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EGCG: Potential application as a protective agent against grass carp reovirus in aquaculture

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Abstract

Grass carp reovirus (GCRV) is the primary cause of grass carp haemorrhagic disease. The major catechin in green tea, (–)-epigallocatechin-3-gallate (EGCG), has been found to have anti-GCRV activity in the *C. idellus* kidney cell line (CIK). The aim of this study was to test the potential application of EGCG as an anti-GCRV agent in aquaculture. Here, we demonstrate that various concentrations (99%, 50% and 35%) of EGCG could inhibit GCRV infectivity. EGCG (50%) + GCRV treatment significantly reduced the number of dead fish at 1-, 2-, 3-, 4 -and 5-day post-challenge compared with the negative control (GCRV challenge without EGCG treatment). The safety of EGCG compound products on cell survival was studied using four fish cell lines; we did not detect a significant change in cell viability within 24 hours of EGCG incubation. We also evaluated toxicity and concentrations of malondialdehyde (MDA), glutathione (GSH) and lysozyme (LZM) in the grass carp, and the results showed that even a high dose of EGCG did not induce toxicity. Following EGCG compound injection, the concentration of MDA decreased and the concentration of GSH and LZM increased compared with the control groups. We also detected EGCG concentration in grass carp plasma and kidney using HPLC with electrochemical detection after intraperitoneal injection at a dose of 150 mg/kg. The concentration of EGCG in the plasma and kidney reached the highest levels (20 μg/ml and 1.5 μg/ml) about 12 hr after injection and then decreased. Overall, EGCG is a safe, effective product that could inhibit GCRV infection and improve immunoactivity in aquaculture.

KEYWORDS
epigallocatechin-3-gallate, Grass carp reovirus, grass carp, Green tea

1 | INTRODUCTION

The grass carp *Ctenopharyngodon idellus* is one of the most popular cultured fish in Asian countries, especially in China (Zhang, He, & Wang, 2017). In 2016, production in China was 5.9 million tons, the highest of all freshwater fish species (Bureau of Fisheries, 2017; Wang et al., 2018). However, recent severe outbreaks of grass carp haemorrhagic disease have led to heavy losses to aquaculture in China. The disease is caused by grass carp reovirus (GCRV), part of the genus Aquareovirus and of the family Reoviridae (International Committee on Taxonomy of Viruses 1991; Nibert & Duncan, 2013). GCRV is a double-stranded RNA (dsRNA) virus with a double-layered protein capsid (Franki et al., 1991), and the genome is composed of eleven segments of dsRNA. It encodes seven structural proteins (VP1-VP7) and five non-structural proteins (NS16, NS26, NS31, NS38 and NS80) (Attoui et al., 2002; Cheng et al., 2010; Zhang, Jin, Fang, Hui, & Zhou, 2010).

There are three different genotypes of GCRV represented by isolates GCRV-873 and GCRV-JX0901 (genotype I), GCRV-HZ08 (genotype II) and HGDRV (genotype III) (Ma et al., 2014) and to date

Wang and Chen contributed equally to this work.

there are no effective therapies against multiple genotypes of GCRV infection. Therefore, effective drugs still need to be developed for this disease.

After it has bound to the membrane protein, the virus enters by penetration and receptor-mediated endocytosis (Denisova et al., 1999). Our previous study identified the laminin receptor (LamR) as a potential interaction partner for the outer capsid protein VP5 of GCRV (Wang, Yu, Li, & Lu, 2016). Tachibana, Koga, Fujimura, & Yamada, 2004; reported that expression of LamR confers (–)-epigallocatechin-3-gallate (EGCG) responsiveness to cancer cells at physiologically relevant concentrations. Our previous research showed that purified EGCG can inhibit GCRV infection in CIK cells (Wang, Liu, Yu, & Lu, 2016).

EGCG is the major active polyphenolic catechin in green tea leaves and accounts for approximately 59% of the total catechins (Steinmann, Buer, Pietschmann, & Steinmann, 2013). Several groups (Calland, Dubuisson, Rouillé, & Séron, 2012; Chen, Pamu, Cui, Chan, & Dou, 2012; Ciesek et al., 2011) have identified that EGCG can inhibit hepatitis C virus (HCV) infection and it has also been shown to be a potent entry inhibitor. EGCG has been identified as a potent inhibitor of many different viruses, such as enterovirus (Steinmann et al., 2013), influenza (Song, Lee, & Seong, 2005), Epstein–Barr virus (EBV) (Hsieh, Wu, Chow, Tsai, & Chang, 2003), hepatitis B virus (HBV) (Grimm, Thimme, & Blum, 2011) and White spot syndrome virus (WSSV) (Wang, Sun, & Zhu, 2017).

Various antiviral mechanisms have been reported for EGCG. It can directly bind to the viral particle and may also reduce virus uptake by inhibiting endocytosis or by modulating the endosomal pH, which could in turn impair pH-dependent viral fusion (Tong et al., 2017; Zhong, Hu, Shu, Gao, & Xiong, 2015).

In this study, we evaluate whether different concentrations of an EGCG commercial compound can protect grass carp against GCRV infection. We also investigate the safety and feasibility of EGCG as antiviral agent in aquaculture.

2 | MATERIAL AND METHODS

2.1 | Animals, cell lines and virus

The GCRV-JX01 strain of grass carp was collected from an aquaculture farm (Jiangxi Province, China) and maintained in the laboratory. *Ctenopharyngodon idellus* kidney (CIK) cells were harvested and grown in medium M199 supplemented with 10% inactivated foetal calf serum (Gibco BRL. USA). Cells were used for stimulation or infections (He, Xu, Yang, Xu, & Lu, 2011) on the following cells lines stored in our laboratory: *C. idellus* kidney cell line (CIK), grass carp ovary (GCO), fathead minnow (*Pimephales promelas*, FHM) and *Epithelioma papulosum cyprinid* (EPC).

2.2 | Chemicals and apparatus

Standards of EGCG were purchased from Selleck.cn (Shang Hai, China); other concentrations (99%, 50% and 35%) were purchased from Tian Yuan Company (China). Methanol, acetonitrile and water of HPLC grade were purchased from Sinopharm Chemical Reagent Co., Ltd (Shang Hai, China). L-Ascorbic acid, Na^{2+}-EDTA and the 0.22 μm Nylon syringe filter used for sample filtration were procured from Sang-Gon Chemical Reagents Company (Shang Hai, China). Quantitative PCR was performed in triplicate using a CFX96 real-time PCR system (Bio-rad, USA). High-performance liquid chromatography (HPLC) was performed using a Thermo Ultimate 3000 and Thermo LTQ XL. Data were analysed by Xcalibur 3.0—Foundation 3.0 SP1. Cytofluorimetric assay was performed using a Muse Count & Viability Kit (Millipore).

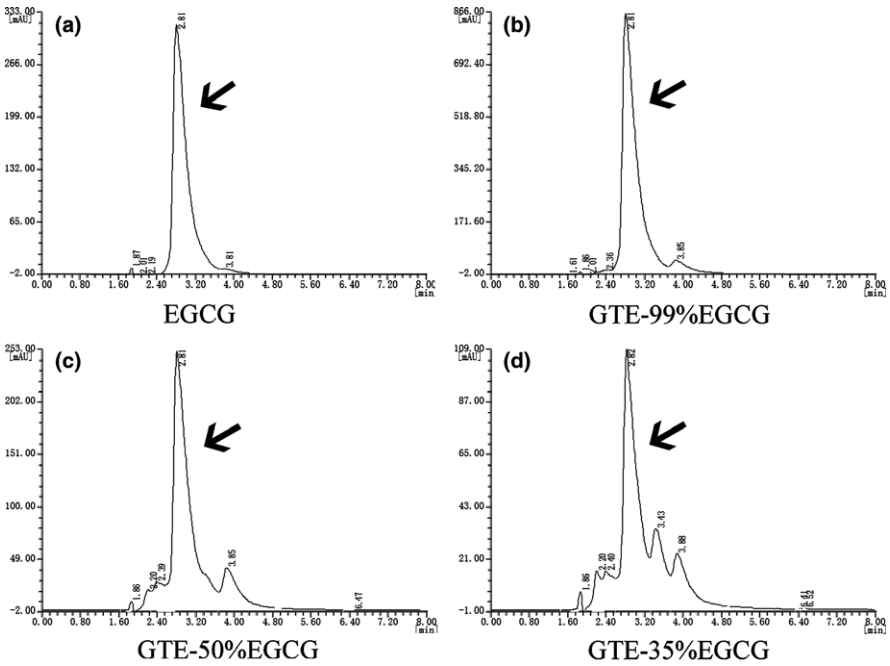

FIGURE 1 Reverse-phase HPLC analysis. (a) purified EGCG; (b) 99% EGCG compounds. (c) 50% EGCG compounds; (d) 35% EGCG compounds. The detection wavelength was 280 nm. Arrows indicate the highest peak

2.3 | Experimental design

2.3.1 | The effect of different concentrations of EGCG compounds on GCRV

CIK cells were grown in six-well plates and incubated until the cell number reached 1×10^6 cells: this was performed in triplicate. The cells were incubated at 27°C without additional CO_2. The cells were then incubated with the three concentrations of EGCG at 27°C for 1 hr. After incubation, cells were exposed to GCRV at a multiplicity of infection of 0.001 for 60 min. Then, the supernatant was replaced with fresh medium, and the infected cells were again incubated at 27°C (Wang, Liu et al., 2016).

The cell monolayers were viewed under a light microscope, and the titre of infectious virus in the growth medium was assayed by real-time PCR (Wang, Liu et al., 2016). A TIANamp Virus DNA/RNA

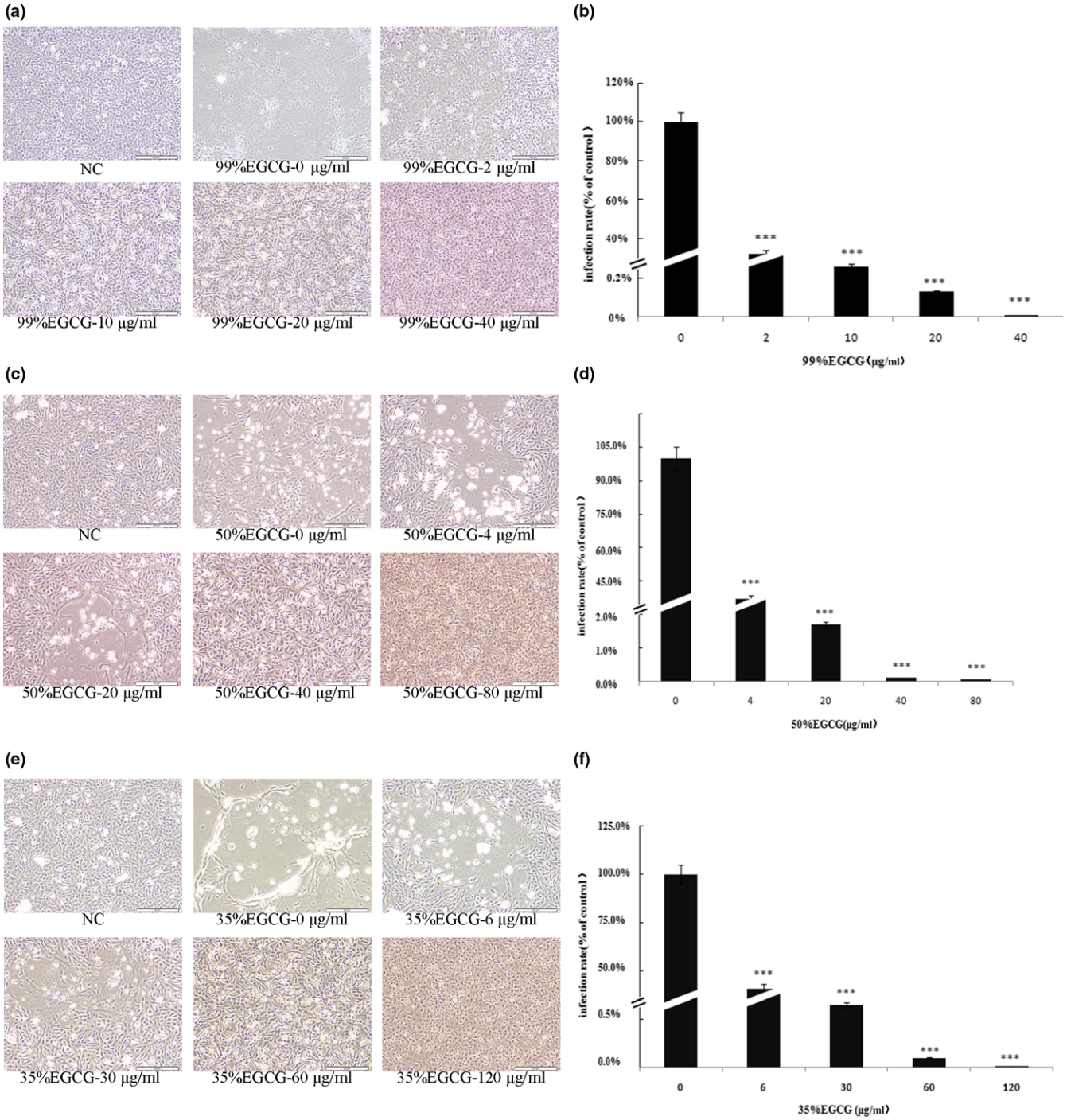

FIGURE 2 CPE due to GCRV infection assessed using a visible light phase microscope. (a,c,e) inhibition of GCRV infection by 99%, 50% and 35% EGCG, respectively, at the indicated concentrations. Bars 200 μm. (b,d,f) real-time PCR assay of virus yield in supernatants. Result is means ± *SD*. from three separate experiments. Asterisks represent a significant difference from the control

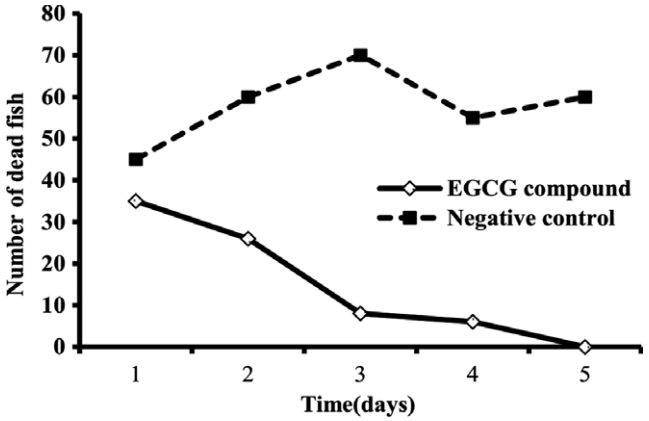

FIGURE 3 Mortality of GCRV-challenged grass carps treated with EGCG (50%). The mortalities were monitored continuously and recorded daily for 5 days

Kit (TIANGEN Biotech Co., Ltd, Beijing) was used to obtain the total RNA of the growth medium. Using a PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan), 200 ng of total RNA was reverse transcribed into cDNA in a 20 μl reaction volume, following the manufacturer's protocol (Yu, Wang, Liu, & Lu, 2016). Quantitative PCR was performed using SYBR® Premix Ex Taq II (TaKaRa). The reaction conditions were as follows: 95°C for 10 min; followed by 39 cycles of 10 s at 95°C, 15 s at 56°C and 20 s at 72°C. The primer sequences used in this study were as follows: JX01 F: 5′-CAAGACCATTCAAGACTC-3′; R: 5′-TCACTCACTTCGACTAAT-3′ (Wang, Li, & Lu, 2013).

2.3.2 | The effects of EGCG on the mortality of GCRV-infected grass carps

Grass carp with an average weight of 200 g were maintained in a fish farm(Shanghai Ocean University fish-breeding farm in Sichuan province). Fish were maintained in aerated water and fed daily with commercial dry feed pellets(water temperature 28 ± 5°C). A total of 1,200 fishes have been divided into two groups. For the infection, fish were intraperitoneally injected with 1 ml of virus at a concentration of 10^6 viral particles/ml. At the same time, the fish of experimental group were fed with artificial feed pellets mixed with the EGCG compound (100 mg/kg, 50% EGCG compound) twice a day for a week. The fish were fed up with the commercial dry feed pellets without EGCG compound as the negative control group. Fish health was monitored daily. Dead fish were counted and removed from the experimental pool. Fish care of animals was in compliance with the guidelines of the Animal Experiment Committee, Shanghai Ocean University.

2.3.3 | The effects of different concentrations of EGCG on the viability of different cells

CIK, GCO, FHM and EPC cells were grown in six-well plates and incubated until the cell number reached 1×10^6: this was performed in triplicate. The cells were incubated at 27°C without additional CO_2. The cells were then incubated with the three concentrations of EGCG at 27°C for 24 hr. A cytofluorimetric assay was performed using a Muse Count & Viability Kit (Millipore). Cell counts were determined using a Muse Cell Analyzer (Millipore) according to the manufacturer's protocol (Wang, Liu et al., 2016).

2.3.4 | Metabolism of EGCG in grass carp

The fish were acclimated at a temperature of 15 ± 2°C and an oxygen level of 6–8 ppm for 30 days at the start of the trial. During the acclimation, fish were fed daily until the day before the trial began. After the acclimation period, 40 juvenile fish of similar size (initial body weight 75 ± 10 g) were transferred into plastic tanks (400 L). The 99% EGCG was dissolved with 0.6% physiological saline and given as an intraperitoneal injection at a concentration of 150 mg/kg (Et & Sinica, 2012).

The fish were anaesthetized with clove oil (50 μl/L) and then blood and kidney samples were collected 0.5, 1, 2, 4, 6, 8, 12, 24 and 96 hr after drug administration, in triplicate (Dar et al., 2017). Blood was taken from the caudal vein using a medical syringe rinsed in 2.7% EDTA solution, and the collected blood was transferred immediately to an Eppendorf tube coated with anticoagulant (EDTA 2.7%). Tissue samples were immediately frozen at −80°C until further analysis (Dar et al., 2017).

A 0.2 g sample of kidney was added to a test tube with 2 ml methanol and 200 μl 0.05% Na^{2+}-EDTA; and a 600 μl blood sample was placed in a separate test tube with 40 μl 20% vitamin C. Each sample was mixed for 2 min, then 4 ml ethyl acetate was added and each sample was vortex mixed for 5 min and then centrifuged at 4500 × g. The supernatant was collected, 2 ml ethyl acetate was added and the supernatant combined and completely dried under nitrogen gas using a Termovap Sample Concentrator at 45°C (Li et al., 2015; Zhu et al., 2018).

Both sets of samples were reconstituted with 500 μl 10% acetonitrile and vortexed for 20 s. Next, 500 μl hexane was added to the samples, which were vortexed for 5 min and centrifuged for 5 min at 12000 × g and the supernatant removed. This procedure was repeated twice and the lower was collected. All samples were filtered through a nylon 0.22 μm syringe and transferred into a vial for HPLC analysis. A 20 μl sample was injected into the HPLC system for each run (Zhu et al., 2018).

2.3.5 | Measurement of toxicity and immune indicators

A total of 180 juvenile fish of similar size were randomly transferred into three plastic tanks (400 L), with 60 in each tank. An intraperitoneal injection of 99% EGCG compound was administered at concentrations of 50 mg/kg, 100 mg/kg and 150 mg/kg. Blood samples were collected 4, 10, 24 and 96 hr post-administration, in triplicate, as described above. After centrifugation at 3,000 *g* for 10 min, the plasma was removed and kept at −80°C until analysis. MDA, GSH and LZM were analysed according to the manufacturer's instructions

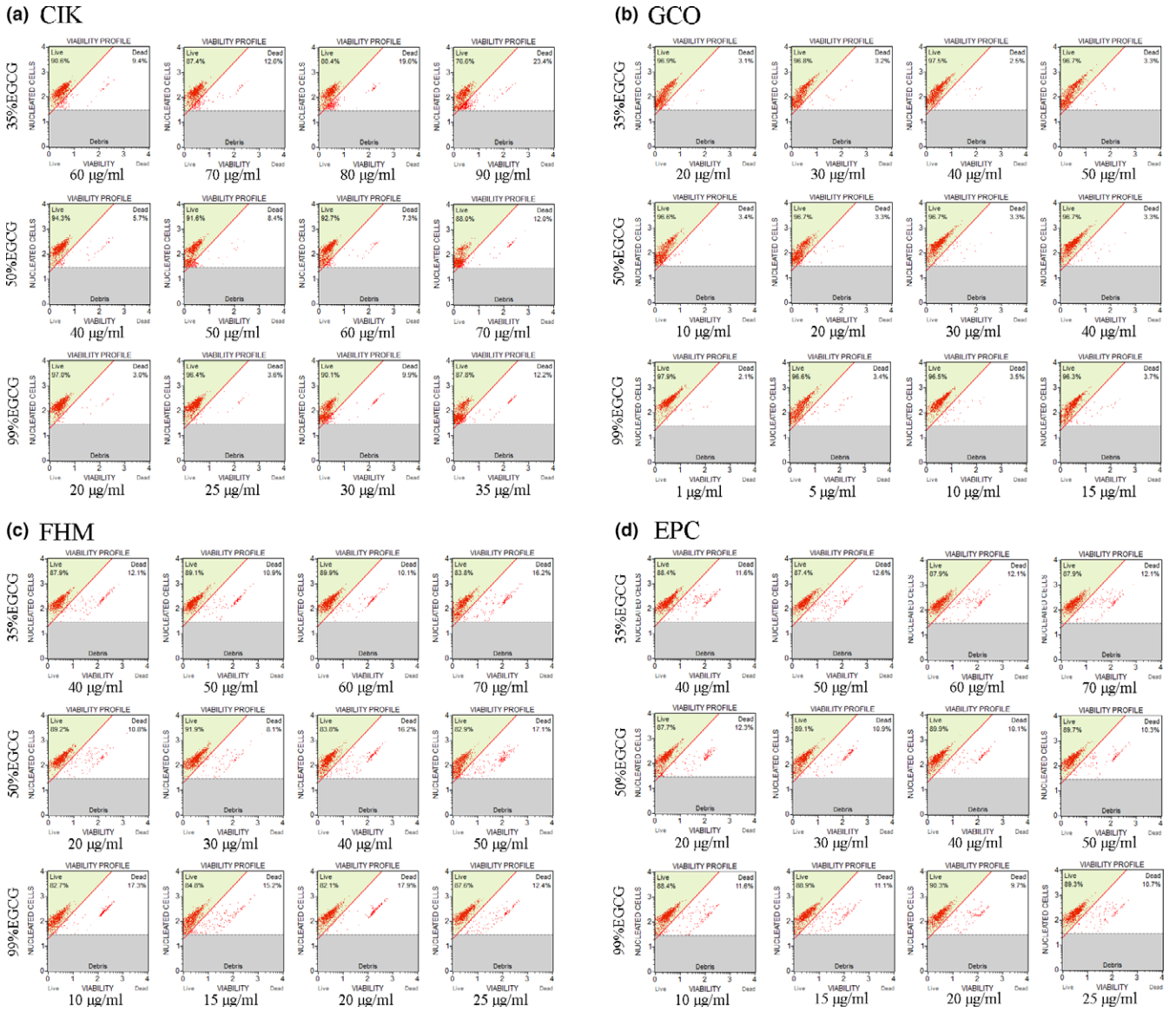

FIGURE 4 Cell viability. Cell viability assessed using MuseTM Count and Viability kit (Millipore, Poland)

(Jiancheng Institute of Biotechnology, Nanjing, China) (Chen et al., 2018; Milev-Milovanovic et al., 2006).

2.4 | Statistical analysis

Experimental data are presented as mean ± standard deviation of independent experiments performed in triplicate. The samples were analysed using one-way ANOVA, and statistical significance was considered at $p < .05$ and $p < .01$. All statistical analyses were performed using SPSS 22.0.

3 | RESULTS

3.1 | HPLC analyses

The structures of EGCG were confirmed by HPLC. Figure 1a shows that the retention time of standard EGCG was 2.81 min. There are many components present in different concentrations of EGCG compounds, including 99% EGCG, 50% EGCG and 35% EGCG. The highest peaks had the same retention time as the standard sample of EGCG (Figure 1b-d). These data indicated that the main component of green tea was EGCG (Wang, Liu et al., 2016).

3.2 | Effect of EGCG against GCRV

We investigated the antiviral protective effects of different concentrations of EGCG against GCRV infection in CIK cells. Observation (Figure 2a,c and e) and real-time PCR (Figure 2b,d and f) were used to determine the culture supernatant. As shown in (Figure 2b,d and f), treatment with all concentrations of EGCG compounds significantly suppressed GCRV. Even concentrations as low as 40 μg/ml compounds (99% EGCG) inhibited almost 100% of the plaque-forming activity of GCRV. These results (Figure 2) suggest that cytopathic

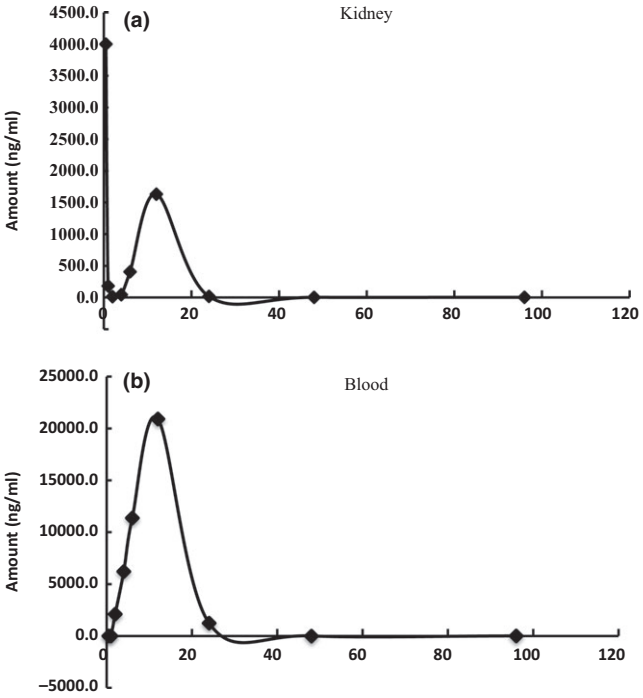

FIGURE 5 Changes in EGCG content over time (a) kidney; (b) blood

effect (CPE) and progeny yield were significantly reduced by various concentrations of EGCG in a dose-dependent manner. The data indicated that treatment with all the concentrations (99%, 50% and 35%) of EGCG significantly reduces the GCRV infection compared with the control group (GCRV only) ($p < .01$). Considering the above results and the cost of dosage, it suggests that 50% EGCG compound maybe more suitable for application. Therefore, 50% EGCG compound were used in the field analysis.

To evaluate the effects of EGCG compound on the mortality of GCRV-infected grass carp, grass carp were challenged with GCRV. Then, the experimental group was fed up with EGCG compound twice a day. As shown in Figure 3, the number of dead grass carp was significantly reduced by the EGCG treatment. Approximately sixty dead fish were recorded in the negative control group at 5-day post-infection, whereas no mortality occurred in the experimental group (Figure 3). All the dead fish infected with GCRV were confirmed by PCR assay (data not shown).

3.3 | Effect of EGCG on the viability of four fish cell lines

To determine the effect of EGCG on fish cell viability, the cytofluorimetric assay was performed using a Muse Count &Viability Kit (Millipore). The four kinds of fish cells were treated with varying

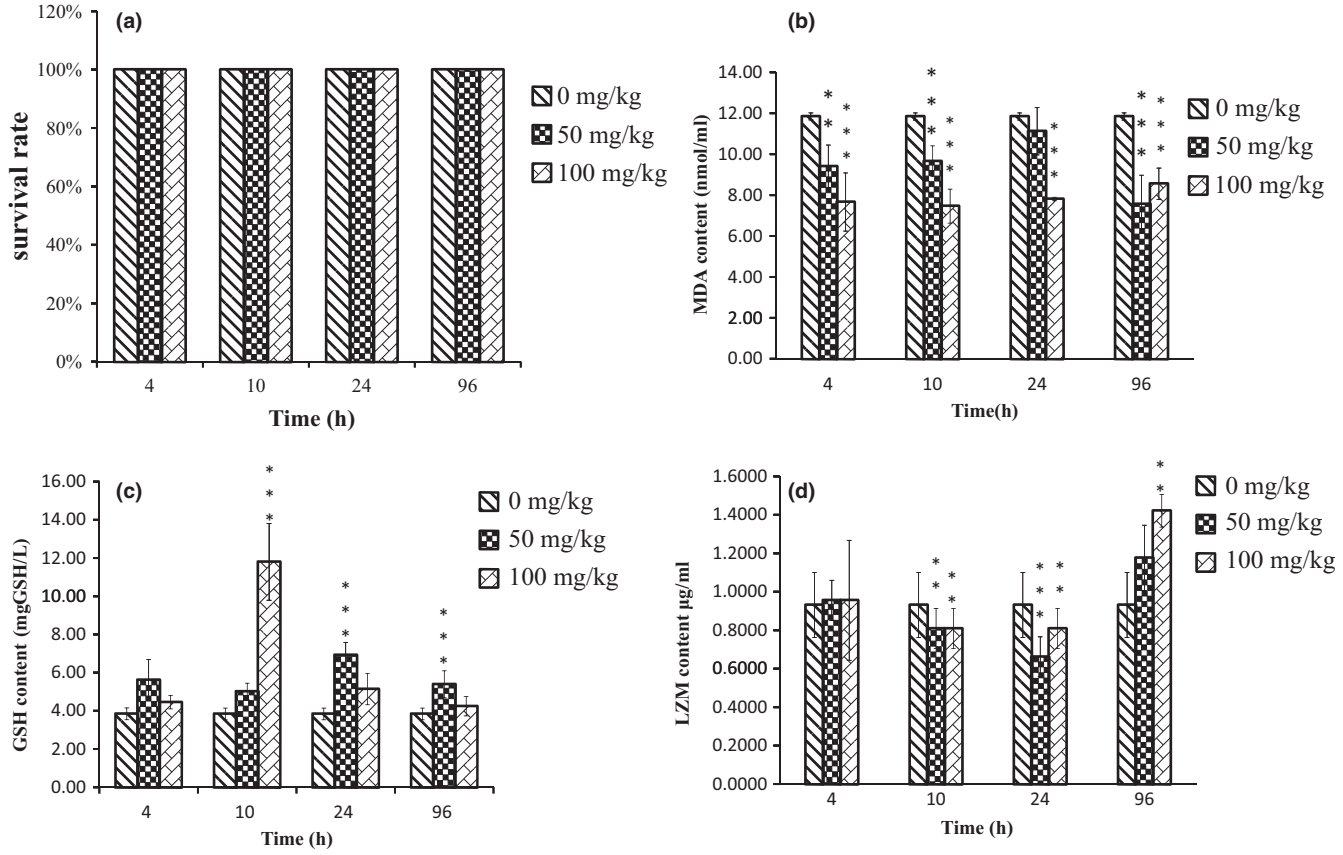

FIGURE 6 Changes in plasma content and activity of various components after injecting different concentrations of EGCG. Each bar represents means ± *SD*. from three separate experiments. Asterisks represent a significant difference from the control ($p < .05$). (a) Survival rate, (b) MDA content, (c) GSH content, (d) LZM content

doses of EGCG (20–90 μg/ml) for 24 hr (Figure 4). The viability of four cell lines after treatment with EGCG at different concentrations (Figure 4) was found to be about 80%–97%. As shown in Figure 4b, GCO showed the best viability among the four tested cell lines.

3.4 | Metabolism of EGCG in the grass carp

We measured the time-course of EGCG concentration in blood and kidney samples after intraperitoneal injection of EGCG. As shown in Figure 5, EGCG concentration increased slowly and reached its highest level about 12 hours after injection for both samples. Within 24 hr, EGCG had disappeared from both blood and kidney.

3.5 | Effect of EGCG on toxicity and immune indicators of grass carp

As shown in Figure 6a, survival did not differ significantly between the control (the group treat with PBS) and treatment groups. Over time, MDA content decreased steadily with increasing concentration of EGCG (Figure 6b). As shown in Figures 6c and 5d, a significant increase in serum LZM activity and GSH content was found in the 100 mg/kg group compared to the other groups at 4, 10, 24 and 96 hr.

4 | DISCUSSION

Several previous studies have shown that EGCG, the main constituent of green tea, has anti-infection properties (Steinmann et al., 2013). Of the 12 types of catechins identified in green tea, EGCG has been the most studied to date. There are three major reasons for this: it accounts for about 65% of the total catechin in green tea; it has the highest biological activity compared with other catechins; and it can regulate the 67-kDa laminin receptor (67LR) on the cell surface (Zhang, Wu, Wang, Wan, & Zhang, 2014). Our previous research (Wang, Yu et al., 2016) identified that the membrane LamR of grass carp could be involved in GCRV infection of CIK cells through its association with the viral outer capsid protein VP5. We therefore investigated whether EGCG can inhibit the infection of CIK cells by GCRV in a dose-dependent manner. However, it is expensive to purify EGCG from green tea as its structure is similar to other components in green tea (Fujiki, Sueoka, Watanabe, & Suganuma, 2015), and therefore, commercial crude EGCG may be a suitable clinical alternative for chemoprevention. However, the effect of these compounds on the antiviral activity of GCRV and the application to aquaculture have not yet been investigated.

Our studies have shown that commercial crude EGCG compounds can inhibit the infectivity of GCRV in CIK cells in a dose-dependent manner (Figure 2). These data confirm our previous research (Wang, Liu et al., 2016; Yu et al., 2016) that EGCG can inhibit GCRV replication by blocking viral adhesion to host cells. Moreover, our data show that commercial EGCG compound (50%) treatment significantly reduced the number of dead fish infected with GCRV at 1-, 2-, 3-, 4- and 5-day post-challenge compared with that of GCRV treatment (Figure 3).

We also found no significant toxicity associated with commercial crude EGCG compounds in four different fish cell lines. In this study, we have shown that EGCG treatment significantly reduced MDA content and increased both GSH and LZM content in grass carp serum. MDA is considered an indicator of oxidative stress in fish (Modesto & Martinez, 2010). LZM is an important antimicrobial effector in fish (Yeh, Chang, Chang, Liu, & Cheng, 2008) and also serves as an opsonin in the complement system for phagocyte activation. GSH (Jia, Cao, Xu, Jeney, & Yin, 2012) is a major antioxidant which helps to reduce lipid oxidation and converts active oxygen molecules into non-toxic compounds. These data indicate that commercial crude EGCG compounds could maintain fish health through protecting the tissue against peroxidation. We also found that commercial crude EGCG compounds can be safely used in the treatment of grass carp. We also used HPLC assay to evaluate the EGCG concentration in grass carp blood and kidney. These data suggest that once-daily dosing of 150 mg/kg was determined to be the rational choice for controlling GCRV infection in grass carp (Liu, Ge, Wang, Cui, & Han, 2014; Swezey et al., 2003; Unno & Takeo, 1995).

Vaccination is currently the primary method for controlling grass carp haemorrhagic disease caused by GCRV infection (Lu, Xu, He, & Li, 2011). However, the only legal vaccine for this disease, licensed for commercial production in 2011, has many limitations; these include poor immunological memory in the grass carp, regional variability, a high cost and difficulty of administration in an aquatic environment (Lu et al., 2011; Sommerset, Krossøy, Biering, & Frost, 2005). Compared with the immunization therapy, commercial EGCG could be used as feed supplement. The characteristics of EGCG compound are very suitable for applying in aquaculture because it is water soluble and exposure to high temperatures such as boiling water does not greatly influence the stability of the molecule. In addition, EGCG is a muti-functional compound, there are many potential applications as a protective agent in aquaculture.

In conclusion, we determined that commercial crude EGCG compounds are able to inhibit GCRV infection. These results can be used for further antiviral research with the aim of protecting the cultured grass carp from GCRV infection. Furthermore, EGCG as a natural agent has low toxicity, even at very high doses, and low impact on the environment.

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REFERENCES

Attoui, H., Fang, Q., Jaafar, F. M., Cantaloube, J. F., Biagini, P., de Micco, P., & de Lamballerie, X. (2002). Common evolutionary origin of aquareoviruses and orthoreoviruses revealed by genome characterization of Golden shiner reovirus, Grass carp reovirus, Striped bass reovirus and golden ide reovirus (genus Aquareovirus, family Reoviridae). *Journal of General Virology*, *83*, 1941–1951. https://doi.org/10.1099/0022-1317-83-8-1941

Bureau of Fisheries, Ministry of Agriculture. (2017). *2016 Aquatic animal health in China*. China Agriculture Press.

Calland, N., Dubuisson, J., Rouillé, Y., & Séron, K. (2012). Hepatitis C virus and natural compounds: A new antiviral approach? *Viruses*, *4*, 2197–2217. https://doi.org/10.3390/v4102197

Chen, D., Pamu, S., Cui, Q., Chan, T. H., & Dou, Q. P. (2012). Novel epigallocatechin gallate (EGCG) analogs activate AMP-activated protein kinase pathway and target cancer stem cells. *Bioorganic and Medicinal Chemistry*, *20*, 3031–3037. https://doi.org/10.1016/j.bmc.2012.03.002

Chen, Y.-J., Wang, X.-Y., Pi, R.-R., Feng, J.-Y., Luo, L., Lin, S.-M., & Wang, D.-S. (2018). Preproinsulin expression, insulin release, and hepatic glucose metabolism after a glucose load in the omnivorous GIFT tilapia Oreochromis niloticus. *Aquaculture*, *482*, 183–192. https://doi.org/10.1016/j.aquaculture.2017.10.001

Cheng, L., Zhu, J., Hui, W. H., Zhang, X., Honig, B., Fang, Q., & Zhou, Z. H. (2010). Backbone model of an aquareovirus virion by cryo-electron microscopy and bioinformatics. *Journal of molecular biology*, *397*, 852–863. https://doi.org/10.1016/j.jmb.2009.12.027

Ciesek, S., von Hahn, T., Colpitts, C. C., Schang, L. M., Friesland, M., Steinmann, J., … Steinmann, E. (2011). The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. *Hepatology*, *54*, 1947–1955. https://doi.org/10.1002/hep.24610

Dar, S. A., Nautiyal, V., Phulia, V., Bhat, I. A., Srivastava, P. P., Sahu, N. P., & Gupta, S. (2017) Determination of benzimidazoles in fish plasma by chromatographic method and their effects on metabolic and antioxidative enzymes activity. *Aquaculture*, *486*, 57–63.

Denisova, E., Dowling, W., LaMonica, R., Shaw, R., Scarlata, S., Ruggeri, F., & Mackow, E. R. (1999). Rotavirus capsid protein VP5* permeabilizes membranes. *Journal of virology*, *73*, 3147–3153.

Et, O., & Sinica, L. (2012) EGCG. 43.

Franki, R. I. B., Fauquet, C. M., Knudson, D. L., & Brown, F. (1991). *Classification and nomenclature of viruses: 5th report of the international committee on taxonomy of viruses. Archives of virology (suppl. 2)*. Vienna, Austria: Springer.

Fujiki, H., Sueoka, E., Watanabe, T., & Suganuma, M. (2015). Synergistic enhancement of anticancer effects on numerous human cancer cell lines treated with the combination of EGCG, other green tea catechins, and anticancer compounds. *Journal of Cancer Research and Clinical Oncology*, *141*, 1511–1522. https://doi.org/10.1007/s00432-014-1899-5

Grimm, D., Thimme, R., & Blum, H. E. (2011). HBV life cycle and novel drug targets. *Hepatology International*, *5*, 644–653. https://doi.org/10.1007/s12072-011-9261-3

He, Y., Xu, H., Yang, Q., Xu, D., & Lu, L. (2011). The use of an in vitro microneutralization assay to evaluate the potential of recombinant VP5 protein as an antigen for vaccinating against Grass carp reovirus. *Virology journal*, *8*, 132. https://doi.org/10.1186/1743-422X-8-132

Hsieh, Y. H., Wu, C. J., Chow, K. P., Tsai, C. L., & Chang, Y. S. (2003). Electroporation-mediated and EBV LMP1-regulated gene therapy in a syngenic mouse tumor model. *Cancer Gene Therapy*, *10*, 626–636. https://doi.org/10.1038/sj.cgt.7700609

Jia, R., Cao, L., Xu, P., Jeney, G., & Yin, G. (2012). In vitro and in vivo hepatoprotective and antioxidant effects of Astragalus polysaccharides against carbon tetrachloride-induced hepatocyte damage in common carp (Cyprinus carpio). *Fish Physiology and Biochemistry*, *38*, 871–881. https://doi.org/10.1007/s10695-011-9575-z

King, A. M., Lefkowitz, E., Adams, M. J., & Carstens, E. B. (Eds.). (2011). *Virus taxonomy: ninth report of the international committee on taxonomy of viruses*. Elsevier.

Li, Y., Zhang, Y., Yang, T., Li, H., Guo, J., Zhao, Q., & Xie, J. (2015). Pharmacokinetics and tissue distribution study of Isovitexin in rats by HPLC-MS/MS. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *991*, 13–20. https://doi.org/10.1016/j.jchromb.2015.04.003

Liu, Y., Ge, J., Wang, M., Cui, L., & Han, B. (2014). Frontier and Future Development of Information Technology in Medicine and Education. *Springer Science & Business Media*, *269*, 1291–1298. https://doi.org/10.1007/978-94-007-7618-0

Lu, L., Xu, H., He, Y., & Li, J. (2011). Protection of grass carp, Ctenopharyngon idellus (Valenciennes), through oral administration of a subunit vaccine against reovirus. *Journal of Fish Diseases*, *34*, 939–942. https://doi.org/10.1111/j.1365-2761.2011.01310.x

Ma, J., Zeng, L., Fan, Y., Zhou, Y., Jiang, N., & Chen, Q. (2014). Significant inhibition of two different genotypes of grass carp reovirus in vitro using multiple shRNAs expression vectors. *Virus Research*, *189*, 47–55. https://doi.org/10.1016/j.virusres.2014.05.009

Milev-Milovanovic, I., Long, S., Wilson, M., Bengten, E., Miller, N. W., & Chinchar, V. G. (2006). Identification and expression analysis of interferon gamma genes in channel catfish. *Immunogenetics*, *58*, 70–80. https://doi.org/10.1007/s00251-006-0081-x

Modesto, K. A., & Martinez, C. B. R. (2010). Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. *Chemosphere*, *78*, 294–299. https://doi.org/10.1016/j.chemosphere.2009.10.047

Nibert, M. L., & Duncan, R. (2013). Bioinformatics of Recent Aqua- and Orthoreovirus Isolates from Fish: Evolutionary Gain or Loss of FAST and Fiber Proteins and Taxonomic Implications. *PLoS ONE*, *8*, e68607. https://doi.org/10.1371/journal.pone.0068607

Sommerset, I., Krossøy, B., Biering, E., & Frost, P. (2005). Vaccines for fish in aquaculture. *Expert Review of Vaccines*, *4*, 89–101.

Song, J. M., Lee, K. H., & Seong, B. L. (2005). Antiviral effect of catechins in green tea on influenza virus. *Antiviral Research*, *68*, 66–74. https://doi.org/10.1016/j.antiviral.2005.06.010

Steinmann, J., Buer, J., Pietschmann, T., & Steinmann, E. (2013). Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *British Journal of Pharmacology*, *168*, 1059–1073. https://doi.org/10.1111/bph.12009

Swezey, R. R., Aldridge, D. E., Levalley, S. E., Crowell, J. A., Hara, Y., & Green, C. E. (2003). Absorption, tissue distribution and elimination of 4-[3 H]-epigallocatechin gallate in Beagle dogs. *International Journal of Toxicology*, *22*, 187–193. https://doi.org/10.1080/10915810305101

Tachibana, H., Koga, K., Fujimura, Y., & Yamada, K. (2004). A receptor for green tea polyphenol EGCG. *Nature Structural and Molecular Biology*, *11*, 380–381. https://doi.org/10.1038/nsmb743

Tong, X., Yang, L., Tang, X., Yang, X., Bao, C., Wang, J., … Tang, M. (2017). Patterns of catabolic capacities and energy metabolism in developing embryos and yolk-sac larvae of turbot (Scophthalmus maximus L.). *Aquaculture*, *481*, 177–183. https://doi.org/10.1016/j.aquaculture.2017.09.004

Unno, T., & Takeo, T. (1995). Absorption of (–)-Epigallocatechin Gallate into the Circulation System of Rats. *Bioscience, Biotechnology, and Biochemistry*, *59*, 1558–1559. https://doi.org/10.1271/bbb.59.1558

Wang, T., Li, J., & Lu, L. (2013). Quantitative in vivo and in vitro characterization of co-infection by two genetically distant grass carp

reoviruses. *Journal of General Virology*, *94*, 1301–1309. https://doi.org/10.1099/vir.0.049965-0

Wang, H., Liu, W., Yu, F., & Lu, L. (2016). Identification of (-)-epigallocatechin-3-gallate as a potential agent for blocking infection by grass carp reovirus. *Archives of Virology*, *161*, 1053–1059. https://doi.org/10.1007/s00705-016-2751-9

Wang, Z., Sun, B., & Zhu, F. (2017). Epigallocatechin-3-gallate inhibit replication of white spot syndrome virus in Scylla paramamosain. *Fish and Shellfish Immunology*, *67*, 612–619. https://doi.org/10.1016/j.fsi.2017.06.050

Wang, Q., Xie, H., Zeng, W., Wang, L., Liu, C., Wu, J., … Bergmann, S. M. (2018). Development of indirect immunofluorescence assay for TCID 50 measurement of grass carp reovirus genotype II without cytopathic effect onto cells. *Microbial Pathogenesis*, *114*, 68–74. https://doi.org/10.1016/j.micpath.2017.11.042

Wang, H., Yu, F., Li, J., & Lu, L. (2016). Laminin receptor is an interacting partner for viral outer capsid protein VP5 in grass carp reovirus infection. *Virology*, *490*, 59–68. https://doi.org/10.1016/j.virol.2016.01.011

Yeh, S. P., Chang, C. A., Chang, C. Y., Liu, C. H., & Cheng, W. (2008). Dietary sodium alginate administration affects fingerling growth and resistance to *Streptococcus* sp. and iridovirus, and juvenile non-specific immune responses of the orange-spotted grouper Epinephelus coioides. *Fish and Shellfish Immunology*, *25*, 19–27. https://doi.org/10.1016/j.fsi.2007.11.011

Yu, F., Wang, H., Liu, W., & Lu, L. (2016). Grass carp Ctenopharyngodon idella Fibulin-4 as a potential interacting partner for grass carp reovirus outer capsid proteins. *Fish and Shellfish Immunology*, *48*, 169–174. https://doi.org/10.1016/j.fsi.2015.11.029

Zhang, A., He, L., & Wang, Y. (2017). Prediction of GCRV virus-host protein interactome based on structural motif-domain interactions. *BMC Bioinformatics*, *18*, 1–13. https://doi.org/10.1186/s12859-017-1500-8

Zhang, X., Jin, L., Fang, Q., Hui, W. H., & Zhou, Z. H. (2010). 3.3 A Cryo-EM structure of a nonenveloped virus reveals a priming mechanism for cell entry. *Cell*, *141*, 472–482. https://doi.org/10.1016/j.cell.2010.03.041

Zhang, L., Wu, S., Wang, D., Wan, X., & Zhang, J. (2014). *Epigallocatechin-3-gallate (EGCG) in or on Nanoparticles: Enhanced Stability and Bioavailability of EGCG Encapsulated in Nanoparticles or Targeted Delivery of Gold Nanoparticles Coated with EGCG* (pp. 131–144). Nanomedicine and Stem Cell Use in Toxicology: Handbook of Nanotoxicology.

Zhong, L., Hu, J., Shu, W., Gao, B., & Xiong, S. (2015). Epigallocatechin-3-gallate opposes HBV-induced incomplete autophagy by enhancing lysosomal acidification, which is unfavorable for HBV replication. *Cell Death and Disease*, *6*, e1770. https://doi.org/10.1038/cddis.2015.136

Zhu, C., Xu, Y., Liu, Z.-H., Wan, X.-C., Li, D.-X., & Tai, L.-L. (2018). The anti-hyperuricemic effect of epigallocatechin-3-gallate (EGCG) on hyperuricemic mice. *Biomedicine and Pharmacotherapy*, *97*, 168–173. https://doi.org/10.1016/j.biopha.2017.10.013

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