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Evaluation of Cytotoxic Activities of Disinfectants on Human Healthy Cells

Basak SIMITCIOGLU
Gaziantep University

Isik Didem KARAGOZ
Gaziantep University

Ibrahim Halil KILIC
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Abstract: The Covid-19 pandemic, including our country, has affected 34 million people around the world and caused high death rates and still is. One of the most important points in this process is disinfection, and both surface and air disinfectants have been used frequently. Disinfectants, which are extremely diversified in the market, have started to create danger while protecting us from the virus. It is known that the disinfectants used prevent the contagion of the virus, but we do not have clear information about whether they have destructive effects on the healthy cells of the people who use them. Therefore, in our study, it was aimed to determine the effects of hand disinfectants produced by various brands on our healthy cells. In our study, first of all, the most widely used disinfectants were obtained from the market, and then the cytotoxic (lethal) activity of these disinfectants on healthy cells was determined by in vitro MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) analysis. As a result of this study, it was observed that apart from the disinfectant containing hypochlorous acid, other disinfectants have cytotoxic activity against healthy human cells. Despite the increasing need during the pandemic process, we recommend that these disinfectant substances, which are put on the market in large numbers and in a wide variety, should be inspected and necessary measures should be taken so that they can be sold after passing through the quality control stages.

Keywords: Disinfectant, Cytotoxic Activity, Toxicity, Covid-19

Introduction

For the last 1.5-2 years, we, as humanity, have been struggling vitally with Covid-19, which is a pandemic epidemic. This new type of coronavirus, which emerged in Wuhan, the capital of the Hubei region of China, on December 1, 2019, was named SARS-COV-2 because it causes an atypical pneumonia and does not respond to drugs and various vaccines. It has been determined that the virus, which can be transmitted from person to person, has increased in the transmission rate in mid-January 2020. Then, virus cases started to be reported in Europe, North America and Asia-Pacific, and a global epidemic was declared by the World Health Organization (WHO) on March 11, 2020. Since then, there have been 195,886,929 confirmed cases of COVID-19, including 4,189,148 deaths, reported to WHO. Coronavirus, which is the causative agent of upper respiratory tract infection, is usually transmitted by close contact with infected people. The route of transmission is mostly through the respiratory route. Today, although vaccination and vaccine production studies continue, social

isolation and personal protection measures against these viruses still maintain their importance. Studies have shown that Covid-19 is sensitive to disinfectants (Beşirbellioğlu, 2007). For this reason, we have given disinfectants an important place in our lives in order to protect against the virus. Recently, these disinfectants are available everywhere we can reach, including our homes, market entrances, pharmacies, hospitals, public transportation vehicles, and their use is quite common. The frequency and misuse of disinfectants has reached a level that threatens human health. So much so that acute and then chronic upper respiratory tract diseases and skin disorders, especially in children, have reached significant levels due to excessive use of disinfectants. While disinfectants used as aerosols increase asthma and allergic reactions; skin disinfectants cause irritation, allergies and eczema. When the studies in the literature are examined, it is seen that there are many clinical cases (Chen, 2020).

Disinfection; It is the whole of the processes carried out to remove microorganisms and prevent contamination in case of suspected presence of disease-causing microorganisms in the environment (Abbasoglu, 2007). Disinfectants; These are chemical substances used for places where pathogenic microorganisms are or suspected to be found, and for devices or materials that may be a source of contamination (Öztürk, 2002). A good disinfectant should be large enough to act against bacteria, viruses, protozoa and fungi. It is also important that it is not corrosive. It should have an antimicrobial effect at low concentrations, be effective at normal temperatures, and should not cause structural defects in the applied area (Metin and Öztürk, 1995; Mısırlı, 2009). Disinfectants are available in different types as high, medium and low level disinfectants according to the active substances in their composition (Favero and Bond, 1991). Disinfectants that eliminate most bacterial spores and kill microorganisms are in the group of high-level disinfectants. In long-term contact with bacterial spores, the spores die, but it should not be used unless necessary due to its toxic effect on human health at the cell and DNA level. This group includes glutaraldehydes (2-3%), hydrogen peroxide (6%), formaldehyde (3-8%), sodium hypochlorite, peracetic acid (0.23%), orthophthalaldehyde (0.55%) (Sagripanti and Bonifacino, 1999; Alev, 2014). Intermediate disinfectants are chemicals that do not affect bacterial spores, but are effective against mycobacteria, non-enveloped viruses and other microorganisms. Those in this group are effective on tuberculosis bacillus and other microorganisms within 10 minutes. Generally, the composition of medium-level disinfectants; ethyl alcohol (70%), isopropyl alcohol (60-90%), phenol compounds (0.4-5%) and iodophor (30-50 ppm free iodine) form (Sagripant and Bonifacino, 1999). Low-level disinfectants, which are considered safer for humans; Although it inactivates vegetative bacteria, some fungi and viruses in 10 minutes, it has no effect against endospores of microorganisms and tuberculosis bacillus. Ethyl alcohol, phenol, iodophor, quaternary ammonium compounds can be given as examples of the compounds in these disinfectants (Sagripant and Bonifacino, 1999). From this point of view, we have planned to reveal the cytotoxic effect, which is the underlying cause of the clinical cases seen. The disinfection process and the content of disinfectants are of fundamental importance in these applications. Depending on the ingredients, the extent of the damage to human health can also vary.

Disinfectants, which have become an indispensable part of our lives due to the Covid-19 pandemic, are chemical products used to purify surfaces with pathogenic microorganisms, and these products are expected to have high efficacy but also not have any side effects. In the fight against Covid-19, disinfectants, which have come to the fore to protect against the virus, have been used in a highly diversified and uncontrolled way to meet the need. Therefore, our concerns about the use of disinfectants have also increased. While these products have a negative effect on the viability of the virus, how do they affect our healthy cells? In this study, which we designed to answer this question, which is important for our health, it was aimed to investigate the cytotoxic effects of disinfectants, which are widely sold in the market, on healthy cells.

Method

Supply of disinfectants

4 different brands of disinfectants sold in the market and frequently preferred were purchased. First disinfectant igredient Hypochlorous acid, second has ethyl alcohol and ammonium derivates, the third one contains isopropyl alcohol and chlorhexidine digluconate (Table1).

Cell culture

In this study, human umbilical vein endothelial cells (HUVEC) cell lines were used (obtained from Gaziantep University, Department of Biology). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM)

medium containing 10% Fetal Bovine Serum (FBS), 1% L-glutamine and 1% penicillin/streptomycin in an incubator at 5% CO_2 and 37°C. The morphological structures of the produced cells were observed daily under an inverted microscope.

Table 1. The main ingredients of the disinfectants used in the study

Dis 1 (HOCl)	Dis 2 (EtDiAl)	Dis 3 (IsoChl)	Dis 4 (EtIso)
Hypochlorous acid	Ethyl Alcohol Didecyldimethylammonium Chloride Alkyldimethylbenzylammonium Chloride	Isoprophyl Alcohol Chlorhexidine Digluconate	Ethyl Alcohol Isoprophyl Alcohol

Cytotoxic Activity Assay

MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability test, which is one of the enzymatic test methods used in the study, is a simple, colorimetric and economical method used to measure cell viability, proliferation or cytotoxicity. Viable cells in a cell population can be detected colorimetrically and quantitatively by the MTT method.This method is based on the principle of fragmentation of the tetrazolium ring of MTT.Formazan formation is only seen in living cells with active mitochondria.This is considered a marker of cell viability and crystals The absorbance values determined spectrophotometrically by dissolving it are associated with the number of viable cells.For the MTT test, HUVEC cells were seeded with 5×103 cells in each well of the 96-well cell dishes and incubated for 24 hours at 37oC in an environment containing 5% CO2.At the end of the incubation, disinfectants were applied to the cells in 5 different in concentration (1, 1/2, 1/4, 1/8, 1/16) have been added. After 15 hours of incubation, 40 µl of MTT dye was added to each well and the cells were incubated for another 4 hours. To solubilize the formazan crystals formed as a result of incubation, 80 µl of DMSO was added to all wells. Then, the color intensity formed in the cells was measured in a spectrophotometer (Thermo Scientific, USA) at a wavelength of 570 nm, and their absorbance was taken. In the experiment, each concentration was repeated in three independent wells (Mossman, 1983). The values read in the spectrophotometer were calculated with the % vitality equation and graphed.

% Viability = [100× (Mean of compound-treated cell absorbance / non-medicated control cell (MO) viability)

In this equation, the viability of the untreated control cell (MO) was assumed to be 100%, and the viability rates of the sample-treated cells were calculated.

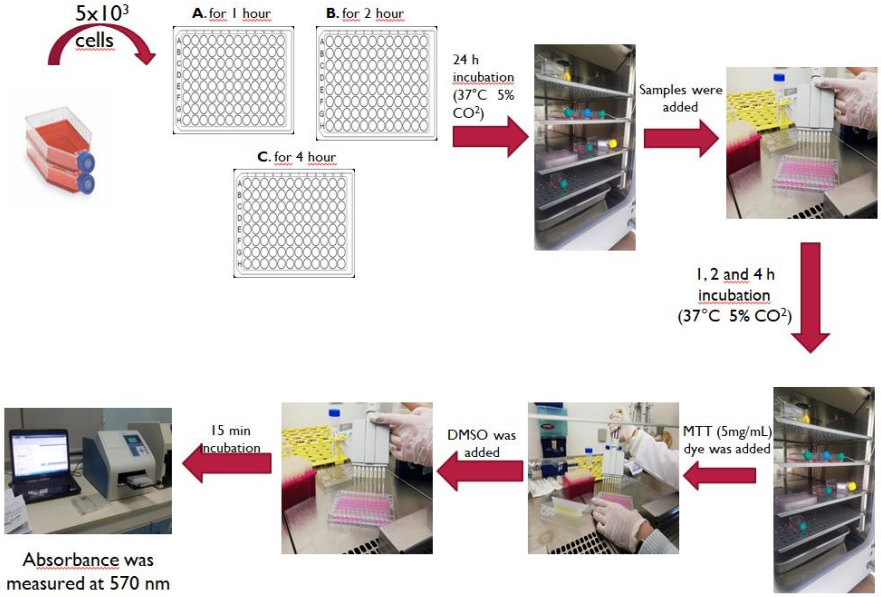

Figure 1. The overall scheme depicting the perform of the MTT assay

Results and Discussion

According to our findings, it was observed that all of the disinfectants with the material in the study had a cytotoxic effect on healthy cells (Figure 2). When the cytotoxic activity results were examined, the group containing ethyl alcohol and ammonium derivatives, which is the second group, showed a serious toxic effect for healthy cells at 3 different hours. In fact, at the end of the 4th hour, all of the cells were dead. Although chlorhexidine and isopropyl alcohol in the 3rd group showed less lethal effect in the first hour at low doses, the viability still decreased to 60%. The situation in the 4th group containing isopropyl and ethyl alcohol is similar to the 3rd group. In the first group, it was observed that the cells remained viable at all concentrations and at all hours. It has been determined that hypochlorous acid in this group does not have a toxic effect on our healthy cells.

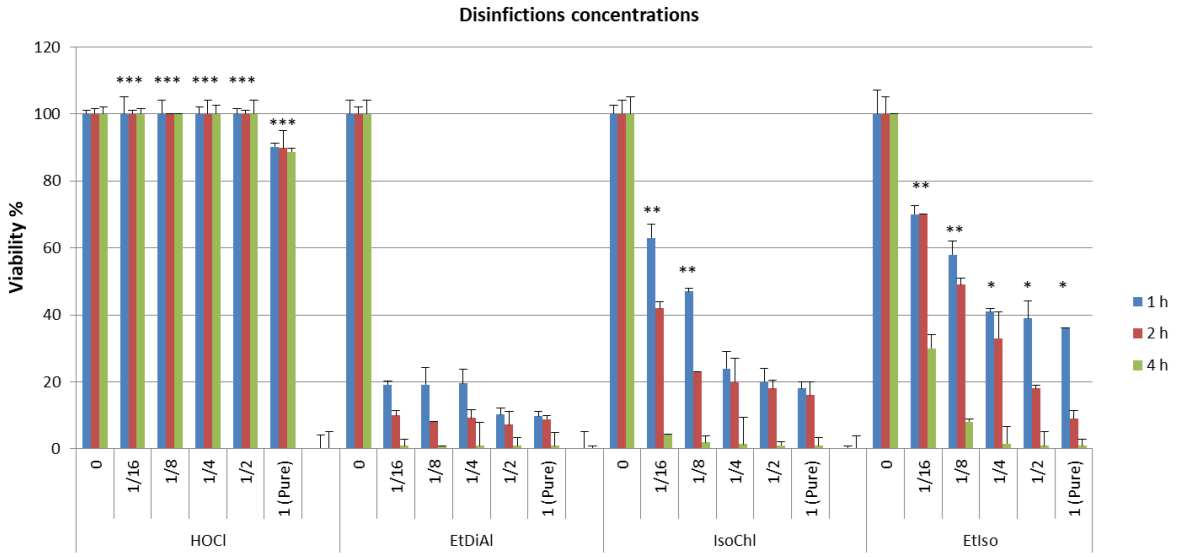

Figure 2. Disinfectants concentration-cell viability on HUVEC cell line

In addition to social isolation in the prevention of COVID-19 disease, the widespread use of masks and disinfectants in the society raises some questions. The fact that disinfectants, which we consider as friendly, are chemical products and contain very harmful components, brings to mind that these products can be harmful to health (Dindarloo et al., 2020). These products, which help us to be protected from viruses, also have the potential to threaten our cells. In this respect, it is very important to observe the effects of disinfectants at the cell level. In this study, it was aimed to observe whether disinfectants obtained from different brands have cytotoxic effects against human umbilical cord vessel endothelial cells, which are healthy cells. According to the data obtained in this study, it is seen that 4 different disinfectants can show lethal results on healthy cells. Commercial forms of these substances, which we frequently use to provide protection against disease-causing agents, have strong cytotoxic activity and therefore may have harmful effects on the human body. No significant reduction in lethality was observed even when diluted at the concentrations sold. This makes us doubt the reliability of disinfectants. When the literature data is examined, it is seen that skin irritation is observed in people who are exposed to disinfectants a lot, and disinfectants trigger asthma disease (Su et al., 2019).

In addition, 50% inhibition on human, monkey and mouse cells in a previous cytotoxicity study with liquid disinfectants (Sagripanti and Bonifacino, 2000) showed that our study also supports the literature. There is information in the literature that alcohol-based disinfectants cause irritant contact dermatitis and allergic contact dermatitis (Wilhelm, 1996; Ale and Maibach, 2014; Tan and Oh., 2020), ammonium salt derivatives cause contact dermatitis and cause lung damage (Ruiz et al., 2011). Also its known that Chlorhexidine Digluconate triggers oxidative stress and organelles damages (Ohnuma et al., 2011). Despite this, it is known that HOCL inactivates viruses, bacteria, endospores, and fungi and is safe for human tissues (including eye, lung, and skin) (Rasmussen et al., 2017). It should be noted that hocl applications have already been approved by FDA for eyelid infection management (Stroman et al., 2017), skin (Abu-Soud et al., 2014), and cosmetics (Lai et al., 2020) with repeated daily exposure for a set period of time.

Conclusion

The non-toxic property of the hocl substance on healthy cells is quite remarkable. While this chemical, which has lethal activity against viruses, can be described as quite safe, other disinfectant chemicals have been found to be harmful to human health.

Recommendations

We think that this study contributes to the development of measures to be taken against COVID-19. Also we believe that the study of HOCl in a dose-dependent manner and with different healthy cell lines should be elaborated, and its effectiveness on corona virus should be evaluated.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors

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Author Information

Basak SIMITCIOGLU
Gaziantep University
Biology Department 27410 Gaziantep/Turkey

Isik Didem KARAGOZ
Gaziantep University
Biology Department 27410 Gaziantep/Turkey
Contact e-mail: *karagoz@gantep.edu.tr*

Ibrahim Halil KILIC
Gaziantep University
Biology Department 27410 Gaziantep/Turkey

Mehmet OZASLAN
Gaziantep University
Biology Department 27410 Gaziantep/Turkey

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