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Original Article

In Vitro Assessment of The Effects of Althaea Officinalis Root Extract on Rotavirus Multiplication

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Abstract

Background and Aims: Medicinal plants possess a variety of beneficial characteristics without causing substantial adverse effects. Antimicrobial activity is one of the qualities, among many others, that have been identified. Althaea officinalis is an annual plant belonging to the Malvaceae family with therapeutic qualities in both the leaves and the roots. Specifically, the effects of Althaea officinalis root extract on rotavirus, which is the most prevalent cause of diarrhea among children, were investigated in this study.

Materials and Methods: The neutral red assay was used to determine whether the extract had any cytotoxic effects on MA-104 cells. TCID50 (50% cell culture infectious dose) and real-time PCR assays were used to investigate the impact of the extract at non-toxic dilutions on human rotavirus.

Results: The maximum non-toxic dilution observed was 6/10 for the extract. When compared to the viral control, the lowest dilution of the extract (1/10) exhibited the highest inhibitory effect, resulting in a 1.03 logarithmic reduction in infectious rotavirus titer (p-value <0.001). Conversely, viral titers were higher at non-toxic dilutions (6/10) than the virus control, with the highest non-toxic dilution (6/10) linked with the most significant logarithmic increase in virus titer (2.54 logarithmic increase). The real-time PCR assay revealed a slight rise in Ct value compared to the viral controls when a dilution of 1/10 was used, similar to the TCID50 assay results.

Conclusion: Althaea officinalis at lower concentrations has mild antiviral effects on rotavirus, which can be due to the high resistance of rotavirus particle structure. However, using higher concentrations of this plant extract has enhanced virus replication.

Keywords: Marshmallow, Medicinal plant, Althaea officinalis, Rotavirus, Root extract

Introduction

Rotavirus is the most common cause of severe diarrhea and acute gastroenteritis in children around the world. Rotavirus infection is more common in children under the age of five, especially between the ages of six months and two years (1). In 2013, the World Health Organization (WHO) reported that over 215,000 children under the age of five died from rotavirus infection, with the vast majority of them living in underdeveloped nations (2). Clinical signs of rotavirus infection range from mild watery diarrhea to acute gastroenteritis, and in extreme cases, it can lead to severe dehydration and death (3).

The major goal of rotavirus gastro-enteritis treatment is to replenish lost fluids and electrolytes due to vomiting and diarrhea (4). Because of the difficulties in treating rotavirus

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infection, such as the lack of effective specific medications and only supportive therapy for infected individuals, new antiviral agents must be developed.Plants have been increasingly used to treat ailments in recent years. Plants and their components, such as extracts and oils, have the potential to replace chemical agents. These molecules also have fewer adverse effects than chemicals (5). Plants have been utilized in medicine since antiquity and are well-known for their potent medicinal properties. Many of these plants have been used to treat ailments that may have been caused by viruses in tradi-tional medicine (6, 7).

Althaea officinalis is a mucilaginous medicinal plant that is extensively distributed worldwide and belongs to the Malvaceae family. It is a perennial plant that has been used for therapeutic purposes since ancient times (8). Various root extracts of Althaea officinalis are effective against cough, throat discomfort, and gastrointestinal mucosa inflammation (9).

In several nations, syrups made from root maceration are used to cure coughs and oral and pharyngeal inflammation. Decoction and tea making are two other ways to use the plant. Constipation is treated with decoction, whereas bronchial catarrh is treated with the infusion (10). Orally administered infusions have been used to treat asthma and as an expectorant (8).

It has also been reported that root extract has antibacterial properties and can be used in mouthwash for topical periodontal prophylactics (11, 12). Other researches have shown that root extract could be used in topical formulations because of its wound-healing properties (12, 13). Extracts of A. officinalis have long been known for their ability to heal chapped skin and reduce inflammation (8). Some research has looked at the antioxidant activity of extracts made from dried plants and flowers (14-16).

Although the antibacterial activity of Althaea officinalis root extract has been documented in various research, the activity of Althaea officinalis root extract against viruses has not been investigated so far. This study now aims to see how the root aqueous extract of this plant affects the rotavirus infection.

Methods

Preparation of the aqueous plant extract: Approximately 50 g of plant materials were boiled for 45 minutes in 500 ml water, then cooled and filtered using Whatman No.1 filter paper. The crude extract was obtained in a vacuum evaporator by eliminating the solvent.

Cells and viruses: The Pasteur Institute of Iran (National Cell Bank of Iran, Tehran, Iran) provided the rhesus monkey kidney cell line (MA-104) for use in this study. The cells were cultured at 37 °C in a humidified environment containing 5% CO2 in DMEM high glucose medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Invitrogen, USA), 2 mM sodium pyruvate (Merck, Germany), 2 mM L-glutamine (Merck, Germany), and 1% Pen-Strep (PenStrep 100X, Bioidea, Iran).

The virus used in this investigation was a proven human rotavirus strain obtained from the Research Center of Pediatric Infectious Diseases at Iran University of Medical Sciences and cultured in MA-104 cells. The standard 50% tissue culture infectious dose (TCID50) assay was used to evaluate the titer of viable progeny.

Determination of cytotoxicity by neutral red uptake assay: In a 96-well microtiter plate, MA-104 cells were seeded at a concentration of 1.5×10^4 cells/well. The culture media were discarded after 24 hours of incubation at 37°C in a humidified environment of 5% CO2, and various dilutions of the extract (1/10 to 10/10) were added to wells. After that, the plate was incubated for another 48 hours at 37°C in a humidified incubator with 5% CO2.

The culture media containing the extract was removed after the incubation period, then neutral red was added to each well at a concentration of 100 µg/ml, and the cells were incubated with the dye for 3 hours. With the addition of a dye release agent to each well, the incorporated dye was subsequently released from the cells. At a test wavelength of 550 nm, the absorbance was measured using a microplate reader. The percentage of viable cells in each well was calculated compared to untreated control cells.

Assessment of antiviral activity: In a 96-well plate, monolayers of MA-104 cells were infected with 100 TCID50/mL of rotavirus solution and incubated for 1 hour at 37°C. The cells were then rinsed with PBS to eliminate any viruses that had not been internalized.
Various non-toxic dilutions of the extract were added to infected MA-104 cells and incubated for an additional 48 hours at 37°C in a 5% CO2 atmosphere.
In this test, cell and virus controls were put in the plate under identical circumstances, and the infectious rotavirus titers were measured using the standard TCID50 assay. Additionally, the real-time polymerase chain reaction (PCR) test was used to evaluate changes in rotavirus viral load.
Real-time PCR assay: Rotavirus genomic RNA was isolated from the supernatants obtained in the antiviral assay using the NucleoSpin RNA Virus Kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions.
The BioFactTM RT Series cDNA Synthesis Kit (BioFact, Daejeon, Korea) was used to reverse transcription of the extracted viral RNA to cDNA according to the manufacturer's instructions. The real-time PCR was carried out in a 25 µL reaction that included 12.5 25 µL of the TransStart Tip Green qPCR SuperMix Kit (TransGen, Beijing, China), 10 pmol of each primer, 4 µL of template cDNA, and 6.5 µL of ddH2O. The forward and reverse primer sequences for the human rotavirus VP6 gene were 5′- CAC CAG CGG TAG CGG CAT TA-3′ and 5′- ATT GTT TCG CTT GCG TCG GC-3′, having an amplicon size of 124 bp. The Rotor-Gene Q instrument (Qiagen, Germany) was used for the assay, and the cycling protocol consisted of an initial denaturation at 95°C for 1 minute, followed by 40 cycles of 95°C for 20 seconds, 58°C for 25 seconds, and 72°C for 25 seconds.
Statistical analysis: The data are presented as the mean of three separate experiments.
The statistical difference between groups was determined using a one-way analysis of variance (ANOVA). Analyses were conducted using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), and statistical significance was defined as a p-value less than 0.05.

Results

To conduct the in vitro antiviral studies, we first identified the dilutions at which the extract could be utilized without causing cell harm, using data from the neutral red uptake assay. On the basis of these findings, dilutions of the extract that displayed less than 10% cytotoxicity were used in the subsequent antiviral experiments. The maximum dilution that was found to be non-toxic was 6/10 (Fig. 1, Table 1).

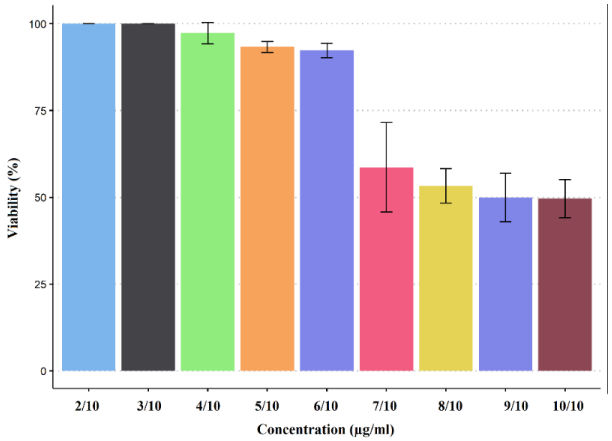

Fig. 1. Cytotoxicity of Althaea officinalis root extract on MA-104 cells.

Table 1. Effects of Althaea officinalis Root Extract on the viability of MA-104 cells

	Dilution	Viability (%) (Mean)	P value
Althaea officinalis root extract	2/10	100	<0.001
	3/10	100	
	4/10	97	
	5/10	93	
	6/10	92	
	7/10	59	
	8/10	53	
	9/10	50	
	10/10	50	

The root extract of Althaea officinalis was investigated for its anti-rotavirus activity using the TCID50 assay. Among the various dilutions of the extract, the lowest dilution (1/10) had the most inhibitory impact, resulting in a 1.03 logarithmic reduction in infectious

rotavirus titer when compared to the viral control (p-value <0.001). On the other hand, infectious rotavirus titers were significantly higher than the virus control at dilutions greater than 3/10 of the extract, with the highest non-toxic dilution (6/10) associated with the highest logarithmic increase in the virus titer compared to the virus controls (2.54 logarithmic increase) (Fig. 2, Table 2).

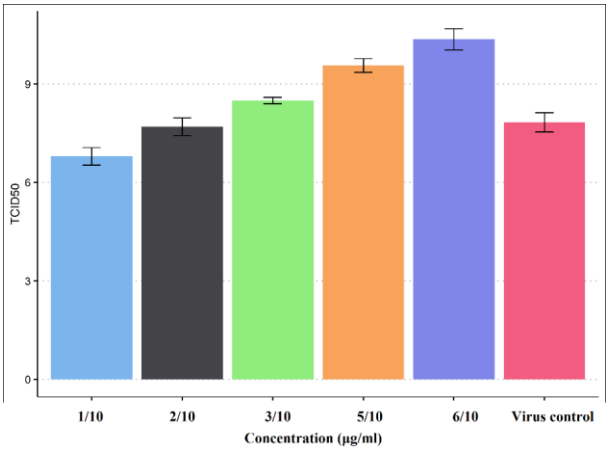

Fig. 2. Effect of different non-toxic dilutions of Althaea officinalis root extract on the infectious rotavirus titer by TCID50 assay.

Table 2. Results of TCID50 assay

	Dilution	Log TCID50/ml (mean ±SD)	P-value
Althaea officinalis root extract	1/10	6.80 ± 0.26	<0.001
	2/10	7.70 ± 0.26	0.5
	3/10	8.50 ± 0.10	0.007
	5/10	9.57 ± 0.21	<0.001
	6/10	10.37 ± 0.32	<0.001
	Virus control	7.83 ± 0.29	-

Real-time PCR analysis was used to corroborate the results of the TCID50 experiment. When a dilution of 1/10 was utilized, the real-time PCR assay indicated a slight increase in Ct value compared to the viral controls, similar to the TCID50 assay results (Fig. 3). Compared to the viral controls, the extract at a dilution of 1/10 resulted in a one-unit increase in the Ct value obtained by real-time PCR. At dilutions 3/10 and 6/10, however, the Ct values were similar to viral control.

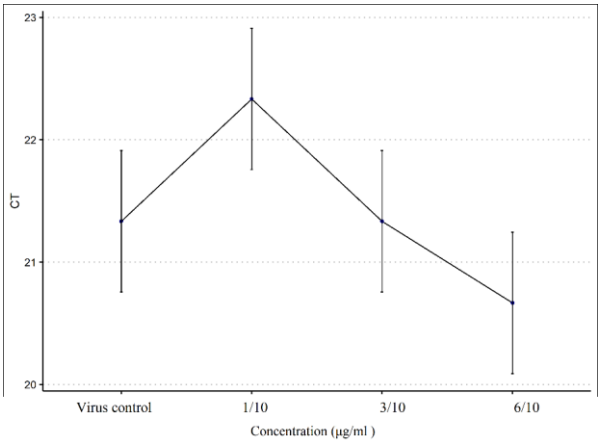

Fig. 3. Real-Time PCR results for treated samples with different dilutions of Althaea officinalis root extract.

Discussion

In recent years, the increasing usage of herbal-based drugs in the treatment of diseases is taking a great interest so, the demand to discover effective natural-based chemotherapeutics ingredients is constantly growing. It is a promising way to efficiently treat diseases by combining both traditional and conventional medicine.

Many compounds have been extracted from the root of Althaea Officinalis, including starch, water-miscible polysaccharides, sucrose, lecithin, tannins, phenols, mucilage, alkaloids, unsaturated fatty acids, arabinogalactans, glucans, arabinans, and galacturorhamnans (9, 15, 17).

The polysaccharides can protect epithelial cells from microbial invasion with their bioadhesive properties (15). The capability to induce chemotaxis of macrophages, T-lymphocytes, and natural killer cells by root-extracted polysaccharides has been recognized (10, 18). The extracted components from the roots of A. Officinalis have an anti-inflammatory effect on burns, boils, and the treatment of respiratory and digestive tract infections and metabolites disorders (19).

Our experimental findings indicate that in the lowest dilution of Althaea Officinalis root extract (1/10), the reduction in infectious rotavirus titer was detected by both real-time PCR and TCID50 assay (p-value <0.001) due to the rotavirus specific properties such as double-layered and non-enveloped capsid.

Conversely, increasing the concentration of the root extract, a significant logarithmic increase in rotavirus titer was observed (2.54 logarithmic increase compared to control). This result indicated a mild antiviral effect of the root extract on rotavirus replication.

Contrarily rotavirus replication was enhanced in high concentration by unknown mechanisms. This may be due to the toxic effect of increased concentrations of the extract on the host cell integrity and the release of more rotavirus progenies by triggering apoptosis.

Numerous studies were done worldwide to reach trustable findings of this extract; for instance, the antifungal effect of ethanolic Althaea Officinalis extract was reported by Rashidi et al. (20). Valiei and et al. were exhibited the antimicrobial activity of root-extracted hexane against some gram-positive and gram-negative bacteria as well as some fungus due to its high content of unsaturated fatty acid (11, 19). The plant polyphenols exert inhibitory effects on microbial growth by producing hydrogen peroxide (11).

The results of Rezaei and et al. showed that Althaea Officinalis extract could be a great candidate for the treatment of gram-positive bacteria, but it is not effective on gram-negative bacteria (12).

Previously, the antiviral effect of the ethanol extract of dried whole Althaea Officinalis plant on some viruses, including herpes type 1 and measles, was confirmed (8, 18).

Conclusion

It should be noted that rotavirus is more resistant to acidic and alkaline conditions of the gastrointestinal tract, and due to the lack of lipid-enveloped coating on the surface of the virion, the composition of this root extract could not affect the viral particle. The results of this study suggest that more studies on the antiviral effect of this extract on enveloped viruses are recommended, and quantitative methods can be helpful to evaluate a precise amount of virus. The extraction method and different solvents, as well as evaluation tests and collection plants from different places, could affect the results.

Acknowledgment

None.

Conflict of interest

No conflict of interest is declared.

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