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Green tea extract reduces viral proliferation and ROS production during Feline Herpesvirus type-1 (FHV-1) infection

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Abstract

Background Feline Herpesvirus type-1 (FHV-1) is a worldwide spread pathogen responsible for viral rhinotracheitis and conjunctivitis in cats that, in the most severe cases, can lead to death. Despite the availability of a variety of antiviral medications to treat this illness, mainly characterized by virostatic drugs that alter DNA replication, their use is often debated. Phytotherapeutic treatments are a little-explored field for FHV-1 infections and reactivations. In this scenario, natural compounds could provide several advantages, such as reduced side effects, less resistance and low toxicity. The purpose of this study was to explore the potential inhibitory effects of the green tea extract (GTE), consisting of 50% of polyphenols, on FHV-1 infection and reactive oxygen species (ROS) production.

Results Crandell-Reese feline kidney (CRFK) cells were treated with different doses of GTE (10–400 µg/mL) during the viral adsorption and throughout the following 24 h. The MTT and $TCID_{50}$ assays were performed to determine the cytotoxicity and the EC_{50} of the extract, determining the amounts of GTE used for the subsequent investigations. The western blot assay showed a drastic reduction in the expression of viral glycoproteins (i.e., gB and gI) after GTE treatment. GTE induced not only a suppression in viral proliferation but also in the phosphorylation of Akt protein, generally involved in viral entry. Moreover, the increase in cell proliferation observed in infected cells upon GTE addition was supported by enhanced expression of Bcl-2 and Bcl-xL anti-apoptotic proteins. Finally, GTE antioxidant activity was evaluated by dichloro-dihydro-fluorescein diacetate (DCFH-DA) and total antioxidant capacity (TAC) assays. The ROS burst observed during FHV-1 infection was mitigated after GTE treatment, leading to a reduction in the oxidative imbalance.

Conclusions Although further clinical trials are necessary, this study demonstrated that the GTE could potentially serve as natural inhibitor of FHV-1 proliferation, by reducing viral entry. Moreover, it is plausible that the extract could inhibit apoptosis by modulating the intrinsic pathway, thus affecting ROS production.

Keywords Feline Herpesvirus type-1, Green tea extract, ROS, Antiviral

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Background

Herpesvirus infections are frequently reported for both humans and animals, with different degrees of severity. Feline Herpesvirus type-1 (FHV-1) is one of most common viruses associated with respiratory disease in domestic and wild felids [1]. Cats of every age and state of health are widely exposed, reaching prevalence rates of up to 97% [2]. FHV-1 is a double strand DNA virus belonging to Alphaherpesvirinae family that replicates in the epithelial cells of both conjunctiva and the upper respiratory tract [3]. Moreover, its tropism for neuronal cells causes lifelong infections in a latent phase (typically in trigeminus ganglia), with the potential of periodic reactivation, thus ensuring its survival in the host [3, 4].

FHV-1 is generally self-resolving in immune-competent cats but could lead to death in immune-depressed and debilitated ones (as cats with chronic diseases and with comorbidities). Significant repercussions can also be observed in kittens that have not completed the vaccination cycle or in wild cats not adequately weaned and, therefore, lacked colostral immunity [5].

Transmission among infected cats and susceptible ones mainly occurs by oral and nasal routes. Typical clinical signs, including sneezing, fever, ocular/nasal secretions (purulent in case of secondary bacterial infection) could be accompanied by oral and skin ulcers, and dermatitis [6]. In severe cases, FHV-1 pathogenic mechanism could lead to blindness, as well as chronic rhinosinusitis and neurological signs [7]. Moreover, this infection is also often associated with feline calicivirus (FCV) and other pathogens, causing multi-agent respiratory syndrome and worsening the clinical picture [8].

Despite the existence of safe and effective vaccines that should be boosted annually to at-risk cats (3 yearly intervals for indoor ones), FHV-1 continues to cause outbreaks, especially in kittens. According to specific guidelines, a supportive treatment consisting of fluids/electrolytes administered by systemic route and broad-spectrum antibiotics to prevent bacterial secondary infections, as well as eye drops/ointments are recommended [8].

The most effective drugs for FHV-1 treatment are characterized by virostatic DNA analogues. These compounds work as DNA polymerase inhibitors and affect chain termination of DNA replication [9]. Although a wide panel of antiviral treatments are available, famciclovir and cidofovir are the first-line treatment strategies to administer in cats with severe FHV-1. Nevertheless, these drugs' effectiveness is doubtful since treatments for FHV-1 infection frequently rely on in vitro research, case-based reports, and veterinarian's personal experience [5]. The ability of viruses to undergo mutations represents a further obstacle in fighting viral infections due to the development of drug resistance [10, 11].

Substances of natural origin have the potential to serve as an alternative to conventional pharmaceuticals in cases of resistance/ineffectiveness, also offering a complement in pharmacological therapy [12]. In recent years, natural remedies derived from medicinal plants have frequently been used for their antiviral activity against many infectious diseases, including herpes simplex virus (HSV), even without any scientific evidence [13].

Green tea-based phytocomplexes, commonly processed from the *Camellia sinensis* plant, are known to possess a wide range of antibacterial and anti-inflammatory properties, due to their polyphenols content known as catechins [14, 15]. Among them, (–)-epigallocatechin gallate (EGCG) has proven to interfere with the viral entry of the human immunodeficiency virus (HIV), HSV, influenza virus, as well as hepatitis C virus (HCV) [16–20]. Recent evidence suggests that viral infections often trigger oxidative stress (OS) in the host cells (as a result of antiviral immune system activation) and contemporary reduces its antioxidant defence [21]. Moreover, increased OS also contributes to the pathogenesis of viral infections as respiratory ones and influenza [22, 23]. Therefore, green tea compounds could be beneficial in enhancing antioxidant defences, thus contributing to preserving cellular health.

Currently, the field of natural remedies against FHV-1 infection is poorly investigated. Therefore, the novelty of this study stands in the investigation of the in vitro effectiveness of GTE against FHV-1.

Results

Green tea extract (GTE) reduces viral titer during FHV-1 infection

CRFK cells were exposed to seven different concentrations of GTE. No significant reduction in cell viability was observed at 10, 25, 50, 75, 100 and 200 µg/mL of the extract, except for the highest dose tested (400 µg/mL, $p<0.05$) (Fig. 1A).

The same GTE concentrations were used during FHV-1 infection for 24 h. Under these conditions, antiviral activity evaluated by $TCID_{50}$ was observed at concentrations higher than 89.59 µg/mL, as evidenced by $EC_{50}=89.59$ µg/mL (Fig. 1B). Real-time PCR on thymidine kinase (TK) gene, involved in the early phases of viral replication, confirmed the efficacy of GTE in reducing viral titers, especially at 100, 200 and 400 µg/mL of GTE ($p<0.0001$, Fig. 1C). As a result of the decrease in the viability when cells were exposed to the highest dose of GTE and due to EC_{50} value, subsequent experiments were carried out using 100 and 200 µg/mL of GTE.

GTE reduces FHV-1-induced apoptosis

In order to assess the efficacy of GTE to protect FHV-1 infected cells from apoptosis, western blot assay was

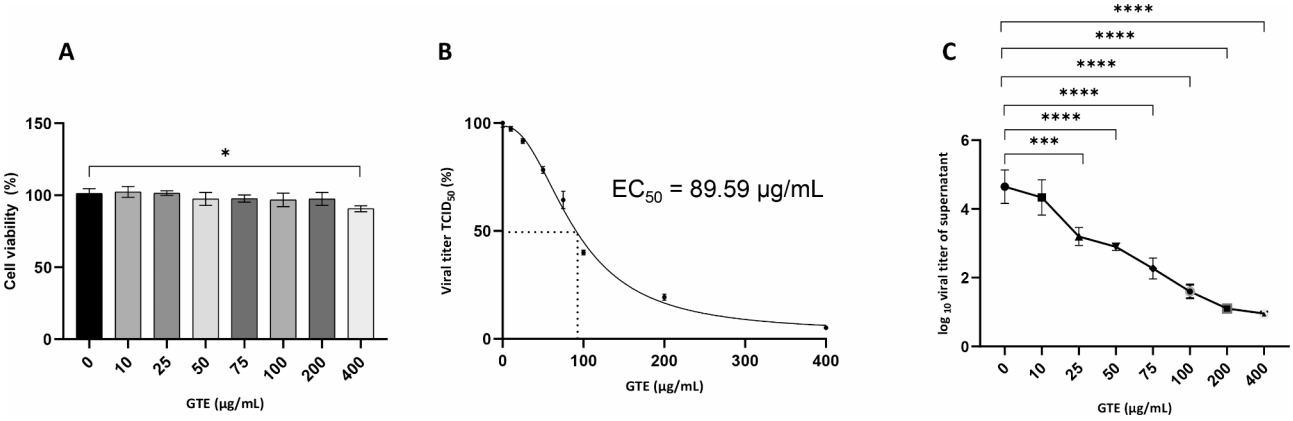

Fig. 1 Antiviral activity of Green tea extract (GTE) against Feline Herpesvirus type-1 (FHV-1). **(A)** CRFK cell viability treated with increasing concentration of GTE; **(B)** EC_{50} of GTE calculated by $TCID_{50}$ assay; **(C)** Real time PCR of FHV-1's thymidine kinase gene conducted on supernatants. (*$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$)

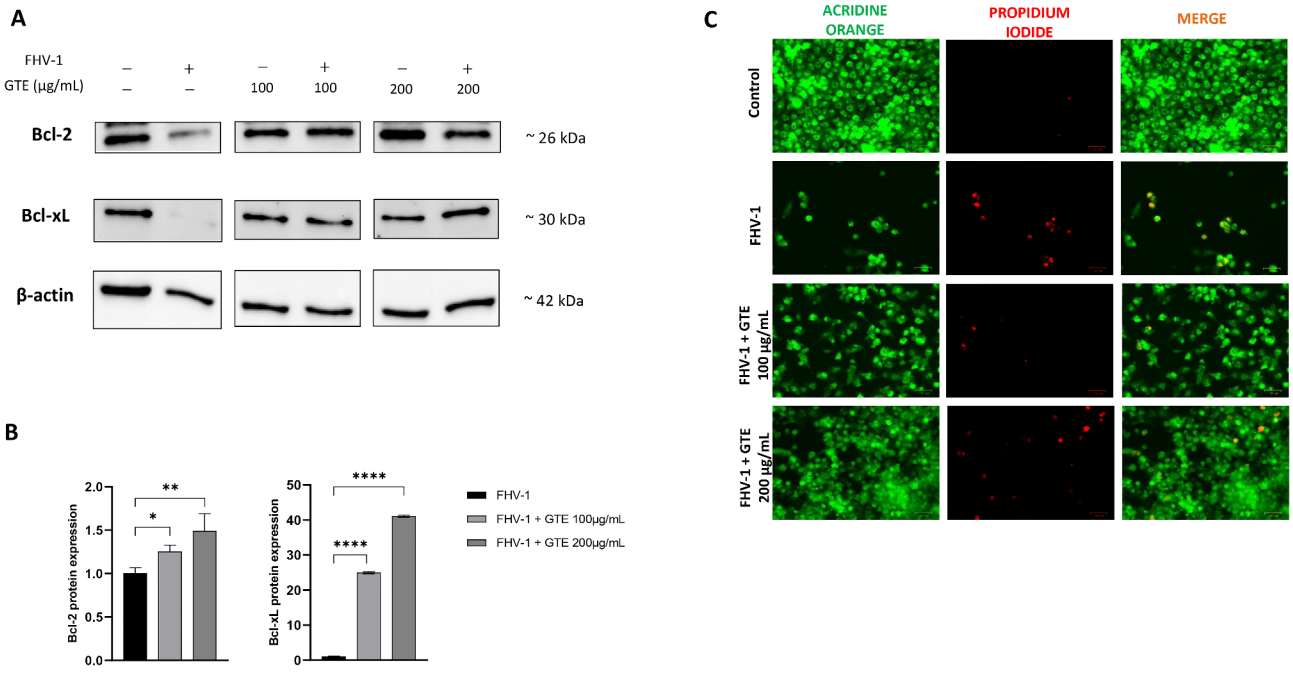

Fig. 2 Green tea extract (GTE) activates the anti-apoptotic pathway during FHV-1 infection. **(A)** Representative western images of Bcl-2, Bcl-xL, and β-actin; **(B)** Densitometric analysis of Bcl-2 and Bcl-xL protein expression normalised toward β-actin. A representative actin has been inserted into the figure. Individual actins from each membrane, as well as full-size membranes, are available in the Supplementary Material. Results were expressed as means ± SD from three independent experiments (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$); (C) Acridine orange (green) and Propidium iodide (red) dual stain, scale bar = 50 µm. Whole blots are available in the Supplementary file (Additional file 1)

performed to evaluate the expression of two anti-apoptotic markers, Bcl-2 and Bcl-xL (Fig. 2A and B), together with Acridine orange (AO)/Propidium Iodide (PI) dual stain (Fig. 2C). Infected cells treated with 200 µg/mL of GTE showed a marked increase in the expression of both Bcl-2 and Bcl-xL proteins ($p < 0.01$ and $p < 0.0001$, Fig. 2B). Regarding the treatment with 100 µg/mL of GTE, a statistically significant increase in Bcl-xL was found ($p < 0.0001$), together with a similar a tendency in the case of Bcl-2 ($p < 0.05$) (Fig. 2B).

PI, which is taken up by nonviable cells and the ones with damaged membranes, was markedly observed in almost all cells infected with FHV-1. Indeed, infected cells exhibited a significant red staining compared to the overall population, indicating an apoptotic/necrotic response to FHV-1 stimulus. However, treatment with GTE at both tested doses increased the vital cell population, thus resulting in markedly less apoptotic cells with respect to their total number.

GTE limits viral entry during FHV-1 infection

To assess whether GTE compromises viral entry and replication, western blot assay on specific proteins was performed. In particular, Akt 1/ 2 and p-Akt involved in viral entry, along with the viral glycoproteins gB and gI (Fig. 3A). GTE showed a significant decrease in p-Akt, gB and gI protein expression ($p<0.0001$) after 24 h of treatment compared to the FHV-1 infected cells (Fig. 3B).

The results from immunofluorescence supported the previous outcomes, indicating the effective suppression of viral replication by GTE. This result was evident from the lower green fluorescence observed in infected cells treated with GTE compared to the FHV-1 infected ones (Fig. 3C).

GTE protects FHV-1 infected cells against ROS production

Since polyphenols in GTE have notable antioxidant capabilities, two distinct assays, namely DCFH-DA and total antioxidant capacity (TAC) assays, were used to detect reactive oxygen species (ROS). When exposed to a high concentration of ROS, DCFH-DA undergoes oxidation and transforms into the green fluorescent dye known as 2'-7'dichlorofluorescein (DCF). During FHV-1 infection, cells exhibited high levels of DCF, indicating an abundance of reactive oxygen species (ROS). In contrast, treatment with GTE at concentrations of 100 and 200 µg/mL resulted in the restoration of fluorescence intensity to levels comparable to the control (Fig. 4A and B). Simultaneously, the oxidative environment caused by FHV-1 infection significantly decreased the Trolox concentration ($p<0.01$), i.e. the antioxidant standard used for TAC assay, and, as a result, exhibited an increased fluorescence ($p<0.01$). The administration of GTE led to an increase in nmols of Trolox, suggesting a recovery in the antioxidant equilibrium in the infected cells to those observed the control groups (Fig. 4C).

Discussion

Despite the existence of vaccinations to control viral spreading and mitigate the severe consequences of infection, FHV-1 remains prevalent among cats. Although a wide panel of antivirals is available, their efficacy is considered controversial [5]. In this scenario, the adoption of natural treatments could represent a valid option to extend the variety of therapeutic alternatives. This strategy may improve the safety and tolerability of the antiviral therapy [12].

Green tea-based products are one of the most used natural compounds employed for their biological activities and have been recognised for their medical applications

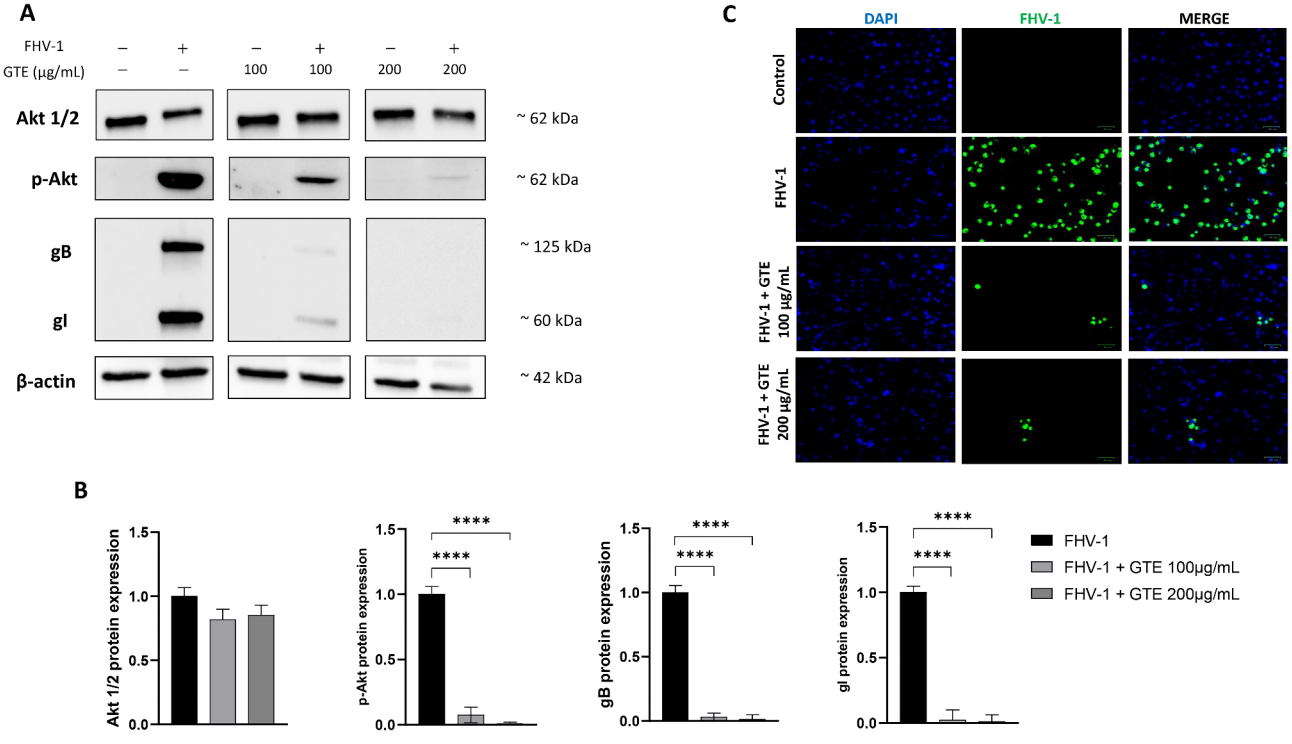

Fig. 3 Green tea extract (GTE) reduces Feline Herpesvirus type-1 entry. **(A)** Representative western images of Akt 1/2, p-Akt, gB, gI, and β-actin; **(B)** Densitometric analysis of p-Akt, gB, and gI protein expression normalised toward β-actin. A representative actin has been inserted into the figure. Individual actins from each membrane, as well as full-size membranes, are available in the Supplementary Material. Results were expressed as means ± SD from three independent experiments (****$p<0.0001$); (C) Immunofluorescence staining of FHV-1 (green) and cell nuclei (DAPI, blue), scale bar = 50 µm. Whole blots are available in the Supplementary file (Additional file 1)

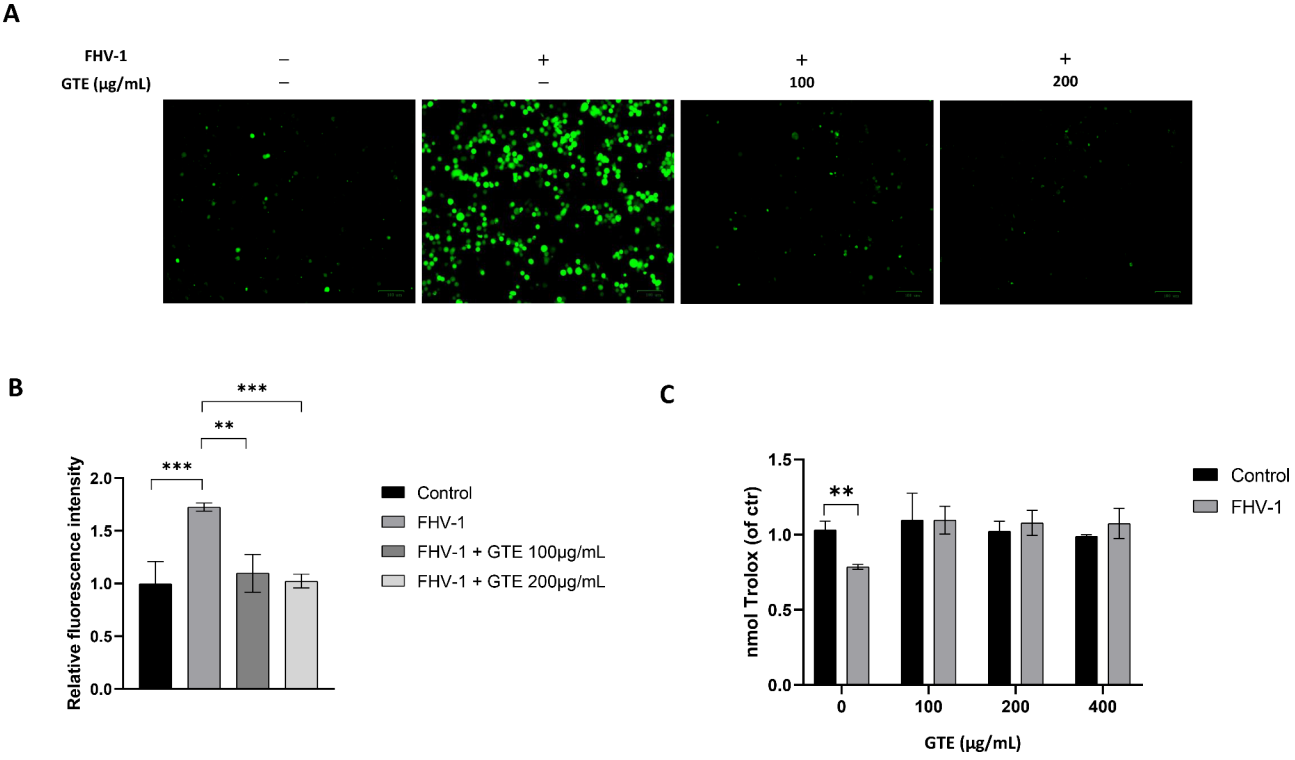

Fig. 4 Green tea extract (GTE) limits ROS production during Feline Herpesvirus type-1 (FHV-1) infection. **(A)** DCFH-DA (2',7'-Dichlorofluorescin diacetate) stain, scale bar = 100 µm; **(B)** Fluorescence intensity quantification of DCFH-DA stain; **(C)** Total antioxidant capacity (TAC) relative to nmol of Trolox. (** $p < 0.01$; *** $p < 0.001$)

for different type of diseases [15, 24]. The antiviral efficacy of the green tea polyphenols against HSV-1, belonging to the same family as FHV-1, has already been proven to effectively block HSV-1 viral particles without influencing Vero cells viability [25]. Phytotherapeutics have not been extensively investigated for FHV-1 treatment despite their widespread use against several human viruses. In the current study, GTE reduced virus proliferation, resulting well tolerated by CRFK cells when supplied with doses lower than 400 µg/mL. Moreover, GTE significantly reduced the viral titers, calculated by $TCID_{50}$, obtaining the EC_{50} around 90 µg/mL.

In this research two pathways' modifications have been investigated to highlight GTE interactions with FHV-1 infection, i.e. apoptosis and PI3K (phosphatidylinositol 3-kinase)/Akt /mechanistic target of rapamycin (mTOR) axis.

Virus infections trigger different types of programmed cell death through distinct signalling pathways, some of them involving caspases activation and thus leading to apoptosis and pyroptosis, others relying on necroptosis and ferroptosis [26]. According to the recent literature, FHV-1 is known to induce apoptosis by down-regulating Bcl-2 and Bcl-xL proteins expression over the 24 h post-infection [27]. The expression of the above-described markers was comparable to control cells when GTE was supplemented. These beneficial effects of GTE may be ascribed to the modulation of the mitochondrial intrinsic pathway of apoptosis. In fact, the Bcl-2 protein family is widely recognised for its role in regulating the intrinsic pathway, with a significant influence on both cell survival and endoplasmic reticulum stress response [28]. Bcl-2 protein family regulates mitochondrial membrane permeabilization, influenced by oxidative balance [29]. In particular, Bcl-2 and Bcl-xL (Bcl-2 family members) inhibit apoptosis by preventing mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release [30]. In this study, an overall rise in oxidative stress was detected in FHV-1 infected cells, accompanied by a decrease in the total antioxidant activity. This condition could compromise both organelles and cell viability, predisposing to death. However, the oxidative damage induced by FHV-1 was lower in GTE treated cells. The antiviral properties of GTE are mostly related to the capacity of polyphenols to function as antioxidants, preserving cellular membranes and inhibiting the binding/invasion of viruses into cells [31]. Therefore, we hypothesise a link between GTE antioxidant activity, and its beneficial effects on cell viability. Indeed, the increased expression of Bcl-2 and Bcl-xL proteins in cells treated with GTE suggests a mitigation of ROS accumulation. Preserving the imbalance between generation and

scavenging of ROS, frequently associated with viral infections [21], is crucial for cellular processes like mitochondrial energy production, host defence, cellular signaling, and the regulation of gene expression [32–34].

Another pathway involved in FHV-1 pathogenesis and modified by GTE was PI3K/Akt/mTOR axis. Recently, its role in FHV-1 entry has been established [35]. In particular, the entry of FHV-1 has been related to the phosphorylation of Akt (p-Akt), that was already observed within few hours post-infection. In the present study, accordingly, infected cells exhibited the over expression of p-Akt (as a result of viral entry and its replication). Furthermore, a dose-dependent disappearance of Akt phosphorylation was detected when GTE was used. This was accompanied by a significant reduction in the expression of viral glycoproteins involved in intra-cellular spreading and fusion, i.e. gB and gI, highlighting the in vitro effectiveness of GTE to contrast viral entry and replication. Further evidence of reduced viral replication in GTE-treated cells was observed in immunofluorescence and real-time PCR. Specifically, we observed reduced levels of TK expression in GTE-treated cells, which in turn led to decreased expression in infected cells. While p-Akt was modulated, Akt expression did not exhibit modification following the treatment, indicating that this protein was not influenced by GTE. This outcome may probably be attributed to the GTE effects on FHV-1 entry. According to the literature, GTE activity could be attributed to two main catechins contained in the green tea, the EGCG and (−)-epicatechin gallate (ECG). In fact, these compounds have already been found to successfully inhibit the proliferation of influenza virus, by altering its viral membrane, and its subtypes [19], as well as Zika virus [36] and chikungunya virus in vitro [37]. Accordingly, it is hypothesised that EGCG and other phenolic compounds contained in the green tea act as antiviral agents by binding the virus and disturbing the endocytic pathway, so limiting the virus's ability to enter cells in the early phases of infection [38]. In fact, it's possible that the GTE polyphenols interfere with heparan sulfate activity of FHV-1, similarly to EGCG effects on HSV-1, influenza virus, HCV, HIV, and many others [39, 40].

Therefore, the hypothesis regarding the mechanism of action of GTE could be dualistic: on the one hand, GTE may counteract the viral entry thanks to the presence of catechins; on the other hand, it is plausible that the extract could inhibit the apoptosis modulating the intrinsic pathway by reducing the oxidative imbalance.

Although these results are encouraging, it is necessary to consider GTE pharmacokinetics in vivo. Previous studies have shown that catechins, and particularly EGCG, exhibit chemical instability, low membrane permeability, as well as rapid metabolism by the organism. As a consequence, their bioavailability is limited [40]. However, the use of the GTE phytocomplex itself can work to improve the stability and synergize and/or potentiate the pharmacological effects of ECGC. Numerous strategies have been improved to address this issue, including the use of delivery methods that can enhance stability [41].One of them has been the synthesis of a lipophilic EGCG derivative with higher affinity towards the cellular membrane [42]. Nevertheless, the possibility of using this extract for topical use or conveyed in the form of ophthalmic drops is not excluded. Therefore, toxicokinetic studies in cats are necessary to assess GTE safety when administered orally and applied at the corneal level.

The search for new compounds, both synthetic and natural, against viruses is a topic of debate very important to counteract viral spreading [43–45]. In fact, apart from a supportive therapy to control symptoms, the effectiveness of antivirals in feline medicine is still debated [5]. In this perspective, natural substances as GTE could be considered an effective alternative therapy against FHV-1 infection, providing usefulness in future pharmaceutical veterinary applications. also contributing to expanding antiviral mechanisms of action to counteract resistance as well as latency.

Conclusion

This study has shown that the tested GTE effectively limits the proliferation of FHV-1 within the host cell in vitro. The mechanism by which this inhibition occurs is probably related to the antioxidant properties of this extract to counteract the apoptotic intrinsic pathway during FHV-1 infection. Thus, natural anti-infective compounds green tea-based might pave the way for defeating the problem of drug resistance as well as provide effective and safe treatment of infections caused by FHV-1.

Methods

Cell culture, viral infection and GTE treatment

Crandell-Rees Feline Kidney (CRFK, ATCC) cells were cultured at 37 °C in 5% CO_2 atmosphere in Dulbecco's Modified Eagle's Medium (DMEM, Corning) containing 10% fetal bovine serum (FBS) and 1% antibiotics (Penicillin-Streptomycin Solution, Corning). FHV-1 strain Ba/91 (MOI 0.5) was added to the permissive cells at 80% confluency. After 2 h adsorption, medium containing virus was replaced by DMEM with FBS 5% and incubated for 24 h. Green tea extract (GTE) with 50% polyphenols (Italfeed) produced by *Camellia sinensis* was dissolved in DMEM, added to the cells, and kept in the medium during the whole experiment, at ascending concentrations (10, 25, 50, 75, 100, 200 and 400 µg/mL), except in the Control cells.

Cytotoxicity evaluation

Cytotoxicity of GTE was assessed by measuring mitochondrial metabolic activity by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) assay, which was conducted in the previously described conditions in 96-well plates [46]. Briefly, MTT powder was dissolved in Roswell Park Memorial Institute (RPMI) medium (0.5 mg/mL), added to each well (100 µL) and incubated at 37 °C. After 4 h, 100 µL of dimethylsulfoxide (DMSO) were added in each well and the plate incubated at 37 °C for 10 min. MTT conversion in formazan was assessed measuring the absorbance at 570 nm using a microplate spectrophotometer (Thermo Fisher Scientific).

Viral replication assessment ($TCID_{50}$ and real time PCR)

After incubation with FHV-1 and ascending concentrations of GTE, the supernatants of each experiment were collected and the Reed-Muench method was used to calculate the viral titer. The obtained data were used to calculate the EC_{50} by GraphPad Prism software. Doses higher than EC_{50} and not significatively influencing the cell viability, were used for the subsequent experiments.

Moreover, DNA isolated from aliquots belonging to the same supernatants, were used for gene expression analysis. A SYBR-green (Bio-Rad) real-time PCR amplifying the TK gene (forward primer: 5' TGTCCGCATTTACA TAGATGG 3'; reverse primer: 5'GGGGTGTTCCTCA CATACAA 3'), was carried out using the CFX96 Touch real-time PCR detection system (Bio-Rad) [47, 48]. Gene expression was measured using a standard curve generated by serial dilution of crude viral DNA.

Western blot analysis

Cell pellets from the experiments previously described were lysed with RIPA buffer supplemented with protease and phosphatase inhibitors (Roche). Equal amounts of total protein (25 µg) were loaded, separated on pre-cast acrylamide gels (Bio-Rad), and blotted on nitrocellulose membranes (Bio-Rad). These latter were incubated overnight with a panel of primary antibodies (1:1000): FHV-1 (Novus biological), Akt (Santa Cruz), p-Akt (Cell Signaling Technology), Bcl-2 (Abcam), Bcl-xL (Cell Signaling Technology), β-Actin (Santa Cruz), and then incubated for 1 h with secondary peroxidase-conjugated antibodies according to the species of the primary ones. Protein bands were detected with a ChemiDoc Imaging System (Bio-Rad) and protein expression levels were assessed by densitometric analysis using Image Lab software (Bio-Rad).

Immunofluorescence

After fixation and permeabilization of cell monolayers properly treated, primary antibody against FHV-1 (Novus Biologicals, mouse monoclonal) was incubated at +4 °C overnight (dilution 1:400). Secondary labelled antibody (Anti-mouse secondary antibody, Alexa Fluor 488) was incubated for 1 h at room temperature (dilution 1:800). Cell nuclei were stained with 4′,6-diamidin-2-fenilindolo (DAPI; Vector Laboratories). ZOE Fluorescent Cell Imaging System (Bio-Rad) was used for images acquisition.

Acridine Orange (AO)/Propidium iodide (PI) staining

Acrydine orange (AO)/propidium iodide (PI) double staining was used to measure apoptosis. After treatments, cell monolayers were washed with PBS (1X), fixed, and incubated with a mix of Acridine orange hemi (zinc chloride) salt (Sigma) and PI (Thermo Fisher) for 15 min. After 3 washes with PBS (1X), images were acquired by ZOE Fluorescent Cell Imaging System (Bio-Rad).

DCFH-DA (2′,7′-Dichlorofluorescin diacetate) assay

Cell monolayers, appropriately treated, were incubated with DCFH-DA (10 µM) at 37 °C. After 30 min, they were washed twice with DMEM and once with PBS (1X). Images were acquired with ZOE Fluorescent Cell Imaging System (Bio-Rad). Subsequently, same cell monolayers were incubated on ice with RIPA buffer and centrifuged at 21,130 x *g* for 10 min at 4 °C. Fluorescence intensity (λex=485 nm; λem=530 nm) was measured in supernatants and normalised to total protein concentration.

Statistical analysis

All experiments were performed in triplicates. The GraphPad software version 8.0.2 was used for statistical analysis. Statistical differences among group were evaluated by ordinary one-way ANOVA. Values of $p<0.05$ were considered statistically significant.

Abbreviations

FHV-1	Feline Herpesvirus type-1
GTE	Green tea extract
ROS	Reactive oxygen species
CRFK	Candrell-Rees Feline Kidney
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazolium bromide
TCID50	Tissue Culture Infectious Dose 50
gB	Glycoprotein B
gI	Glycoprotein I
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
DCHF-DA	Dichloro-dihydro-fluorescein diacetate
TAC	Total antioxidant capacity
FCV	Feline calicivirus
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HCV	Hepatitis C virus
EGCG	(−)-Epigallocatechin gallate
OS	Oxidative stress
PCR	Protein chain reaction
TK	Thymidine kinase
AO	Acridine Orange
PI	Propidium Iodide

PBS	Phosphate-buffered saline
ECG	(–)-Epicatechin gallate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04227-0.

Supplementary Material 1

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Author contributions
Conceptualization, C.L., S.D., and R.C.; methodology, C.L., and G.F.; formal analysis, C.L., and S.D.; investigation, C.L., G.F., and R.E; resources, S.D., and R.C.; data curation, C.L., and S.D.; writing original draft preparation, C.L.; writing—review and editing, C.L.; visualization, S.M., and S.F; supervision, S.D., and R.C.; project administration, S.D., and R.C.; funding acquisition, S.D., and R.C. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Fernandez M, Manzanilla EG, Lloret A, León M, Thibault J-C. Prevalence of feline herpesvirus-1, feline calicivirus, Chlamydophila felis and Mycoplasma felis DNA and associated risk factors in cats in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis. J Feline Med Surg. 2017;19(4):461–9.
2. Amoroso MG, Serra F, Miletti G, Cardillo L, de Martinis C, Marati L et al. A retrospective study of viral molecular prevalences in cats in Southern Italy (Campania Region). Viruses 2022, 14(11).
3. Gaskell R, Dawson S, Radford A, Thiry E. Feline herpesvirus. Vet Res. 2007;38(2):337–54.
4. Townsend WM, Jacobi S, Tai SH, Kiupel M, Wise AG, Maes RK. Ocular and neural distribution of feline herpesvirus-1 during active and latent experimental infection in cats. BMC Vet Res. 2013;9:185.
5. Gould D. Feline herpesvirus-1: ocular manifestations, diagnosis and treatment options. J Feline Med Surg. 2011;13(5):333–46.
6. Gaskell RM, Povey RC. Transmission of feline viral rhinotracheitis. Vet Rec. 1982;111(16):359–62.
7. Stiles J. Ocular manifestations of feline viral diseases. Veterinary J (London England: 1997). 2014;201(2):166–73.
8. Walter J, Foley P, Yason C, Vanderstichel R, Muckle A. Prevalence of feline herpesvirus-1, feline calicivirus, Chlamydia felis, and Bordetella bronchiseptica in a population of shelter cats on Prince Edward Island. Can J Veterinary Res = Revue canadienne de recherche veterinaire. 2020;84(3):181–8.
9. Galle LE. Antiviral therapy for ocular viral disease. Veterinary Clin North Am Small Anim Pract. 2004;34(3):639–53.
10. Piret J, Boivin G. Antiviral resistance in herpes simplex virus and varicella-zoster virus infections: diagnosis and management. Curr Opin Infect Dis. 2016;29(6):654–62.
11. Lampejo T. Influenza and antiviral resistance: an overview. Eur J Clin Microbiol Infect Diseases: Official Publication Eur Soc Clin Microbiol. 2020;39(7):1201–8.
12. Nabi F, Shi D, Wu Q, Baloch DM, Editorial. Treatment of animal diseases with veterinary phytotherapy. Front Veterinary Sci. 2023;10:1171987.
13. Thompson KD. Herbal extracts and compounds active against herpes simplex virus. In: *Advances in Phytomedicine. Volume 2*, edn. Edited by Khan MTH, Ather A: Elsevier; 2006: 65–86.
14. Renzetti A, Betts JW, Fukumoto K, Rutherford RN. Antibacterial green tea catechins from a molecular perspective: mechanisms of action and structure-activity relationships. Food Funct. 2020;11(11):9370–96.
15. Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. Beneficial Properties of Green Tea Catechins. Int J Mol Sci 2020, 21(5).
16. Calland N, Albecka A, Belouzard S, Wychowski C, Duverlie G, Descamps V, et al. (-)-Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry. Hepatology (Baltimore MD). 2012;55(3):720–9.
17. Isaacs CE, Wen GY, Xu W, Jia JH, Rohan L, Corbo C, et al. Epigallocatechin gallate inactivates clinical isolates of herpes simplex virus. Antimicrob Agents Chemother. 2008;52(3):962–70.
18. Akram M, Tahir IM, Shah SMA, Mahmood Z, Altaf A, Ahmad K, et al. Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis, and coxsackievirus: a systematic review. Phytother Res. 2018;32(5):811–22.
19. Song JM, Lee KH, Seong BL. Antiviral effect of catechins in green tea on influenza virus. Antiviral Res. 2005;68(2):66–74.
20. Zhao Y, Jiang F, Liu P, Chen W, Yi K. Catechins containing a galloyl moiety as potential anti-HIV-1 compounds. Drug Discovery Today. 2012;17(11–12):630–5.
21. Amini MA, Karimi J, Talebi SS, Piri H. The Association of COVID-19 and Reactive Oxygen Species Modulator 1 (ROMO1) with oxidative stress. Chonnam Med J. 2022;58(1):1–5.
22. Haque MM, Murale DP, Lee JS. Role of microRNA and Oxidative Stress in Influenza A Virus Pathogenesis. *International journal of molecular sciences* 2020, 21(23).
23. Chen AC, Burr L, McGuckin MA. Oxidative and endoplasmic reticulum stress in respiratory disease. Clin Translational Immunol. 2018;7(6):e1019.
24. Kochman J, Jakubczyk K, Antoniewicz J, Mruk H, Janda K. Health benefits and Chemical composition of Matcha Green Tea: a review. Molecules 2020, 26(1).
25. de Oliveira A, Adams SD, Lee LH, Murray SR, Hsu SD, Hammond JR, et al. Inhibition of herpes simplex virus type 1 with the modified green tea polyphenol palmitoyl-epigallocatechin gallate. Food Chem Toxicology: Int J Published Br Industrial Biol Res Association. 2013;52:207–15.
26. Verburg SG, Lelievre RM, Westerveld MJ, Inkol JM, Sun YL, Workenhe ST. Viral-mediated activation and inhibition of programmed cell death. PLoS Pathog. 2022;18(8):e1010718.
27. Ferrara G, Longobardi C, Sgadari MF, Restucci B, Iovane G, Ciarcia R, et al. Apoptosis is mediated by FeHV-1 through the intrinsic pathway and interacts with the autophagic process. Virol J. 2023;20(1):295.
28. Hatok J, Racay P. Bcl-2 family proteins: master regulators of cell survival. Biomol Concepts. 2016;7(4):259–70.
29. Wu CC, Bratton SB. Regulation of the intrinsic apoptosis pathway by reactive oxygen species. Antioxid Redox Signal. 2013;19(6):546–58.
30. Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 2008;18(4):157–64.
31. Friedman M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res. 2007;51(1):116–34.
32. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol. 2011;194(1):7–15.
33. Sun L, Wang X, Saredy J, Yuan Z, Yang X, Wang H. Innate-adaptive immunity interplay and redox regulation in immune response. Redox Biol. 2020;37:101759.
34. Sinha K, Das J, Pal PB, Sil PC. Oxidative stress: the mitochondria-dependent and mitochondria-independent pathways of apoptosis. Arch Toxicol. 2013;87(7):1157–80.
35. Ferrara G, Longobardi C, Damiano S, Ciarcia R, Pagnini U, Montagnaro S. Modifications of the PI3K/Akt/mTOR axis during FeHV-1 infection in permissive cells. Front Veterinary Sci. 2023;10:1157350.
36. Carneiro BM, Batista MN, Braga ACS, Nogueira ML, Rahal P. The green tea molecule EGCG inhibits Zika virus entry. Virology. 2016;496:215–8.

37. Weber C, Sliva K, von Rhein C, Kümmerer BM, Schnierle BS. The green tea catechin, epigallocatechin gallate inhibits Chikungunya virus infection. Antiviral Res. 2015;113:1–3.
38. Vázquez-Calvo Á, Jiménez de Oya N, Martín-Acebes MA, Garcia-Moruno E, Saiz JC. Antiviral properties of the Natural polyphenols Delphinidin and Epigallocatechin Gallate against the Flaviviruses West Nile Virus, Zika Virus, and Dengue Virus. Front Microbiol. 2017;8:1314.
39. Colpitts CC, Schang LM. A small molecule inhibits virion attachment to heparan sulfate- or sialic acid-containing glycans. J Virol. 2014;88(14):7806–17.
40. Maeda K, Yokoyama N, Fujita K, Maejima M, Mikami T. Heparin-binding activity of feline herpesvirus type 1 glycoproteins. Virus Res. 1997;52(2):169–76.
41. Dai W, Ruan C, Zhang Y, Wang J, Han J, Shao Z, et al. Bioavailability enhancement of EGCG by structural modification and nano-delivery: a review. J Funct Foods. 2020;65:103732.
42. Minnelli C, Galeazzi R, Laudadio E, Amici A, Rusciano D, Armeni T, et al. Monoalkylated Epigallocatechin-3-gallate (C18-EGCG) as Novel Lipophilic EGCG Derivative: characterization and antioxidant evaluation. Antioxidants. 2020;9(3):208.
43. Di Micco S, Rahimova R, Sala M, Scala MC, Vivenzio G, Musella S, et al. Rational design of the zonulin inhibitor AT1001 derivatives as potential anti SARS-CoV-2. Eur J Med Chem. 2022;244:114857.
44. Sumon MAA, Asseri AH, Molla MHR, Aljahdali MO, Hasan MR, Rahman MA, et al. Identification of natural antiviral drug candidates against Tilapia Lake Virus: computational drug design approaches. PLoS ONE. 2023;18(11):e0287944.
45. Cerracchio C, Del Sorbo L, Serra F, Staropoli A, Amoroso MG, Vinale F, et al. Fungal metabolite 6-pentyl-α-pyrone reduces canine coronavirus infection. Heliyon. 2024;10(6):e28351.
46. Forte IM, Indovina P, Montagnaro S, Costa A, Iannuzzi CA, Capone F et al. The Oncolytic Caprine Herpesvirus 1 (CpHV-1) induces apoptosis and synergizes with cisplatin in Mesothelioma Cell lines: a new potential Virotherapy Approach. Viruses 2021, 13(12).
47. Persico P, Roccabianca P, Corona A, Vercelli A, Cornegliani L. Detection of feline herpes virus 1 via polymerase chain reaction and immunohistochemistry in cats with ulcerative facial dermatitis, eosinophilic granuloma complex reaction patterns and mosquito bite hypersensitivity. Vet Dermatol. 2011;22(6):521–7.
48. Ferrara G, Sgadari M, Longobardi C, Iovane G, Pagnini U, Montagnaro S. Autophagy up-regulation upon FeHV-1 infection on permissive cells. Front Veterinary Sci. 2023;10:1174681.

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