular, *Crotalus horridus* and *Influenzinum* exhibited very less antiviral effect while *Pyrogenium* had no antiviral activity against TiLV infection.

Homeopathy medicine has cured Canine Distemper virus infected Dogs and it also showed faster recovery against the virus (Naveenkumar *et al*., 2019). The corneal ulcer has been recovered using homeopathy medicines for dogs when compared with other medicines (Neto *et al*., 2018). In the present study, homeopathy medicine incorporated into the feed has provided very minimal protection against fish TiLV infection. The combination of different homeopathy medicines with different potency could be used for the effective control of aquatic virus (Raj *et al*., 2020).

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