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Review Article

Urea: A Low-cost Disinfectant for Prolonged Activity in Combating Spread of COVID-19

Vishal Paul[1], Chandrashekharaiah PS[1], Rajesh Nandru[2], Shyam Prasad[1], Santosh Kodgire[1], Shivbachan Kushwaha[1]*, Debanjan Sanyal[1] and Santanu Dasgupta[2]

[1]Reliance Industries Ltd, Jamnagar, India

[2]Reliance Industries Ltd, Reliance Corporate Park, Ghansoli, Navi Mumbai, India

***Corresponding Author:** Shivbachan Kushwaha, Research and Development, Reliance Industries Ltd, Gagva, Jamnagar, Gujarat, India.

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Abstract

Disinfection is a primary means to control a spread of any disease caused by infectious microorganisms. The never seen before COVID-19 pandemic has created a havoc in public health. The COVID-19 virus which persists on human body parts and environment acts as a new source of infection. The personal and environmental sanitization along with other social measures needs to be practised to prevent the rapid spread of COVID-19. Disinfection of public places and open environment requires large quantity of disinfectants and due to this it remains a costly approach. Already, there have been a plenty of disinfectants available in the market which are mostly based on alcohols and phenols but have limited applications owing to their higher costs. Hence, there is a need for identification of bulk commodity chemicals which have biocidal properties. Urea is one of the commodity chemicals which has biocidal property due to its protein and RNA denaturation ability. In most of the literature it has been mentioned that 7-8% urea is safe to use on human skin and found effective against many enveloped and non-enveloped viruses. Also, at 50-60 °C temperature and pH 3-9, urea was found stable for more than 2 months. Because of all these properties it can be a potential low-cost disinfectant for COVID-19 control. In this review, a systematic study was done to assess the antiviral, stability, and compatibility properties of urea to use as an ideal surface or personal care disinfectant agent for control of COVID-19.

Keywords: Antiviral; COVID-19; Disinfectant; Protein Denaturant; Urea

Abbreviations

CI2: Chymotrypsin Inhibitor 2 Protein; DU: Denatured State; HCoV: Human Coronavirus; MERS: Middle East Respiratory Syndrome; NaOCl: Sodium Hypochlorite; NMF: Natural Moisturising Factor; PV: Poliovirus; QACs: Quaternary Ammonium Compounds; RH: Relative Humidity; SARS: Severe Acute Respiratory Syndrome; Tm: Melting Temperature; WC: Watson and Crick

Introduction

The corona viruses are large group of viruses which target mainly the respiratory systems of humans. Previously two viruses from the same family have caused global outbreak. The first one was severe acute respiratory syndrome (SARS) observed in the year 2002 and the second outbreak was middle east respiratory syndrome (MERS), which was reported in the year 2012. Now in the year 2019, the COVID-19 outbreak caused by SARS-CoV-2 virus is also belongs to the same group. The disease was first reported in China and expeditiously spread across the globe. The COVID-19 had caused considerable mortality and economic crisis throughout the world. The virus has killed millions of people worldwide because of quick transmission between human beings. Because of these reasons in March 2020 WHO declared COVID-19 as a pandemic [1].

The virus is mutating continuously, and the new versions of virus is creating the stir in public life.

SARS-CoV-2 are large, enveloped viruses with pleomorphic spherical shape and contains RNA as genetic material [2]. The envelope of the virus consists of a lipid bilayer and on to this spike like projections made up of glycoproteins are anchored [2]. The nucleocapsid is made up of multiple copies of the protein, which are bound to the single-stranded positive-sense RNA genome [3]. The lipid bilayer envelope, membrane proteins and nucleocapsid protects the virus when it is outside the host cell [4].

Based on current evidence, the COVID-19 disease is mostly transmitted through contact routes and respiratory droplets [5]. Since the virus is spreading rapidly through contacts from individual to family and then to community the cases are increasing worldwide. As the numbers of positive cases are increasing day by day, the Hospitals are overloaded with large number of patients. The global leaders are advising for controlling the contact transmission and to practise social distancing (Physical) to flatten the infection curve. Contamination of frequently touch surfaces in healthcare settings are considered as a potential source of viral transmission. Data on the contact time required for transmission of SARS-CoV-2 from contaminated surfaces to hands of human beings are not available. Whereas the study on influenza-A virus shown that a contact time of 5 seconds is enough to transfer 31.6% of the viral load to the hands [6]. In this pandemic situation it is essential to disinfect the surface to reduce the transmission. WHO is continuously highlighting the importance of environmental disinfection, as one of the preventive measure. At the current scenario the disinfection of public places like hospitals, bus stands, railway stations, houses of positive patients and nearby areas are extremely important to control the spread. In this review, we are discussing about various properties of urea which makes it an effective disinfectant agent against COVID-19.

Role of disinfectants in controlling the spread of COVID-19

Disinfection is the process of eliminating pathogenic microorganisms (bacteria, fungi, and virus), from inanimate objects or surfaces [7] and the agents used for this process are called disinfectants. The disinfectants are the first line of defensive agents against the spread of pathogen infection [8]. It has been claimed that the coronaviruses can transmit from contaminated dry surfaces to humans after close contact [9]. One millilitre (ml) sputum of positive COVID-19 patient was found to contain ~10^8 viral copies [10] and many reports claims that, the persistence vary with the inoculum load and observed longer persistence at higher inoculum. In a recent review on persistence of coronaviruses on inanimate surfaces [11] indicate that, the endemic human coronavirus (HCoV) strain 229E can remain infectious for 2 hours to 9 days on various types of materials and influenced by temperature and relative humidity. It was found that at room temperature with 50% relative humidity (RH), HCoV-229E persists better as compared to 30% RH [12]. The temperature of 30°C or 40°C was known to reduce the persistence of highly pathogenic Middle East Respiratory Syndrome (MERS) coronavirus. WHO recommends to ensure that environmental cleaning and disinfection procedures are followed consistently and correctly. Thorough cleaning of environmental surfaces with water and detergent and applying commonly used hospital-level disinfectants (sodium hypochlorite) are effective procedures as explained in WHO, Interim guidance published on 25 January 2020. The various classes of disinfectants, mode of action and their limitations which are registered under EPA and recommended against SARS - CoV-2 are discussed here. For usage guidelines and safety procedure refer to the EPA web site.

- **Halogens:** Based disinfectants include chlorine and iodine compounds. These are broad spectrum substances effective against almost all groups such as bacteria, mycobacteria, fungi, and virus (enveloped and non-enveloped) [13]. At higher concentration (2500 ppm) chlorine compounds acts as sporicidal [14]. They denature proteins because of their electronegative properties and affects the enzymatic systems [15]. Examples are Sodium hypochlorite (household bleach) and sodium chlorite ($NaClO_2$). Sodium hypochlorite (NaOCl) is widely used chlorine disinfectant. The biocidal activity mainly depends on the amount of active chlorine present in the preparation [14]. Higher concentrations are corrosive, irritating to the eyes, skin and mucus membranes [14]. These compounds are sensitive to light, highly reactive and when chlorine compounds are mixed with acid or ammonia, releases toxic chlorine gas [16]. As per WHO, Geneva Annex 2014, the typical house hold bleach with final concentration of 0.05% can effectively kill the SARS-CoV-2 virus with contact time of 10 min or more. The recent published data also suggest that 0.1% concentration of sodium hypochlorite is enough to kill the SARS-CoV-2 virus with a contact time of 1 min [11].

- **Oxidizing agents:** Are broad spectrum antimicrobial compounds. They kill the microbe by denaturing proteins and lipids [15]. These are very effective on hard surfaces and equipment's. However, the concentrated forms are irritant and may damage the clothes. Examples: hydrogen peroxide (H_2O_2) and peroxyacetic acid (CH_3CO_3H).
- **Alcohols:** Are also broad-spectrum disinfectants which mainly denature the proteins and cause cell lysis by damaging cell membrane [17]. One important consideration is the higher concentration of ethanol >95-100% is not effective against the microbes, some quantity of moisture is required for its effectiveness, hence 70-90% has been recommended [17]. Some of the limitations are, they evaporate quickly, flammable and not effective in presence of organic matter [17]. Examples: Ethanol (C_2H_5OH) and 1, 2-Hexanediol ($C_6H_{14}O_2$). For disinfection of small surfaces ethanol (62%-71%) was found to be the best [11]. WHO recommends 70% concentration of ethanol for disinfecting small surfaces contaminated by SARS-CoV-2.
- **Acids:** This group acts on hydrogen bonds of nucleic acid, precipitate the protein and change the pH of the environment there by kill all the pathogenic microbes. The concentrated acid solutions are toxic, irritants, corrosive, and cause chemical burns because of this the use is limited. The antimicrobial property of these agents is highly pH dependent and this also acts as one of the limiting factors for extensive use [15]. Examples: Citric acid ($C_6H_8O_7$), octanoic acid ($C_8H_{16}O_2$), L-Lactic acid ($C_3H_6O_3$), glycolic acid ($C_2H_4O_3$), and hypochlorous acid (HClO).
- **Phenolics (C_6H_6O):** These group of compounds act by denaturing proteins and inactivating membrane bound enzymes [14,16]. Prolonged exposure of phenolics compound cause skin irritation [14]. Concentrations over 2% are highly toxic to all animals, especially cats.
- **Quaternary Ammonium Compounds (QACs):** Are cationic compounds interact with negatively charged ions found on cell walls of microorganisms and bound irreversibly to phospholipids. Kills the microorganism by denaturing proteins, affecting cell membrane, and permeability of the cells. These compounds are effective against fungi, Gram-negative bacteria, and enveloped viruses. QACs are considered as sporostatic but not sporicidal [15,18], found active at neutral to alkaline pH, loose their activity at acidic pH and not effective on non-enveloped viruses. QACs generally get inactivated by organic matter, soaps, and detergents. Example: Benzalkonium chloride ($C_{22}H_{42}ClNO$).

Urea as disinfectant

The various properties of urea which makes it an effective disinfectant are reviewed here.

Urea as a protein denaturant

Urea is one of the most used chaotropic agent used for protein denaturation [19]. This property of urea makes it an antiviral agent. According to a study by Takashi., *et al*. (2008), urea at 0-2 M concentration causes destruction of the tertiary structure, results in mildly denatured state (DU) of proteins, with a little amount of secondary structures [20]. Urea at the concentration of 2-4 M was found to act further by reducing secondary structure and increasing radius of gyration to a maximum possible value. At 4 M concentration, the polypeptide chain was denatured to an extent that side chains became highly mobile. Increasing the urea to 8 M concentration had caused high amount of denaturation state [20].

In another study, it was reported that urea affects the tertiary structure of protein by both direct and indirect ways. Urea directly binds to the peptide groups through hydrogen bonds and competes with the native interaction of protein. Whereas indirectly it affects the protein by altering the solvent environment [21]. Urea interacts with both polar as well as nonpolar components of proteins [22]. The various investigations and studies have provided the molecular level understanding of protein denaturation by urea [23]. Urea also enhances the aqueous solubility of proteins and weakens the hydrophobic interactions which results in exposure of larger number of non-polar side chains of protein molecule and denaturation occurs.

In a simulation study, it was found that the preferential solvation of peptide bonds in protein by urea was due to Van der Waals interactions and hydrophobic hydration [24]. In hydrophobic hydration the water was constrained, and it was displaced by the urea molecule. There are several studies on electrostatic activity of urea with protein [25]. In electrostatic interaction it was observed that, the urea preferentially attaches to the polar

groups and charged chains and destabilizes the polypeptide helix structure of protein. Bennion and Daggett [26], found that 8 M urea was directly affecting the chymotrypsin inhibitor 2 protein (CI2) structure by attaching to the polar moieties through hydrogen bonds and indirectly accelerating the protein unfolding by creating the perturbation in water structure and dynamics.

Urea as a RNA denaturant

Urea affects the hydrogen bond interactions between base pairs of RNA [27]. In a simulation study it was found that urea involves in stacking reaction with purine bases and causes denaturation [28]. The denaturation study conducted with various concentration of urea (0, 6 and 8 M) and 22-nucleotide RNA hairpin P5GA5 (22nRNAP5GA5) structure revealed that the base pair disruption was observed at 8 M urea concentration in 20 nano seconds [29]. The sample treated with 8 M urea shown the decrease in the fraction of intact hydrogen bonds in stem region from 0.71 (control) to 0.46. As the concentration of urea increases from 0 to 8 M, the loss of Watson and Crick (WC) hydrogen bonds were observed. The breakage of hydrogen bonds resulted in increased hydrogen bond donor-acceptor distance from 3 A° (control) to 6 A° (6 M urea) and then to 10 A° (8 M urea). The increased donor-acceptor distance had significantly affected the structure of RNA in 20 nano seconds (ns). Based on this study it was confirmed that, the urea induced denaturation of RNA was due to the loss of WC hydrogen bonds in the nonspecific bases.

In another study the effect of urea (2.04, 4.08, and 6.01 M) was investigated using hyper stable RNA tetra loop [30]. In this study, the results described the free energy dependence denaturation, such as effect of urea on the free energy landscape and pairwise energies (van der Waals/ electrostatics) interactions. With increase in urea concentration (0 > 2.04 > 4.08 > 6.01 M) the decrease in melting temperature (Tm) and the increase in cooperativity for the melting curve was observed. The lowest Tm of 376 ± 1 was observed at 6 M as compared to control (399 ± 1). The protein and RNA denaturation properties of urea can be exploited to use urea as a sanitizer to control the spread of COVID-19. The primarily infection and spread can be restricted by using the effective concentrations.

Urea as an antiviral agent

The enveloped viruses (ex. SARS-CoV-2) can be inactivated by using active biocidal substances like alcohol which has the property of inactivating enveloped viruses [31]. However, aqueous solution of urea has been reported to have the virucidal activity and was proven effective against multiple active viruses such as polio virus and was tested in monkey's [32].

Ionidis., *et al*. [33], tested the formulation of 3.69% w/w 2-propanol, 69.39% w/w ethanol, 2.0% citric acid, and 2.0% urea against several non-enveloped viruses like polyomavirus SV40, Murine norovirus (MNV), poliovirus (PV), Aleutian disease virus (AdV) and enveloped viruses strain Elstree, bovine viral diarrhoea virus (BVDV) and vaccinia virus. In clean conditions the above combination was found to inactivate non-enveloped viruses effectively within 30 seconds. Whereas, it took 15 seconds to inactivate enveloped viruses. The formulation of 2.0% uric acid and 2.0% citric acid had increased the activity against the broad-spectrum viruses [33]. The table 1 summarises the effectiveness of urea solutions in water or in combination with alcohol as an antiviral agent against various enveloped and non-enveloped viruses.

As per Buckland [36], 2 M (12%) urea solution had shown

Sr. No.	Formulation With additive	Viruses	Virucidal time	Reference
1	69.39% w/w ethanol, 2.0% citric acid and 2.0% urea	(Enveloped) vaccinia virus strain Elstree and BVDV	15 seconds	[33]
2	69.39% w/w ethanol, 2.0% citric acid and 2.0% urea	(Non-enveloped) polyomavirus SV40, MNV, PV, and AdV	30 seconds in clean and 60 seconds with Foetal Calf Serum (FCS)	[33]
3	7.5% urea	Influenza Type C	1 hour	[34]
4	7.2 M urea	Polio Virus	60 seconds	[35]

5	8 M urea with 0.15 M sodium chloride containing 0·01 M phosphate Buffer at pH 7·0	Influenza A_2	60 seconds	[36]
6	2 M urea in 0.1 M potassium phosphate buffer at pH 7	Influenza A	30 minutes	[36]
7	40% urea solution	Yellow fever virus	20 minutes	[37]

Table 1: Formulations containing urea solutions tested against various viruses.

antiviral activity against Influenza-A virus in 30 min. This can be considered as active concentration against enveloped viruses. Cooper [35], reported that 7.2 M urea initially induces the changes in poliovirus surface, and it leads to advanced damage to poliovirus particle. This rupture resulted in release of RNA from virus, and which has affected the infectivity [35]. Prasad., *et al*. [34], investigated the effect of 7.5% urea against Influenza type-C. The different temperatures and urea concentration have shown clear effect on Influenza type-C virus replications and it had reduced the titre value [34]. The urea at 40% concentration had inactivated yellow fever virus in 20 minutes of time frame [37]. Therefore, use of urea as a disinfectant is evaluated by various researchers for virucidal activity.

Before categorisation and recommendation of any specific disinfectant, they were artificially tested for effectiveness on viability of viruses. Quality tests of disinfectants achieving at least titre reduction factor of 4 log10 (RF of 4) against BVDV and vaccinia virus are considered as a suitable disinfectant against all enveloped viruses (virucidal limited spectrum) [38]. According to DVV/RKI Guidelines, disinfectants inactivating AdV, poliovirus (PV), polyomavirus SV40 and 2015 MNV also can claim as effective antiviral agents against all viruses (virucidal) [39].

Advantages of using urea as disinfectant

The above-mentioned chemical disinfectants have one or the other limitations and get degraded very fast. These disinfectants need to be applied frequently to keep the surface free from pathogenic microbes. The surface disinfection of large public areas by these disinfectants would be very costly and labor intensive. The urea is a commonly available commodity. The price of the technical grade urea in India ranges from 20 to 30 INR per kg. Presently, as per government of India department of fertilizer, India's current production of urea is 240 lakhs metric tonnes and imports ~80 lakh metric tonnes. In the previous section the antiviral and protein denaturation properties of urea were discussed. The other properties such as compatibility (material and skin) and stability which makes urea as a superior disinfectant over the other chemical disinfectants are briefly discussed in the below section.

Compatibility of urea with various materials

Urea is compatible with majority of commonly used metallic, plastic and elastic materials. Compatibility of urea is good with commonly used metals like aluminium and iron (Table 2). Compatibility of urea is excellent with few plastics and good with others except nylon where the compatibility is poor (Table2).

Compatibility of Urea	Metals					Plastics and Elastomers					
	Aluminium	Cast/ Ductile iron	304 Steel	316 Steel	Carbon steel	Fluoro-elasto-mer	polypro-pylene	Nylon	Polytet-rafluo-roethyl-ene	Polyvi-nylidene fluoride	Ure-thane
Excellent (A)						A	A		A	A	
Good (B)	B	B	B	B							B
Fair to Poor (C)								C			
No data (-)					-						

Table 2: Compatibility of urea with commonly used metallic, plastic, and elastic surfaces [40].

Skin compatibility

Urea is a natural substance and an active part of our natural moisturising factor (NMF), found in the surface layer of our skin which functions to keep our skin hydrated, protected and working efficiently. Urea makes up 7% of our natural moisturising factor, which decreases with age [41]. Hydrophilic (water-loving) property of urea hold water molecules and keep our skin moist. It has a very high-water content, which helps to reduce the amount of water loss through the skin. Topical formulations containing urea lesser than 10% be used as skin moisturizer, whereas higher than 10% exert a keratolytic action [42]. There are multiple skin care products containing urea, which are available in the market. Some of them are 1) Derma Feet Urea 40% (With Emulgel Urea 40% moisturize, flexible, and decrease the thickness of the skin), 2) Eucerin Urea Repair PLUS 10% Urea Foot Cream, 3) VECTEM Tractopon 30% Urea, 4) LACTOVIT Lacto-Urea Body Milk, 5) GLAAN Urea 10% Lotion.

Limited studies are available on dermal exposure urea and their effects. Majority of the studies conducted on human dermal exposure mention that up to 60% dose, does not create skin irritation, inflammation or trans-epidermal water loss [43]. In these studies, skin irritation was studied based on visual inspection whereas the effects on the skin barrier properties were evaluated by trans-epidermal water loss study and electrical capacitance/conductance assessment was used to study skin hydration properties. However, other studies have shown that a 20% urea formulation did produce skin irritation and swelling [44]. Interestingly, in these studies, where urea was shown to develop skin irritation, petroleum jelly was present in the preparations. According to previous studies, it has been mentioned that the penetration of urea into human skin strongly dependant on the vehicle of carrier used [44].

Prolonged activity

Chemically urea is a diamide of carbonic acid and has the capability to form intramolecular dipoles similar to the water molecule [45]. As a result, urea is readily soluble in polar solvents (water and alcohol) and insoluble in non-polar solvents such as ether or chloroform. Urea is a very stable molecule with a half-life (t½) of approximately 40 years at 25°C [46]. Urea can be stored as solid at room temperature and solution can be prepared as and when required.

Stability study of urea on various surfaces is not available but there are reports available on volatility data from soil. Urea in soil gets hydrolysed by urease and then releases volatile ammonia. Urea hydrolysis process in soil is urease dependent and influenced by soil temperature and pH (Tables 3 and 4).

Days	Temperature: 60 °F	Temperature: 75 °F	Temperature: 90 °F
4	2% of N volatilized	4% of N volatilized	5% of N volatilized
8	7% of N volatilized	12% of N volatilized	19% of N volatilized

Table 3: Effect of soil temperature on urea stability [47].

Days	Soil pH- 5.0	Soil pH- 6.0	Soil pH- 7.0
4	1% of added N volatilized	5% of added N volatilized	18% of added N volatilized
8	8% of added N volatilized	12% of added N volatilized	30% of added N volatilized

Table 4: Effect of soil pH on urea stability [47].

The study suggests that a few days of warm temperatures or high pH was required to degrade the urea in soil. The data reported in table 3 and 4 indicate that, the added urea in soil is quite stable, with only 19% and 30% volatilization of nitrogen in a span of 8 days at 90 °F temperature and pH 7.0 respectively. These results confirm that the urea is quite stable for few days and degrades slowly.

Urea solutions can be stabilized to prevent the degradation and to maintain the activity. According to Mooshammer [48], the formation of cyanate from urea can be avoided by buffering the urea solution at lower pH. The stability study with various concentrations of urea solution (2.5% - 20%) at 298.15 K – 333.15 K temperature and 3.11- 9.67 pH showed that, the urea was more stable between 4-8 pH range and the stability decreases with increasing temperature irrespective of pH [49]. Within the above given experimental range of temperature and initial urea concentration, the urea degradation was found lowest when the urea solution pH was adjusted to 6.0 using lactate buffer [49]. Phosphoric (H_3PO_4) acid also can be used as a stabilizing agent. Nattakan., *et al.* [49], in their study found that, the 20% nitrogen containing urea solution can be preserved for 5-6 months by adding 0.39% H_3PO_4. The

H_3PO_4 was mainly acting as a pH regulator and found to keep the pH of the solution below the critical point of 7.5 and thereby it has prevented the degradation [50]. In another study it was found that, the rate of degradation was inversely proportional to the concentration of urea solution. As the concentration of urea was increased, the rate of degradation was decreased. These findings indicate the importance of the reverse reaction in preventing the degradation of concentrated urea solution [49]. There are many reports on the usage of triacetin to prevent the degradation of urea [51]. Triacetin is an ester of glycerol and acetic acid. Whenever pH of urea solution increases due to degradation, the ester bonds in triacetin breaks down and releases the acetic acid which lowers the pH, there by prevents the degradation of urea. Current method of stabilization of urea containing formulations is mostly done by using polysaccharide starch. Because of the non-sandiness nature of polysaccharides, the pH can be adjusted as desired making its use more popular for this purpose [51].

From the overall literature study, it is evident that in spite of varied pH and temperature, the rate of urea degradation is very slow. This property of urea is critical from the point of virucidal activity for prolonged durations. Further, the degradation of urea can also be reduced or even prevented by addition of many stabilizing agents that can play role in extending the virucidal activity.

Conclusion

The COVID-19, global pandemic needs to be addressed in a multi-pronged approach. Environmental disinfection, social distancing and hand hygiene practices are primarily recommended by WHO and respective governments to contain the spread of virus before and after vaccination. Disinfection is one of the major contributors in flattening the pandemic curve and effective in reducing the spread. With the surge in demand, the availability of effective disinfectant with prolonged activity is a challenge for personal, hospitals as well as for public place sanitisation. In this context urea can be used as disinfectant owing to virucidal activity pertaining to its protein and RNA denaturation properties. In contrast to commonly used disinfectants, urea doesn't get evaporated and degraded immediately. The higher stability of urea keeps it active for prolonged duration until it is washed off or denatured. In addition to this, urea in low concentrations can also be used in combination with alcohol-based sanitizers for increasing the effectiveness of hand sanitizers against non-enveloped viruses. Based on the literature study, 7.2 M and 8 M urea can kill influenza A2 virus and polio virus in 60 seconds respectively. Since SARS-CoV-2 is similar to influenza viruses, 7.2 M concentration of urea solution could be sufficient to denature SARS-CoV-2. As a disinfectant, urea can be extremely cost-effective, and it can be prepared in house with minimum knowledge. Therefore, because of all these properties urea can be recommended as a safe, low cost, stable, and effective disinfectant for controlling the spread of COVID-19.

Authors' Contributions

As a team, all the authors have equally contributed for writing review article titled "Urea: A Low-Cost Disinfectant for prolonged activity in Combating Spread of COVID-19".

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Conflicts of Interest Statement

All the authors do not have any conflicts of interest.

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