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Screening Antiviral Activity of *Moringa oliefera* L. Leaves Against Foot and Mouth Disease Virus

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Abstract: Foot and mouth disease is one of very devastating veterinary disease caused by Foot and mouth disease virus. So far no reasonable anti-FMDV agents are available and vaccines are also not providing sufficient prevention. In the present study, ethanolic leaves extract of *Moringa Oleifera* at different concentrations was utilized to evaluate anti-FMDV potential. The results indicated that six concentrations 1 µg/ml up to 100 µg/ml were considerably safe for BHK-21 cell culture as cell survival was above 50% whereas two concentrations 200 µg/ml and 400 µg/ml were resulted in cell survival below 50% so these two concentrations were cytotoxic for BHK-21 cells. Thus plant could play important role in the development of anti-FMDV agents.

Key words: Antiviral Activity · FMDV · *Moringa oleifera* · MTT Assay

INTRODUCTION

Foot and mouth disease (FMD) is terribly communicable disease caused by a virus named *picornavirus* [1]. Generally, this disease is transmitted among hoofed domestic and wild animals through entire cattle herds and therefore significantly affecting economy of developing countries. In a study conducted in Ethopia, it has been reported that FMD is a pandemic disease resulting in huge financially viable loss in the country. Rural communities of Ethiopia are reported to be greatly affected as their livelihood depends upon livestock [2]. Another study indicated very high mortality rate among animals including goat, sheep and pigs during the outbreak in 2012 in Egypt [3].

The natural spread of the virus can be direct or indirect, in direct way it can be caused by direct contact of animals excreting viruses and indirectly it can be caused by contacting with certain materials which are contaminated with this virus such as milk, excretory waste and by inhalation of air in which virus froth has been drooped [4]. These viruses are deadly threatening that these virus droplets can travel to maximum long distances while maintaining their pathogenicity. These viruses are capable of producing symptoms in infected animals just within 24 hours of exposure [5]. The infections phases are distributed in two phases i.e. first, is previremic phase in which infection starts in nasopharynx region and second, is viremic phase in which infection spread towards larynx, elevating virus burden and leading pulmonary infection affecting entire lung region and conclusively lesions in mouth cavity and feet region along with high grade fever persists [1].

It is crucial to understand the pathogenicity and cytotoxicity of FMDV, it can be attained by knowing the structure of this virus. Picornavirus is small non capsulated virus. The capsid of this virus is composed of 60 clones of every four structural proteins. The capsid is outlined by single strand RNA and covalently bound with viral protein. FMDV structural proteins are capable of providing this virus increased stability, cluster association, strong bond with spotted cell, infection

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causing specificity and thus decreasing immunity. This virus possesses phenomenal feature f variability in external capsid proteins. Therefore, FMDV is such a hugely incurable infection that it's threatening globally for productivity deprivation and animal health affairs. Annihilation of FMDV can be achieved by striking vaccination or by natural sources [6].

Nature has endowed us with number of medicinal plants which possess magical therapeutic agents that can be utilized for number of diseased states. Moringa Oliefera is a plant belonging to a family Moringaceae which is hugely cultivated throughout the world. Common names of this plant are drumstick tree, horse radish tree and suhanjna. All parts of the plant are immensely nutrimental including bark, leaf, root, seeds, gum, pod, fruit and flowers [7]. Antiviral activity of Moringa oliefera has been stated earlier against much publicized diseases i.e. HSV, HIV, EBV, influenza, yellow fever virus and polio virus.

The phytochemicals in *Moringa oliefera* are of highly significance. The bark is enriched in alkaloids, resins, mucilages, sterols, terpenes, triterpenoids and ashes. The seeds possess fixed oil (Moringa oil) 60 % and white solid mass 40%.This Moringa oil is composed myristic acid, stearic acid, palmitic acid, oleic acid and behenic acid. The constituents of leaves are aspartic acid, glutamic acid, serine, glycine, threonine, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, tryptophan, cystine and methionine and β-carotenes. Flower and fruit contain seven recognized and reported aminoacids. The stem is supplied with β- sitosterol, vanillin and oclacosanoic acid [8]. Pods and leaves of Moringa Oliefera yielded with large amount of vitamin C, carotene, calcium, potassium, flavonoids and phenols which are beneficial to play role as antioxidants and antivirals [9].

MATERIAL AND METHODS

The plants were collected from Lahore, Pakistan, air dried and identified from Herbarium, The University of Punjab. The extract was prepared with ethanol using Soxhlet apparatus (CG-1368*)* [10]. A cell culture media (M-199) was used for developing stock solution of 40 mg/ml and later it was sterilized by filtration technique. M-199 cell culture media was accounted for two fold serial dilution of the plant, eight concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12 µg/ml, 6 µg/ml and 1 µg/ml were formulated.

BHK-21 cell line was utilized for the growth of FMDV. The virus was used at the titre of 10^6 $TCID_{50}$ [11].

Cytotoxicity and Antiviral Assay: 96-well microtiter plate was used for the growth of BHK-21 confluent monolayers. All the concentrations were used in triplicates wells. The plate was incubated at 37° C with 5% CO_2 for 48 hours. The antiviral assay was also performed in the same manner except that FMDV was also added to each concentration.

For cytotoxicity, the "Positive control" was BHK-21 cells and cell culture media, while the "Negative Control" was BHK-21 cells, cell culture and DMSO (20%). In case of antiviral assay, the "Positive control" was BHK-21 cells and cell culture media, while the "Negative Control" was BHK-21 cells, cell culture and FMDV. The cytotoxic and antiviral assays were performed by MTT assay [12] and cells viability and cytotoxicity were evaluated respectively.

Statistical Analysis: The cell survival percentage (CSP) was calculated for cytotoxic and antiviral activity of ethanolic extract of *Moringa Oleifera*. The statistical analysis was done by applying Statistical Packages for Social Sciences (SPSS) – 19. Means ± S.D were used for expressing results. The analysis of results was done by applying Analysis of Variance (ANOVA) [13]. Cut off value was considered as $p < 0.05$.

RESULTS

Cytotoxic assay of *Moringa Oleifera* ethanolic leaves extract showed cell survival as 92%, 87%, 83%, 80%, 88%, 75%, 38% and 17% at concentrations 1 µg/ml, 6 µg/ml, 12 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml respectively. At 200 µg/ml, there is twofold decline in CSP than initial concentrations. The results had mentioned that six concentrations 1 µg/ml upto 100 µg/ml were considerably safe for BHK-21 cell culture as cell survival was above 50% whereas two concentrations 200 µg/ml and 400 µg/ml were resulted in cell survival below 50% so these two concentrations were cytotoxic for BHK-21 cells. The reason of cytotoxicity at higher concentration might be presence of cytotoxic phyto-constituents in the extract.

Antiviral assay of *Moringa Oleifera* alcoholic leaves extracts at above eight concentrations had revealed cell survival percentages in the range of 14% to 74% (Table 2). The results had indicated that first six concentrations up to 100 ìg /ml had antiviral activity against FMDV as cell survival was found above 50% whereas 200 µg/ml and 400 µg/ml did not resulted in antiviral activity. Cytotoxic concentrations of *Moringa Oleifera* spoiled the BHK-21

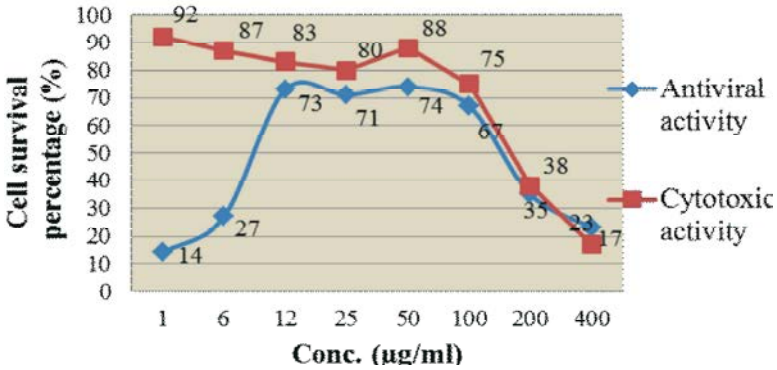

Fig. 1: Comparison of cytotoxic and antiviral activity of *Moringa Oleifera* leaves ethanolic extract

Table 1: Cytotoxic activity of ethanolic leaves extract of *Moringa Oleifera* for BHK-21 cells

Sr No.	Concentrations (µg/ml)	Mean OD ± SD	Cell survival percentage
1	1	1.176±0.031	92
2	6	1.119±0.007	87
3	12	1.073±0.029	83
4	25	1.038±0.091	80
5	50	1.130±0.142	88
6	100	0.981±0.018	75
7	200	0.558±0.046	38
8	400	0.312±0.027	17

Table 2: Antiviral activity of ethanolic leaves extract of *Moringa Oleifera* against *FMDV*

Sr No.	Concentrations (µg/ml)	Mean OD ± SD	Cell Survival Percentage
1	1	0.278 ±0.018	14
2	6	0.428±0.054	27
3	12	0.958±0.081	73
4	25	0.935±0.131	71
5	50	0.969±0.023	74
6	100	0.889±0.005	67
7	200	0.520±0.067	35
8	400	0.382±0.088	23

cells in both antiviral and cytotoxicity assays so FMDV did not get access to viable cells as a result unable to replicate in antiviral assay.

Cell Survival Percentages of *Moringa Oleifera* ethanolic leaves extract for cytotoxic and antiviral assay are represented in Table 1 and 2 respectively. Comparison of cytotoxic and antiviral activity of extract is graphically represented in Figure 1.

DISCUSSION

FMD is global disease spread through importation of live animals and visitors from infected countries and FMD is offering main hindrance to the development of agriculture by affecting animals [14]. The virus could also be toxic to health of human being as they may be transmitted to milk and other food items [15]. Thus there is dire need to establish anti-FMDV agents.

In the present investigation, *Moringa Oleifera* ethanolic leaves extract had shown significant antiviral activity against *FMDV*. In another study, the ethanolic extract of leaves of *Moringa Oleifera* had shown antiviral activity against HSV at 100 ± 5.3 ug/ml and cytotoxic activity at 875 ± 35 µg/ml [16]. In the present study ethanolic *Moringa Oleifera* leaves extract was also effective against FMDV at 100 µg/ml. Niaziminin is one of the thiocarbamate compound present in *Moringa Oleifera* leaves that had shown considerable antiviral activity against Epstein bar virus (RNA virus) [17]. In the present study, it might be Niaziminin or any other member of thiocarbamate group that exerted its antiviral activity against FMDV.

In cytotoxic assay, ethanolic extract was toxic to the cells at higher concentration (At or above 100 µg / ml). The results are in accordance with another study in Thailand where ethanolic and water extracts of *Moringa Oleifera* showed cytotoxicity at concentration above than 100 µg/ml for cancer cells COR L-23 and PC3 and normal cells 10FS [18]. Cytotoxicity of MO aqueous leaves extracts on Hella cells causes extremely high death rate of cells at 100 µg/ml [19]. Different studies had reported that *Moringa Oleifera* contains phenolic compounds which possess cytotoxic activity at higher concentration [7, 20]. It might be these phenolic compounds due to which the extract in the present study exerted its cytotoxic effects.

Moringa Oleifera leaves has shown antiviral potential for Equine herpes virus (Double stranded DNA virus), Herpes simplex virus (Double stranded DNA virus), Epstein bar virus (Double stranded DNA virus), Hepatitis virus (Ds DNA virus), Rhinovirus (+ sense ss RNA virus), HIV (Retro RNA virus) and *FMDV* (+ sense ss RNA virus). So it is quite evident that *Moringa Oleifera* leaves have broad spectrum antiviral activity. The antiviral activity may be due to niaziminin and cytotoxic activity may be due to phenolic compounds at

higher concentrations. It was reported that *Moringa Oleifera* contains saponins, tannins, alkaloids, terpenes, flavonoids and carbohydrates [21].This plant is a great source of vitamin A, B, C, D, E and K. It contains vital minerals and approximate 40 antioxidant sources [22]. Zinc, selenium and vitamin E are present in *Moringa Oleifera* [23, 24] that had been reported in another study as potent antioxidant supplement to improve the management of clinical signs of FMD infection in sheep [25].

The extracts obtained from Moringa oliefera seeds were assessed for possessing antifungal and cytotoxic activities. The activity was highly sensitive for herpes simplex and lower activity was recorded for polio virus [8]. Moringa oliefera was also examined for its antiviral activity against poliomyelitis virus.The extract from powdered leaves of the plant was used and cytotoxicity was evaluated by cytopathic assay on cell lines. The extract was inhibiting the virus selectively and possesses potential antiviral effects.

Earlier, antiviral properties of Moringa Oliefera extracts were examined against yellow fever virus, poliomyelitis virus and human immunodeficiency virus. Three different methods were used to evaluate these three RNA virus strains. Phytochemistry shows the presence of saponins, alkaloids, glycosides, tannins, flavonoids, carbohydrates, reducing sugar, resins and proteins. Methanloic extracts showed the best antiviral activities, it was concluded that extracts were selective for inhibiting specific strain of virus. This plant secured potent antiviral activities and could be used as novel or lead compound for antiviral drugs [9].

CONCLUSION

In the present in- vitro investigation ethanolic extract of *Moringa Oleifera* leaves showed remarkable anti-FMDV activity. Thus the results of present findings suggest that antiviral activity of Moringa oliefera could pose a great deal of significance in future for FMD and certain other viral diseases. Further studies are necessary to evaluate its antiviral potential using in-vivo investigations.

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