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ORIGINAL PAPER

Inactivation of Adenovirus in Water by Natural and Synthetic Compounds

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Abstract

Millions of people use contaminated water sources for direct consumption. Chlorine is the most widely disinfection product but can produce toxic by-products. In this context, natural and synthetic compounds can be an alternative to water disinfection. Therefore, the aim of this study was to assess the inactivation of human adenovirus by N-chlorotaurine (NCT), bromamine-T (BAT) and Grape seed extract (GSE) in water. Distilled water artificially contaminated with recombinant human adenovirus type 5 (rAdV-GFP) was treated with different concentrations of each compound for up to 120 min, and viral infectivity was assessed by fluorescence microscopy. The decrease in activity of the compounds in the presence of organic matter was evaluated in water supplemented with peptone. As results, NCT and GSE inactivated approximately 2.5 $\log_{10}$ of adenovirus after 120 min. With BAT, more than 4.0 $\log_{10}$ decrease was observed within 10 min. The oxidative activity of 1% BAT decreased by 50% in 0.5% peptone within a few minutes, while the reduction was only 30% for 1% NCT in 5% peptone after 60 min. Organic matter had no effect on the activity of GSE. Moreover, the minimal concentration of BAT and GSE to kill viruses was lower than that known to kill human cells. It was concluded that the three compounds have potential to be used for water disinfection for drinking or reuse purposes.

Keywords N-chlorotaurine · Bromamine-T · Grape seed extract · Water disinfection · Adenovirus

Introduction

Nowadays more than 660 million people do not have access to safe and potable water for consumption. Most of them use water collected from wells, rivers and lakes for their daily needs, often without any treatment (UN 2015). However, these sources might be contaminated by enteric pathogens derived from inadequate waste sanitation. Nearly 80% of waste generated worldwide is discharged into water bodies without any treatment (UN 2015).

Diarrhea is the main disease related to contaminated drinking water, causing 1.3 million deaths per year, above all in developing countries (WHO 2018). Microbial contamination of water is the major cause of diarrhea, and enteric viruses are highlighted since they usually are more resilient in environment than bacteria (Ashbolt 2015; La Rosa et al. 2012). Among them, adenovirus is considered a viral fecal contamination indicator and is frequently studied in disinfection process due to its high resistance to traditional water treatments (Bofill-Mas et al. 2013; Reynolds et al. 2008; LeChevallier and Au 2004).

Chlorine is the disinfectant most widely used due to its high efficiency and low price, but its use in raw water is dangerous because the interaction with organic matter can produce toxic by-products (LeChevallier and Au 2004). In this context, natural and synthetic compounds can be an alternative to water disinfection, mostly for those that drink directly from water bodies. The potential of three compounds to be used for water disinfection was studied in this work: N-chlorotaurine (NCT), bromamine-T (BAT) and grape seed extract (GSE).

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N-chlorotaurine (Cl–HN–CH_2–CH_2–SO_3H) is an oxidant present in human granulocytes and monocytes resulting from the reaction of N-chlorinated compounds with the amino acid taurine. The ability to synthesize NCT as a sodium salt allowed to advance studies, mostly using aqueous solution at 1% (*w*/*v*) (Gottardi et al. 2013a; Gottardi and Nagl 2002). NCT has microbicidal action against bacteria, fungi, protozoa and viruses (Gottardi and Nagl 2010; Romanowski et al. 2006; Nagl et al. 1998a). Besides that, NCT has anti-inflammatory action in vitro and high tolerability, being used in several clinical studies (Gottardi and Nagl 2010). Furthermore, NCT has mild reactivity compared to chlorine and hypochlorous acid and does not lead to toxic by-products such as trichloramines, and its cytotoxicity is markedly lower (Gottardi and Nagl 2013, 2010; Kontny et al. 2006).

Bromamine-T ($[CH_3–C_6H_4–SO_2–N–Br]^- Na^+$) is a synthetic compound originated by the reaction of amino groups with hypohalogenic acids (Gottardi et al. 2014; Gottardi and Nagl 2013b). Few studies assessing bacteria and fungi inactivation were already performed (Walczewska et al. 2017; Gottardi and Nagl 2013b), and no studies were reported regarding its virucidal ability. However, it is known that BAT is a more stable and stronger oxidant than NCT. Usually, bromine compounds are more efficient than chlorine compounds on microbial inactivation in the absence of significant amounts of organic matter (Gottardi et al. 2014). Despite the data gap on bromamines toxicity, clinical investigations showed good tolerability on the skin during treatment of acne and herpes zoster (Kyriakopoulos et al. 2016; Marcinkiewicz et al. 2008). Recently, it was found that the relation between the concentration of bromamines including BAT toxic to human body cells compared with that toxic to bacteria (biocompatibility index) is very good (Walczewska et al. 2017). Anti-inflammatory activity in vitro was also demonstrated for BAT (Walczewska et al. 2017; Gottardi and Nagl 2013b).

In recent years, the interest in using natural products to control and prevent human enteric virus transmission has increased (D'Souza 2014). Grape seeds are a by-product of wine industry, and the grape seeds extract has antimicrobial and antioxidative activity mostly due to the high phenolic content. Studies showed that GSE inhibits bacterial growth and has virucidal activity (Adámez et al. 2012, Su and D'Souza 2011; Jayaprakasha et al. 2003). Furthermore, no toxicity or side effects were observed after 4-week consumption by mice and humans (Sano 2017; Brown et al. 2010).

Although the use of NCT, BAT and GSE in water disinfection remains to be investigated, these compounds may become promising as chlorine alternative mainly in a water crisis scenario, since they have broad-spectrum microbicidal, anti-inflammatory or antioxidative activity, and may present lower cytotoxicity and reduced toxic by-products formation. In this context, the aim of this study was to assess the virucidal activity of NCT, BAT and GSE on human adenovirus in water. Aiming to apply these compounds for water treatment, their decrease in activity in the presence of organic matter was evaluated as well.

Methods

Chemicals

N-chlorotaurine (NCT) sodium salt was prepared according to Gottardi and Nagl (2002). NCT has a molecular weight of 181.57 g/mol and is highly soluble in water. The crystalline sodium salt presents a stability of 1 year at 4 °C and at least 2 years at − 20 °C. The 1% solution can be stored for 1 year at 4 °C, too, and for 3 weeks at 20 °C (Gottardi and Nagl 2002). For viral inactivation assays, NCT was dissolved in distilled water at concentrations ranging from 2.0 to 0.05% (*w*/*v*).

Bromamine-T (BAT) was synthesized from dibromamine T according to Nair, Lalithakumari and Senan (1978). BAT has a molecular weight of 300.84 g/mol and is promptly soluble in water, presenting high stability even at room temperature when protected from light. For viral inactivation assays, BAT was dissolved in distilled water at concentrations ranging from 0.02 to 0.005% (*w*/*v*).

Grape seed extract (GSE) was kindly donated by OptiPure, Chemco Industries (Los Angeles, USA). According to the manufacturer, GSE has not less than 95% of flavonol content. For viral inactivation assays, GSE was dissolved at high concentration (4% - *w*/*v*) in pure dimethyl sulfoxide (DMSO) and diluted in distilled water to concentrations ranging from 0.02 to 0.005% (*w*/*v*).

Cell Line and Virus Strain

HEK293 cell line (Human Embryonic Kidney cells) was used for viral inactivation assays. For cell cultivation, DMEM plus 10% (*v*/*v*) fetal bovine serum (FBS) and 1% of 1M Hepes salt solution was used. The cells were grown in 180 cm^2 flasks and maintained at 37 °C under 5% CO_2 atmosphere.

Recombinant human adenovirus serotype 5 that expresses GFP (rAdV-GFP) was used as a model. This virus contains the *GFP* gene replacing the *E1* gene and only replicates in HEK293 cells since *E1* gene is integrated in the cell genome. Fluorescent cells infected by rAdV-GFP can be counted by fluorescence microscopy after a 24 h post-infection period.

Experimental Design of Virus Inactivation

For human adenovirus inactivation assays, tubes were prepared containing 1.3 mL of distilled water spiked with approximately 4.0×10^6 FFU of rAdV-GFP. Also, 1.3 mL of compounds was added to reach the final concentrations of interest (NCT: 1.0, 0.5 and 0.25%; BAT and GSE: 0.01, 0.005 and 0.0025%—*w*/*v*). After incubation at 20 °C, samples of 500 µL were taken at time 0, 15, 30, 60 and 120 min. Shorter times were used when necessary. Aliquots were placed into 500 µL of respective stop solutions that inactivate the test compounds (NCT and BAT: 1% methionine/ 1% histidine aqueous solution; GSE: DMEM with 15% FBS) to warrant exact incubation times. The respective compound solvent was used as negative control. For inactivation controls, stop solutions were added to compounds, and rAdV-GFP was added at last.

Virus Infectivity Assessment

To assess rAdV-GFP infectivity, a protocol previously described by Garcia, Nascimento and Barardi (2015) was followed. HEK293A cells were cultivated in 48-well plates at a concentration of 1.5×10^5 cells/well. After 24 h, the culture medium was removed and 100 µL of diluted samples were added in duplicate. For dilution of samples, 50 µL were added to 450 µL of DMEM with 1% antibiotic solution [(PS) 100 U penicillin G /mL; streptomycin sulfate, 100 µg/mL]. Plates were incubated for 1 h to provide viral adsorption, and 400 µL of maintenance medium was added per well (DMEM, 2% FBS, 1% Hepes and 1% PS). After 24 h of incubation, the supernatant was aspirated, the cells were immediately counted by fluorescence microscope at 100× magnification, and the virus titer was expressed in Focus Forming Units per mL (FFU/mL).

Decrease in Activity of Test Compounds in the Presence of Organic Matter

To understand how NCT, BAT and GSE might perform in natural water disinfection, the decrease in activity of these compounds in the presence of organic matter was measured. For this purpose, peptone (Peptone of enzymatic digest from casein, Sigma, Buchs, Switzerland) was used to simulate organic matter in water. Peptone was selected since it is a simple and reproducible means of organic matter and the main component to formulate artificial sewage according to the Organization for Economic Co-operation and Development (OECD 2010).

Oxidative capacity of NCT and BAT was measured by iodometric titration (Clescerl et al. 1999). Both compounds were used in two concentrations (1.0 and 2.0%), whereby 1% NCT or BAT was tested in 0.5, 1.0, and 2.0% protein and 2% in 1.0, 2.0 and 4.0% protein.

For this assay, 500 µL of peptone were added to 500 µL of NCT or BAT. After incubation times of 0, 1, 2, 5, 10, 15, 30, 60 min at 20 °C, 10 mL of distilled water were added, followed by 200 µL of acetic acid (50% solution), and potassium iodate (KI) at molar excess. The oxidative capacity was immediately measured using a High Performance Potentiometric Titrator (TIM960—Radiometer Analytical). Results were expressed in percentage of oxidation capacity.

For GSE, the decrease in total phenolic content in the presence of peptone was measured first. The Folin–Ciocalteau protocol was used (Singleton et al. 1999), with adaptations for 96 well plates. Briefly, in each well was added 6.25 µL of peptone (0.4%) and 6.25 µL of GSE (final concentration of 0.02%). After incubation times of 0, 5, 15, 30, 60, 90 and 120 min at 20 °C, 50 µL of ultrapure water and 12.5 µL of Folin–Ciocalteau reagent (Sigma) were added. After 6 min, 100 µL of 7% sodium carbonate ($NaCO_3$) solution and 100 µL of ultrapure water were added. The plate was incubated for 90 min in the dark, and the absorbance was measured spectrophotometrically at 720 nm (SpectraMax M2 – Molecular Devices). An analytical curve was done using gallic acid aqueous solution between 25 and 250 mg/mL. Results were expressed in milligrams of gallic acid equivalent (mg GAE).

Free radical scavenging activity of GSE was also measured using the DPPH (2,2-difenil-1-picrilhidrazil) protocol described by Brand-Williams, Cuvelier and Berset (1995) and adapted by Sánchez-Moreno, Larrauri and Saura-Calixto (1998). Briefly, 20 µL of peptone (0.1%) and 20 µL of GSE (final concentration at 0.01%) were added in microtubes. After incubation times of 0, 15, 30, 60, 90 and 120 min at 20 °C, 1560 µL of DPPH (methanolic solution at 60 µM) was added and incubated for 60 min in the dark. 340 µL was transferred to a 96-well quartz plate, and the absorbance was measured spectrophotometrically at 515 nm. Results are expressed as percentage of free radical scavenging, represented by the total of DPPH neutralized and calculated by the following formula:

$$\% \text{ Scavenging} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right] \times 100$$

where A_{sample} is the absorbance of the test sample and A_{control} is the absorbance of the control.

The influence of pH on the decrease in activity was evaluated for all compounds, using peptone at pH 6.8 and 2.5. Acidic peptone was adjusted using 0.1N HCl.

Data Analysis

Three independent replicates were conducted in all experiments and the data are presented as mean values and SD. Levene's test was used to check the homoscedasticity. Student's *t* test for two groups and one-way analysis of variance (ANOVA) with Bonferroni's multiple comparison test for more groups were used to test for differences between test and control groups. p values < 0.05 were considered significant. All analyses were performed using IBM SPSS 19.0 software. Figures were designed in GraphPad 6.0 software.

Results

Viral Inactivation by Compounds

NCT at concentrations of 0.25, 0.5 and 1.0% reduced rAdV-GFP FFU by 1.22, 2.04 and 2.51 $\log_{10}$, respectively, after 120 min of treatment (Fig. 1a). BAT was used at a concentration hundred times lower than NCT and, at the highest concentration tested (0.01%), rAdV-GFP completely lost its infectivity (Fig. 1b). Thus, incubation times were shortened, and the FFU count decreased ≥ 4.73 $\log_{10}$ by 0.01% BAT after 10 min. At lower concentrations of 0.005 and 0.0025%, BAT caused a decrease of 2.41 and 0.40 $\log_{10}$, respectively, after 30 min (Fig. 1b). GSE lowered the FFU count by 2.51, 1.53 and 0.71 $\log_{10}$ at concentrations of 0.01, 0.005 and 0.0025%, respectively (Fig. 1c). Inactivation controls showed that virus replication by the HEK293 cells was not inhibited by mixtures of the test compounds and their inactivation substances.

Consumption of Activity of Test Compounds in the Presence of Organic Matter

Aiming to apply these compounds as disinfectants for less purified water sources, their loss of activity by organic matter was determined. NCT showed a slight decrease in activity after 60 min in 2% peptone, losing approximately 20% of its oxidation capacity (Fig. 2). Due to this high stability, 1% NCT was tested with a higher peptone concentration (5%), wherein it lost 30% of its oxidative capacity at the final sampling time. No significant difference was observed using peptone in acid (pH 2.5) or neutral (pH 6.8) conditions (Fig. 2). With 2% NCT, the percentage of loss of oxidation capacity was lower when comparing the same peptone concentration.

BAT, by contrast, lost oxidation capacity very fast in the presence of organic matter. The decrease exceeded 50% after 2 min using the two highest peptone concentrations and was lower for 2% BAT than for 1%, comparing

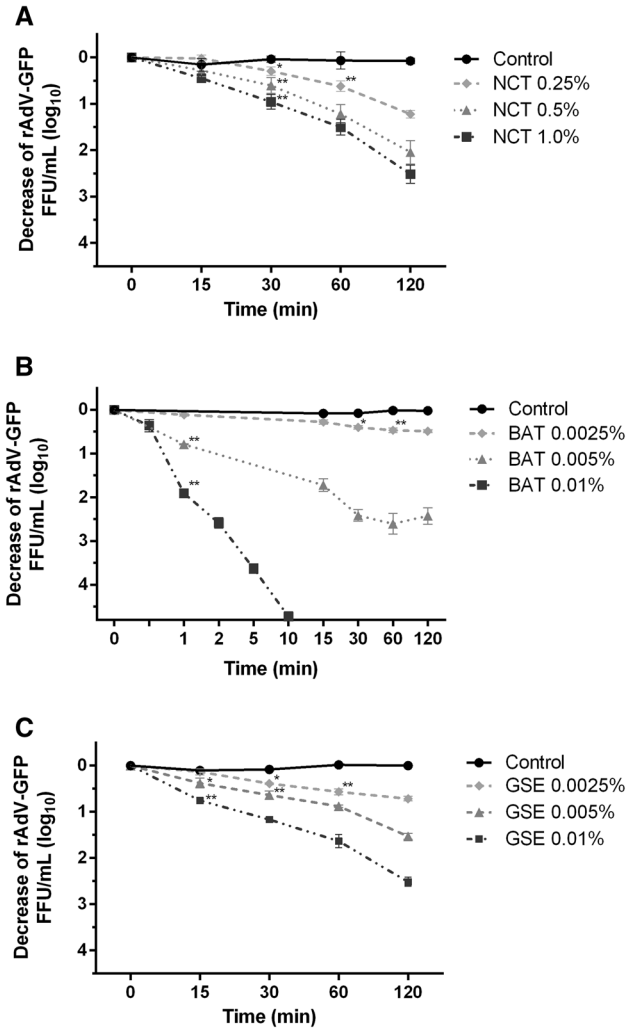

Fig. 1 Reduction of rAdV-GFP infectivity (log10) in distilled water treated with different concentrations of **a** NCT, **b** BAT and **c** GSE at 20 °C. Mean values ± SD of three independent experiments. *$p < 0.05$ *versus* controls; **$p < 0.01$ *versus* controls

the same concentration of peptone (Fig. 3). However, some oxidation capacity remained over time, probably due to the limited reduction capacity of the proteinaceous material. Acidic condition (pH 2.5) accelerated the BAT decrease compared to neutral one (pH 6.8) (Fig. 3).

For GSE, the total phenolic content was used as parameter to evaluate the influence of organic matter. Figure 4 shows that the total phenolic content remained unchanged (approximately 150 mg GAE) for all period evaluated and with no influence of acid or neutral conditions. Free radical scavenging activity of GSE also remained unchanged during the 120 min of analysis (Fig. 5). Interestingly, the scavenging capacity of GSE in acidic peptone was lower than in neutral one.

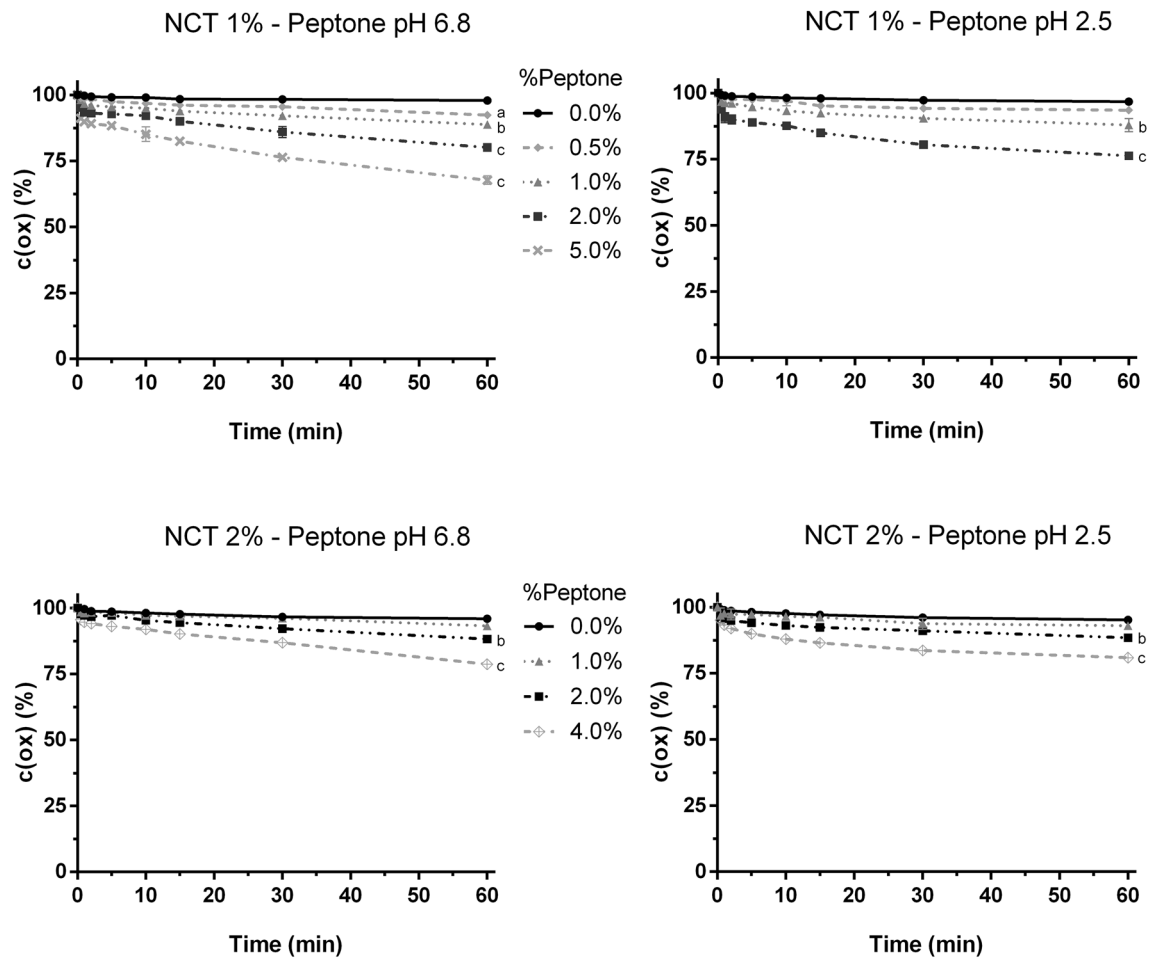

Fig. 2 Decrease in NCT oxidation capacity c(ox) in different concentrations of peptone at pH 6.8 and 2.5 at 20 °C. Mean values ± SD of three independent experiments. **a** $p < 0.05$ at time 60 min, versus control; **b** $p < 0.05$ at time 15 min, versus control; **c** $p < 0.05$ at time 2 min or before, versus control

Discussion

The microbicidal activity of NCT is well known. Gottardi and Nagl (2010) showed that NCT at 1% had a considerable killing effect on bacteria (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) and protozoa (*Acanthamoeba* spp., *Leishmania* spp. and *Trichomonas vaginalis*). After 1 h of treatment, a decrease of more than 4.0 $\log_{10}$ of viable microorganisms was observed. For fungi inactivation by NCT, about 2–8 h were required (Gottardi and Nagl 2010).

Virucidal activity of NCT at 1% was also described, showing 99.9% (3 $\log_{10}$ or more) of inactivation after 15 min of treatment for herpes simplex virus, influenza virus and HIV-1 (Gottardi and Nagl 2010; Dudani et al. 2008; Nagl et al. 1998a). For human adenovirus 5, a 1.5 $\log_{10}$ decrease was observed, which is lower than the 4 $\log_{10}$ decrease after 1 h at pH 7 and 20 °C obtained in previous studies (Gottardi and Nagl 2010; Nagl et al. 1998a). The slower inactivation curve of adenovirus in the present study might be caused by the different viral strain and by the higher pH, since the pH of 1% NCT adjusts to 8 in distilled water (Gottardi and Nagl 2002). According to Romanowski et al. (2006) and Uchio, Inoue and Kadonosono (2010), the virucidal effect of NCT was also observed against several clinically relevant adenovirus serotypes with slight variations among the strains.

Studies on microbial inactivation by BAT are incipient. However, BAT is an analogue of chloramine-T (CAT) whose microbicidal, including virucidal activity, has been well described (Gowda et al. 1986). Nonetheless, it is known that bromine compounds have higher reactivity and consequently higher inactivation rates than chloramine compounds. Gottardi, Klotz and Nagl (2014) showed that *E. coli* and *S. aureus* were reduced by 4 $\log_{10}$ after 20 min in contact with 0.0003% BAT. For CAT, concentrations 10–25 times higher were necessary to achieve the same result. Another work showed that BAT at 0.0025% was efficient in inactivation of *Candida albicans, S. aureus* and *P. aeruginosa* after 1 h

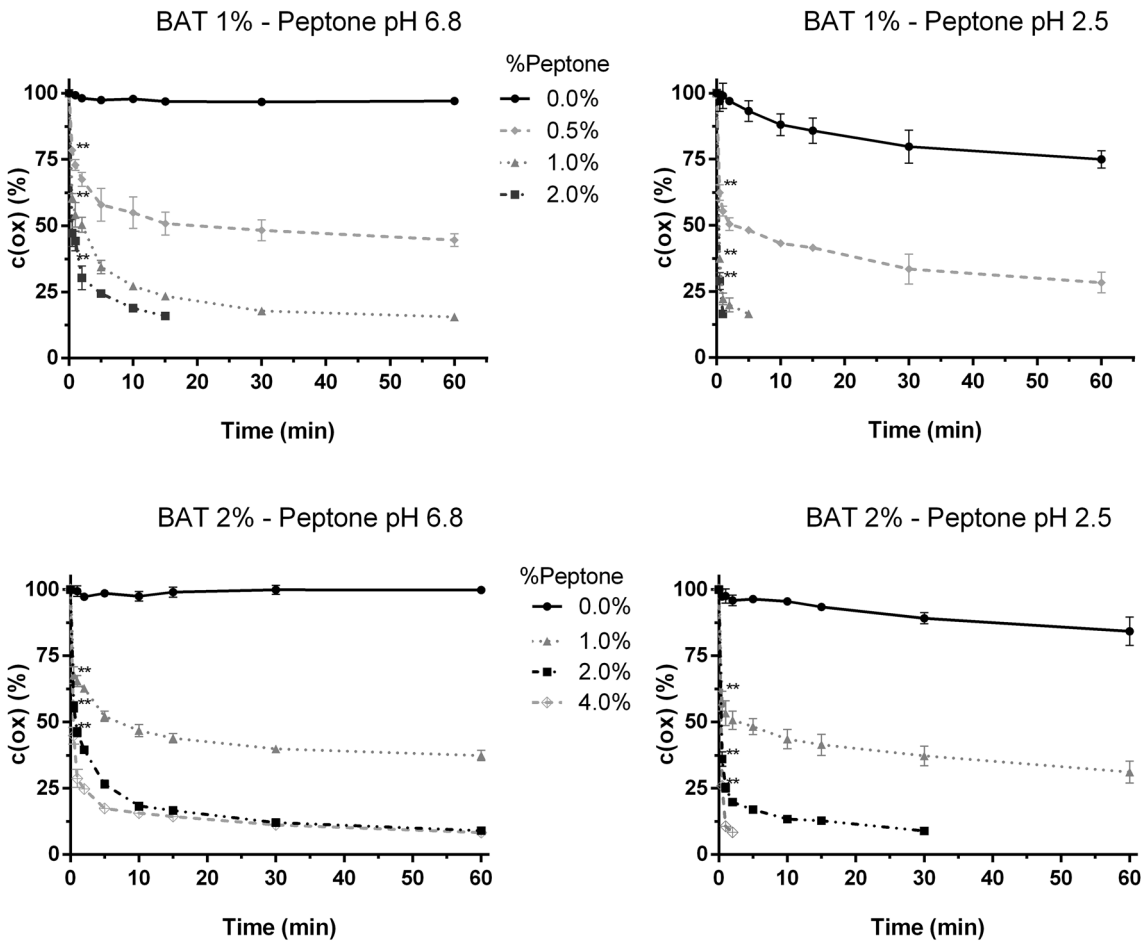

Fig. 3 Decrease in BAT oxidation capacity c(ox) in different concentrations of peptone at pH 6.8 and 2.5 at 20 °C. Mean values ± SD of three independent experiments. $^{**}p < 0.01$ *versus* controls

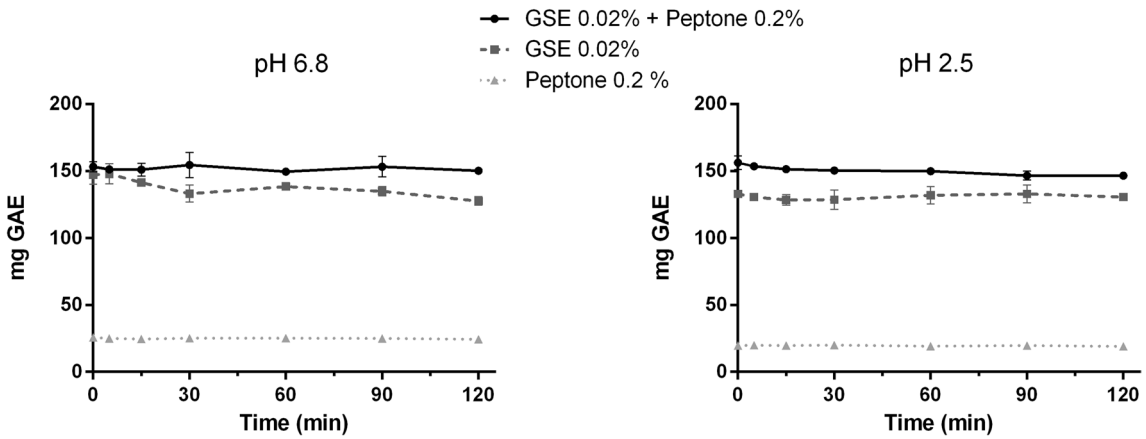

Fig. 4 GSE total phenolic content (mg GAE) in the presence and absence of peptone at pH 6.8 and 2.5 at 20 °C. Mean values ± SD of three independent experiments

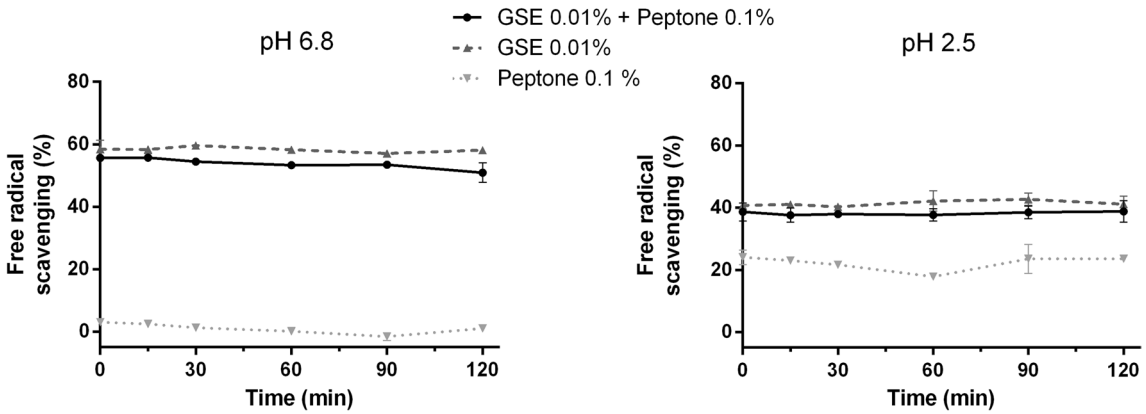

Fig. 5 Free radical scavenging (%) of GSE in the presence and absence of peptone at pH 6.8 and 2.5 at 20 °C. Mean values ± SD of three independent experiments

of treatment (Walczewska et al. 2017). However, there is no study evaluating viral inactivation by BAT. Thus, this is the first work that showed a human adenovirus inactivation of 4 $\log_{10}$ within 10 min by BAT, using a concentration of 0.01%.

Antibacterial activity of GSE is well described. Several studies showed that the extract can inhibit growth of *Bacillus* spp., *S. aureus, E. coli, P. aeruginosa, Enterococcus faecium* and *E. faecalis* (Adámez et al. 2012; Corrales et al. 2009; Jayaprakasha et al. 2003). However, there are few works evaluating the GSE virucidal activity. Li et al. (2012) observed a loss of 3 $\log_{10}$ of murine norovirus infectivity and 1 $\log_{10}$ in genomic copies of human norovirus GII.4 after 1 h of GSE 0.1% treatment. Feline calicivirus was inactivated after 2 h using concentrations of 0.025%. Murine norovirus, MS2 and hepatitis A virus were more resistant, showing inactivation of 1–2 $\log_{10}$ using GSE between 0.025 and 0.1% (Joshi et al. 2015, Su and D'Souza 2011). In the present investigation, we observed a similar inactivation rate for adenovirus, however, by a tenfold lower concentration of GSE. Although adenoviruses normally show higher resistance against chemical disinfectants, differences in sensitivity could be found for several serotypes (Sauerbrei et al. 2004). Regarding the solvent used for GSE, previous studies used phosphate buffer or an ethanolic solution, while we used the universal solvent DMSO. Although in all assays the final DMSO concentration was lower than 0.25%, which is considered non-cytotoxic, an additive effect with GSE cannot be excluded.

As a mechanism of action of NCT and BAT for virus inactivation, oxidation and chlorination/bromination of viral capsid proteins and other viral proteins must be assumed according to the chemical reactions of these agents (Gottardi et al. 2013a; Gottardi and Nagl 2013b). Oxidative inactivation of key viral proteins of fiber and hexon of adenovirus type 5 has been demonstrated by N,N-dichloro-2,2-dimethyltaurine, a dimethylated analogue of NCT (Yoon et al. 2011). Thus, the virus will not be able to attach, infect cells and to multiply.

The mechanism of action of GSE against viruses is not well known. It has already been described that non-enveloped viruses are more resistant to phytocompounds (Bright and Gilling 2016). Phenolic compounds with high affinity to proteins could interfere with viral attachment to cells (Jakobek 2015). Su and D'Souza (2011) showed that the infectivity decreases when viruses are treated with GSE prior to cell attachment. Li et al. (2012) described a capsid degradation, but another study showed no morphological changes of viruses after GSE treatment (Joshi et al. 2015).

Regarding the decrease in oxidation capacity in the presence of organic matter, the relatively high stability of NCT compared to BAT was also observed in a previous study, which can be explained by the higher reactivity of BAT (Gottardi and Nagl 2013b). The present work tested higher peptone concentrations and longer time aiming to apply these compounds in natural water disinfection. Equivalent results were also observed in relation to pH variation, again showing hardly a difference with NCT and a slightly more rapid decrease with BAT at acidic pH (Gottardi and Nagl 2013b). The slower decrease in the oxidation capacity of 2% NCT or BAT compared to 1% at the same protein content can be explained in that the portion of the total reducing capacity of protein becomes lower with higher NCT and BAT concentrations.

For GSE, studies demonstrated that there was no difference on murine norovirus inactivation in tap water and water with distinct chemical oxygen demand (between 500 and 1500 mg/L); however, the virucidal activity of GSE was decreased in the presence of milk (Joshi et al. 2015). Here we found no change in the total phenolic content or in the free radical scavenging activity of GSE in the presence of peptone. Since the Folin–Ciocalteau method is used for protein measurement as well, the control containing

only peptone presented a basal quantification in mg GAE. The peptone also may interfere in the DPPH method. It is true that these interferences may provide an inaccurate quantification of total phenolic content and free radical scavenging capacity, but there was no change over time for both GSE in the presence and in the absence of peptone, which indicates high stability of GSE in this setting. Some studies showed that acid pH may cause structural changes and loss of oxidative stability on phytocompounds, which could explain the lesser free radical scavenging capacity of GSE in the presence of peptone at pH 2.5.

Regarding tolerability of the agents used in this study, preconditions are promising. Su and D'Souza (2011) showed that a GSE concentration below 0.04% was non-cytotoxic for CRFK, RAW264.7 and FRhK4 cells. Also, there are studies that evaluated GSE ingestion by mice and humans without toxicity or side effects (Sano 2017; Brown et al. 2010).

Regarding BAT cytotoxicity, a study using concentrations of 0.01% (approximately 300 µM) or below showed no change of J774.A1 cell viability in vitro (Walczewska et al. 2017). *In vivo*, however, another bromamine (N-bromotaurine) with similar activity was tolerated at up to 1% on the skin (Walczewska et al. 2017; Kyriakopoulos et al. 2016; Marcinkiewicz et al. 2008).

For NCT, it is known that cells tolerate concentrations up to 0.01% in vitro (approximately 500 µM) (Geiger et al. 2009; Kontny et al. 2006). However, there are several clinical studies using NCT at 1% (55 mM) in different body regions (e.g., urinary tract, skin, nasal mucosa and eyes) with minimum side effects (Gottardi and Nagl 2010; Romanowski et al. 2006; Hofer et al. 2003; Nagl et al. 2003, 2000, 1998b).

Of note, pilot experiments showed that the minimal concentration of BAT and GSE to inactivate viruses was lower than that to kill human cells in vitro (results not shown). However, further studies are necessary to accurately confirm the cytotoxicity outcomes.

The results obtained in this study showed the potential of some natural and synthetic compounds for water disinfection. However, more studies must be conducted to safely apply GSE, BAT or NCT for drinking water purposes. Also, the viral inactivation should be tested in several water sources, with distinct organic matter content and in larger scales. Besides that, by-products formation should be investigated in water treated with these compounds. Also, benefits of ingestion can be considered and evaluated, such as the already described anti-inflammatory (NCT and BAT) and antioxidative (GSE) properties (D'Souza 2014; Gottardi and Nagl 2013b, 2010). We believe that the tested compounds have a promising potential to be used especially in emergency cases where water sources are scarce and extremely needed to ensure health maintenance, such as during environmental disasters, remote and poor regions.

Conclusion

NCT inactivated adenovirus in water and has high stability in the presence of protein. Thus, it has the potential to disinfect water with high organic matter content, such as wastewater. BAT was the most efficient agent on adenovirus inactivation. However, it rapidly lost oxidation capacity in the presence of organic matter. Therefore, it has the potential to be used as a disinfectant for previously treated water, such as drinking water. GSE inactivated adenovirus and was stable in the presence of protein. This indicates a high potential to be used for disinfection of both drinking and wastewater.

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