جـــامـعـة الـــشـرق الأوسـط
MIDDLE EAST UNIVERSITY
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Evaluation of the Antiviral Potential of Green Tea-Mediated Zinc Oxide Nanoparticles Against Peste des Petits Ruminants Virus (PPRV)

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تقييم الفاعلية المضادة للفيروسات لجسيمات أكسيد الزنك النانوية المحضّرة حيويًا من مستخلص الشاي الأخضر ضد فيروس طاعون المجترات الصغيرة (PPRV)

إعداد

نبأ عباس توفيق العلو

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قُدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير
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Thesis Committee Decision

This Thesis, titled "**Evaluation of The Antiviral Potential of Green Tea-Mediated Zinc Oxide Nanoparticles Against Peste des Petits Ruminants Virus (PPRV)**" By **Naba Abbas Tawfiq Alallo** Researcher Was Successfully Defended and Approved On 02/12/2025.

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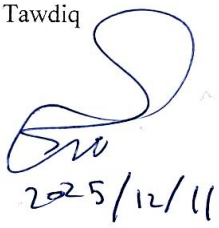

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By The Grace Of Almighty God, I have been Gifted with The Wisdom, Patience and Strength to Accomplish This Task for Which I Thanks. I couldn't do it without His grace and mercy.

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I wish to avail this occasion to present my heartfelt thanks to my educated friends, professors, and others in the organization which provided me with their advice, guidance and support and particularly, my educated friends. I want to devout my achievement to my family. My success is possible because of their trust, care, and support. I was able to gain the strength to live and reach this moment with their blessings and support.2025

Naba ALallo

Dedication

To those who helped and supported me,

To those who instilled in me ambition and nurtured it with love and sacrifice,

To those whose prayers were the reason for my success...

To my father, who sacrificed everything in life for my happiness,

who was a light that shone before me and supported me in times of hardship -

You were my inspiration, my guide, and my support.

Thanks to your faith in me, I was able to achieve this accomplishment.

To my mother, the compassionate and loving heart,

whose prayers paved the way for my success,

whose countless care and sacrifices transformed me into who I am today -

Nothing would have been possible without you.

Dedicated to my loving family, who have always showered me with warmth, tenderness, and support.

You were the light that illuminated my path and the hands that lifted me up above every challenge.

With all my love and gratitude, I dedicate this work to my parents and family, praying to God to bless you and reward you for your sacrifices, and to make you a beacon for me on my journey through life and knowledge.

2025

Naba ALallo

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List of Abbreviations

ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ANOVA	Analysis of Variance
CD150	Cluster of Differentiation 150 (SLAM receptor)
CPE	Cytopathic Effect
DMEM	DMEM – Dulbecco's Modified Eagle Medium
DNA	DNA – Deoxyribonucleic Acid
EGCG	EGCG – Epigallocatechin-3-gallate
FAO	FAO – Food and Agriculture Organization
FBS	Fetal Bovine Serum
GT	Green Tea
GT-Zno-NPs	Green Tea-Synthesize Zinc oxide Nanoparticles
HCV	Hepatitis C Virus
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HSV	Herpes Simplex Virus
IC50	Half Maximal Inhibitory Concentration
MOI	Multiplicity of Infection
NPs	Nanoparticles
OIE	World Organisation for Animal Health
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PPE	Personal Protective Equipment
PPR	Peste des Petits Ruminants
PPRV	Peste des Petits Ruminants Virus
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SARS	Severe Acute Respiratory Syndrome
SD	Standard Deviation
SLAM	Signaling Lymphocytic Activation Molecule
SPSS	Statistical Package for the Social Sciences
TCID50	Tissue Culture Infectious Dose 50

USA	United States of America
USD	United States Dollar
UV	Ultraviolet
XRD	X-ray Diffraction
ZnO-NPs	Zinc Oxide Nanoparticles

Evaluation of the Antiviral Potential of Green Tea-Mediated Zinc Oxide Nanoparticles Against Peste des Petits Ruminants Virus (PPRV)

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Mohammad Jamal Saadeh

Abstract

Introduction: Peste des petits ruminants' virus (PPRV) causes high morbidity and mortality in small ruminants, and current live-attenuated vaccines are limited by heat instability and incomplete protection against emerging strains. There is a need for complementary antiviral agents that can be used alongside vaccination in endemic, resource-limited settings. **Objectives:** This study aimed to evaluate the in vitro antiviral activity of green tea–mediated zinc oxide nanoparticles (GT–ZnO NPs) against PPRV. It also sought to characterize their physicochemical properties and explore their predicted safety profile. **Methods:** GT–ZnO NPs were synthesized using green tea extract and characterized by UV–Vis spectroscopy and X-ray diffraction to confirm nanoparticle formation and crystalline structure. Antiviral activity was assessed in Vero cells using virucidal reduction and pre-treatment assays, with viral titers quantified as $TCID_{50}$/mL and IC_{50} estimated from dose–response curves; ADMET predictions were generated using the NanoSolveIT platform. **Results:** GT–ZnO NPs showed a characteristic UV–Vis absorption peak around 350 nm and a wurtzite hexagonal structure with nanoscale crystallite size. Virucidal assays demonstrated a clear, concentration-dependent reduction in PPRV infectivity, with up to ~5-$\log_{10}$ reduction at 25 µg/mL and an IC_{50} of approximately 5.03 µg/mL, whereas pre-treatment of Vero cells produced no significant protection, indicating a primarily direct virus-inactivation mechanism. ADMET modeling suggested moderate absorption, renal clearance, and no predicted hepatotoxicity or genotoxicity at effective concentrations. **Conclusions:** GT–ZnO NPs exhibit potent, dose-dependent virucidal activity against PPRV at relatively low concentrations while maintaining a favorable predicted safety profile. These findings support GT–ZnO NPs as promising candidates for further in vivo evaluation as adjunct antiviral tools in veterinary PPRV control programs

Keywords: antiviral activity, green tea, zinc oxide nanoparticles, PPRV, and in vitro study.

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الملخص

مقدمة: يُسبب فيروس طاعون المجترات الصغيرة (PPRV) معدلات اعتلال ونفوق عالية لدى المجترات الصغيرة، كما أن اللقاحات الحية المُضعفة الحالية محدودة بسبب عدم استقرارها الحراري وعدم اكتمال حمايتها من السلالات الناشئة. هناك حاجة إلى عوامل مضادة للفيروسات مُكملة يُمكن استخدامها مع التطعيم في البيئات الموبوءة محدودة الموارد. الأهداف: هدفت هذه الدراسة إلى تقييم النشاط المضاد للفيروسات في المختبر لجسيمات أكسيد الزنك النانوية المُتحوّطة بالشاي الأخضر (GT-ZnO NPs) ضد فيروس طاعون المجترات الصغيرة. كما سعت إلى توصيف خصائصها الفيزيائية والكيميائية واستكشاف ملف سلامتها المُتوقع. الطريقة: تم تصنيع جسيمات أكسيد الزنك النانوية المُتحوّطة بالشاي الأخضر باستخدام مستخلص الشاي الأخضر، وتمت مقارنتها باستخدام مطيافية الأشعة فوق البنفسجية والمرئية وحيود الأشعة السينية لتأكيد تكوين الجسيمات النانوية وبنيتها البلورية. تم تقييم النشاط المضاد للفيروسات في خلايا فيرو باستخدام اختبارات التخفيض القاتل للفيروسات واختبارات ما قبل المعالجة، حيث تم تحديد عيارات الفيروسات بـ $TCID_{50}/mL$ وIC_{50} المقدرة من منحنيات الاستجابة للجرعة؛ وتم توليد تنبؤات ADMET باستخدام منصة NanoSolveIT. النتائج: أظهرت جسيمات نانوية GT-ZnO ذروة امتصاص مميزة للأشعة فوق البنفسجية والمرئية عند حوالي 350 نانومتر، وبنية سداسية من الوورتزيت بحجم بلورة نانوية. أظهرت الاختبارات القاتلة للفيروسات انخفاضًا واضحًا يعتمد على التركيز في عدوى فيروس PPRV، مع انخفاض يصل إلى $\sim 5-\log_{10}$ عند 25 ميكروغرام/مل، وIC_{50} يبلغ حوالي 5.03 ميكروغرام/مل، في حين لم تُنتج المعالجة المسبقة لخلايا فيرو أي حماية تُذكر، مما يشير إلى آلية تعطيل فيروس مباشرة في المقام الأول. أشارت نمذجة ADMET إلى امتصاص معتدل، وتصفية كلوية، وعدم وجود سمية كبدية أو جينية متوقعة عند التركيزات الفعالة. الاستنتاجات: تُظهر جزيئات أكسيد الزنك النانوية (GT-ZnO) نشاطًا فعالًا مبيدًا للفيروسات يعتمد على الجرعة ضد فيروس طاعون المجترات الصغيرة (PPRV) عند تركيزات منخفضة نسبيًا، مع الحفاظ على مستوى سلامة متوقع إيجابي. تدعم هذه النتائج جزيئات أكسيد الزنك النانوية (GT-ZnO) كمرشحات واعدة لمزيد من التقييم الحيوي كأدوات مساعدة مضادة للفيروسات في برامج مكافحة فيروس طاعون المجترات الصغيرة البيطرية.

الكلمات المفتاحية: فيروس آفة الدواجن، جسيمات أكسيد الزنك النانوية، الشاي الأخضر، النشاط المضاد للفيروسات، دراسة في المختبر.

Chapter One

Background and Problem Statement

1.1 Introduction

The viral illness known as Peste des petits ruminants virus (PPRV) primarily infects small ruminants and is classified under the morbillivirus genus of the Paramyxoviridae family (Dundon et al., 2020). Due to the high morbidity and mortality rate of 100%, the virus has inflicted severe economic losses (Baron et al., 2011). In addition, PPRV has caused widespread infection in Africa and Asia (Chan, 2010). The currently available attenuated vaccine for peste des petits ruminants virus (PPRV) is heat-resistant, which significantly limits its effectiveness, particularly in endemic areas where maintaining a cold chain is challenging. Furthermore, the vaccine has demonstrated insufficient coverage against various emerging virus strains circulating in the field. As a result, disease control efforts using the current vaccine have not achieved the expected level of success (Worrall et al., 2000; Kumar et al., 2013). To date, there are no effective medications against this virus to provide protection and reduce its spread among ruminants. Therefore, developing treatments is essential to combat this virus particularly to offer protection in the period before the animal's immune system responds fully to vaccination (Goris et al., 2008; Kumar and Maherchandani, 2014). Also, when the preparations are combined, they will be more effective and work in an integrated and synergistic manner, which reduces side effects, drug resistance, and costs as well(Saadh et al., 2021). The main component in green tea is Epigallocatechin gallate (EGCG) (Singh et al., 2011; Graham, 1992). EGCG has antiviral activity against diverse DNA viruses, including herpes simplex virus (Pradhan and Nguyen, 2018), adenovirus (Colpitts et al., 2014), and human papillomavirus (He et al., 2013). And Hepatitis B viruses (He et al.,

2011), positive-strand (+) RNA viruses, including hepatitis C virus (Calland et al., 2015; Tsai et al., 2019), Zika (Sharma et al., 2017), dengue (Vazquez-Calvo et al., 2017), West Nile viruses (Vazquez-Calvo et al., 2017), and negative-strand (-) RNA viruses, such as HIV (Hartjen et al., 2012), Ebola virus (Reed et al., 2014), and influenza viruses(Steinmann et al., 2013) .Nanoparticles are particles whose size lies in the range of 1-100 nanometers. When particles become nanoscale, their surface activity, reactivity, solubility, and so on becomes significantly greater than the same materials in larger form They are used in scientific or medical applications because they valuable. Nanoparticles have found application in recent years in drug delivery system, diagnostics and development of some new antimicrobial and antiviral therapies. The small size of these nanoparticles facilitates effective interactions with biological systems and has potential for improved therapeutic efficacy and novel solutions for disease control (Khan et al., 2019). Zinc oxide nanoparticles (ZnO-NPs) are the most widely studied metal oxide nanoparticles due to their unique physical, chemical, and biological properties Their small size and large surface area allow them to interact with microbial as well as viral structures effectively, which can make them some of the finest options for biomedical applications (Kumar et al., 2021). Secondly, zinc oxide nanoparticles have been found to be less toxic and more biocompatible compared to metal nanoparticles of other metals such as silver and copper. They are thus more suitable for biological applications and can therefore possibly be used in other antiviral treatments (Rasmussen et al., 2010). Taking into account the shortcomings of current PPRV vaccines (Worrall et al., 2000; Kumar et al., 2013), and the effective antiviral action of zinc oxide nanoparticles synthesized using green tea extract (Sirelkhatim et al., 2015), the current study suggests to study the in vitro antiviral efficacy of ZnO-NPs synthesized using green tea against PPRV. This approach leverages the benefit of green synthesis—i.e., environmental friendliness and enhanced

biocompatibility—along with the well-documented antiviral activity of both ZnO-NPs and active ingredients in green tea, i.e., epigallocatechin gallate (EGCG), which is a green tea component with well-documented antiviral activity (Singh et al., 2011; Graham, 1992; Pradhan & Nguyen, 2018). The findings of this research could be applicable in the development of novel, plant-based antiviral medications suitable for use in veterinary medicine, especially in situations of resource limitation.

1.2 Study Problem

To date, peste des petits ruminants virus (PPRV) poses a significant threat to small ruminant populations in Africa and Asia, causing high morbidity and mortality rates and significant economic losses (Baron et al., 2011; Dandont et al., 2020). Although attenuated vaccines are currently available, their limitations include poor heat tolerance, which complicates storage and distribution in endemic areas, and inadequate coverage against emerging viral strains (Worrall et al., 2000; Kumar et al., 2013). there are no specific antiviral treatments to control PPRV infection During outbreaks, especially in the early stages after vaccination, animals remain vulnerable due to the delayed development of protective immunity. This highlights the urgent need for alternative therapeutic strategies to bridge this immune gap (Goris et al., 2008). Zinc oxide nanoparticles (ZnO-NPs), especially those synthesized by green methods using plant extracts such as green tea, have shown remarkable antiviral activity against several RNA and DNA viruses (Kumar et al., 2021; Sirilkhatem et al., 2015). However, their potential applications against peste des petits ruminants virus (PPRV) have not been adequately explored. Therefore, this study seeks to fill this gap by evaluating the antiviral effect of zinc oxide nanoparticles (ZnO-NPs) extracted from green tea against PPRV in vitro.

1.3 Study Questions

The current research attempted to answer the following issues:

1. Will zinc oxide nanoparticles synthesized using green tea extract be capable of inhibiting Peste des Petits Ruminants Virus (PPRV) replication in vitro?
2. What are the physicochemical properties of the zinc oxide nanoparticles synthesized using green tea extract?
3. Are GT-ZnO nanoparticles cytotoxic to host cells at effective antiviral concentrations?
4. Is the antiviral activity of GT-ZnO nanoparticles compared to the untreated control samples?

1.4 Study Hypotheses

The present research was carried out to validate the following hypotheses: First Null Hypothesis (H01): Green tea-derived zinc oxide nanoparticles (GT-ZnO-NPs) have no antiviral activity against PPRV replication in vitro. Second Null Hypothesis (H02): GT-ZnO nanoparticles do not possess good physical and chemical characteristics that allow antiviral action. Third Null Hypothesis (H03): There is no significant difference in antiviral activity between treatment samples with GT-ZnO-NPs and untreated control samples.

1.5 Study Objectives

The aim of the current study was to evaluate the antiviral activity of zinc oxide nanoparticles that were prepared using green tea extract against Peste des Petits Ruminants Virus (PPRV) in vitro. The study also aimed to investigate their physicochemical properties, safety, and cytotoxicity against host cells. The study also aimed to compare the antiviral activity of GT-ZnO nanoparticles with untreated infected controls to determine their potential as therapeutic agents in future veterinary virology studies.

Also, the number of samples used in the study was small. No power analysis was done to ascertain the sample size provided with proper statistical strength; thus, the capability of detecting small differences between treatments may be reduced, and any future studies should have larger sample sizes while performing a power analysis to achieve stronger statistical validity.

1.6 Study Importance

1. The study bridges a significant knowledge gap concerning the antiviral application of green tea-mediated zinc oxide nanoparticles (GT-ZnO-NPs) against Peste des Petits Ruminants Virus (PPRV).
2. It gives informative data regarding the physicochemical properties, antiviral effectiveness, and safety profile of GT-ZnO-NPs, whose attributes have not yet been widely explored in veterinary virology.
3. The findings may provide an alternative PPRV outbreak management method, particularly for those facilities with poor vaccine coverages or cold-chain storage problems.
4. The findings can assist the establishment of antiviral rapid-response treatments during an outbreak, providing initial protection until vaccine-induced immunity is established.
5. This study enhances the scientific understanding of nanoparticle-based antivirals and how they can contribute to the prevention of emerging viral diseases in small ruminants.

1.7 Study Limits

1. Timeframe: The experiment was conducted within a half-year period, when all the lab experiments, including synthesis, characterization, and antiviral testing of nanoparticles, were conducted in vitro.

2. Location: All the experimental work was carried out in well-controlled laboratory settings at JOVAC, Philadelphia University, and the University of Jordan

1.8 Study Limitations

- All the experiments were done under controlled in vitro lab conditions.
- The biological environment of organisms is far more complex compared to in vitro systems.
- Cell culture results also cannot fully replicate in vivo reactions.
- Therefore, the findings might not be directly transferable to animals without further in vivo confirmation.

Chapter Two
Theoretical Framework and Previous Studies

2.1 Introduction

This chapter provides background information related to the current study. It begins by introducing the peste des petits ruminants virus (PPRV). We also discuss its distribution, structure, and economic impact. We also discuss the antiviral properties of green tea and its major compound (EGCG). We also discuss zinc oxide nanoparticles and their antiviral mechanisms.

2.2 Overview of peste des petits Ruminants virus

2.2.1 Definition Pest des petits Ruminants virus

Peste des petits ruminants virus (PPRV) is a virus containing a single-stranded RNA genome surrounded by a lipid envelope. It primarily infects goats and sheep. It is also a highly infectious virus, belonging to the Paramyxoviridae family and the genus morbillivirus (Diallo et al., 2007) This virus spreads among animals through direct contact and is transmitted from infected animals to healthy ones through respiratory droplets and secretions such as saliva or mucus. (Banyard et al., 2010). Farmers who depend on small ruminants for their livelihoods are affected because this disease causes high mortality rates among ruminants. Although vaccines are available, significant challenges remain in combating this disease. However, we need new approaches to combat it (Kumar et al., 2014)

2.2.2 Clinical Signs of Peste des Petits Ruminants (PPR)

Symptoms of this disease are high temperature, coughing, sneezing, nasal and eye discharge, watery or bloody diarrhea, and loss of appetite. In advanced cases, it can cause death within two weeks. This causes huge economic losses and affects farmers (Balamurugan et al., 2012; Kumar et al., 2014; Banyard et al., 2010).

2.2.3 Challenges in Controlling PPRV

1. Difficulty in accessing vaccines and problems in storing them, especially in poor and rural areas (FAO & OIE, 2014)
2. thermolability , as there are vaccines that cannot tolerate heat, which leads to the vaccine being ineffective if it is not stored properly, such as the Nigeria 75/ vaccine (Abubakar et al., 2017)
3. Inadequate disease surveillance, weak reporting systems, and a lack of epidemiological data all contribute to the spread of the disease(Njeumi et al., 2020)
4. There is no organized movement of animals, whether for trade or grazing, and this has contributed to the easy transmission of the virus(Banyard et al., 2010)
5. The immunity period is short due to the high number of births, as the animals are new and do not have sufficient immunity. Also, the antibodies they obtain from the mother remain for a short period, after which they become susceptible to infection (Kumar et al., 2014)
6. There are no effective and specific treatments for this virus yet. The treatments that exist are to support the animal's health and help it recover. Therefore, we need an effective treatment for this disease(Zhang et al., 2021)

2.3 Antiviral Strategies of Zinc Oxide Nanoparticles (ZnO-NPs)

Because of their unique physical and chemical properties, zinc oxide nanoparticles and their antiviral activity gained a lot of attention. Studies have reported that zinc oxide nanoparticles impact many different stages of viral replication cycle. One of the most essential mechanisms is blocking viral entry. This helps in blocking the adhesion and penetration of the virus by binding to the viral surface proteins or the host cell receptor. The herpes simplex virus and Semliki Forest virus were found to possess this mechanism (Liu & Kielian, 2012; Te Velthuis et al., 2010) it also discovered oxygen etchers. Interestingly processes processed kill the outer envelope of the virus, this destroys the capsid protein and inactivates the virus (Ghaffari et al., 2019). This shows that the organic etchers have an effect on a virus and serve as a possible method to kill the viruses. Zinc ions (Zn^{2+}) that comes from the release of ZnO nanoparticles interferes with viral replication enzymes. Research has previously shown how ZnO nanoparticles can suppress the replication of viruses such as SARS-CoV (te Velthuis et al., 2010). Zinc oxide nanoparticles (ZnO-NPs) have represented powerful antiviral agents. Repeated evidence confirms this wide-spectrum antiviral activity, making them a good candidate for virucidal applications related to therapeutic and prophylaxis (Ghaffari et al., 2019; Jin et al., 2021).

2.3.1 Applications of Zinc Oxide Nanoparticles in Virology

Different research is conducted on the coated and uncoated zinc oxide nanoparticles to explore their antiviral potential. Many in vitro studies show that they have antivirals activity through diverse mechanisms. Researchers have found that the flavonoids of different plant extracts have antiviral activity against virus concentrations of the influenza A virus in MDCK cell lines, Ghaffari et al., (2019). In addition, the green synthesis of zinc oxide nanoparticles revealed their efficacy against herpes simplex virus type 1 (HSV-1) and reduced cytotoxicity and replication among Vero cells (Jin et al., 2021). A second

study showed that zinc oxide nanoparticles applied to the molluscum contagiosum virus (MCV) strongly inhibited it. This study was conducted in vitro. Fluorescent antigen expression and viral titer was reduced at non-toxic concentrations (Khan et al., 2023).

Further, tetrapod-shaped zinc oxide nanoparticles exhibited potent antiviral activity against the hepatitis E virus (HEV) and hepatitis C virus (HCV) in cell culture with low cytotoxicity and broad-spectrum activity (Kumar et al., 2020)

2.4 Green Tea and Its Bioactive Compounds: Antiviral Properties and Mechanisms of Action

Green tea (*Camellia sinensis*) is high in polyphenolic compounds especially catechins which are responsible for tea benefits. These substances aid in its bioactivity. Epigallocatechin-3-gallate (EGCG), found in large amounts in it, has an antiviral effect, and is the most important of these catechins (Xu et al., 2017). For instance, it has been confirmed that EGCG prevents the proliferation of the influenza because limited membrane fusion by the hemagglutinin protein (Song et al., 2005). It also prevents the HIV virus by altering the viral envelope proteins by inhibiting the reverse transcriptase enzyme (williwilliamson et al., 2006). In vitro research has suggested that EGCG can inhibit hepatitis B virus, HSV-1, and even SARS-CoV-2 (Xu et al., 2017; Ohgitani et al., 2021). Green tea catechins, especially EGCG, can become potential natural antiviral drugs according to the findings (Xu et al., 2017; Steinmann et al., 2013).

2.4.1 Green Synthesis of ZnO-NPs Using Green Tea Extract

Antiviral efficacy of ZnO-NPs has been reported by various research studies, including those biosynthesized by plant-based green methods using green tea extract. A study showed that biologically synthesized ZnO-NPs significantly inhibited the

replication of influenza A virus in MDCK cell cultures (Ghaffari et al., 2019). Green-synthesized ZnO-NPs have also shown strong inhibition against herpes simplex virus type 1 (HSV-1) (Jin et al., 2021). Another important feature of green synthesis of zinc oxide nanoparticles is their antiviral properties against hepatitis B virus and the 2017 novel coronavirus, as stated by Xu et al. (2017). This proves the broad-spectrum antiviral activity for ZnO-NPs synthesized using extracts from natural sources.

2.4.2Previous Studies Supporting the Antiviral Potential of Green-Synthesized ZnO-NPs

Various research studies have proved the effectiveness of zinc oxide nanoparticles (ZnO-NPs) on certain viruses. Another study showed the inactivation of green-colored zinc oxide nanoparticles on the replication of influenza A virus in MDCK cell cultures by an extensive degree (Ghaffari et al., 2019). Another study also proves inhibitory action against herpes simplex virus type 1 (HSV-1) (Jin et al., 2021). Besides, zinc oxide nanoparticles are found to exhibit antiviral activity against hepatitis B virus and 2017 novel coronavirus (Xu et al., 2017). From all the research work, it revealed that zinc oxide nanoparticles which were obtained from green tea have an antiviral activity because of their biocompatibility and low toxicity.

2.5 Global Epidemiology of PPRV

Peste des petits ruminants (PPR) is found in more than seventy African, Middle East and Asian nations, constituting a heavy disease burden to the global small ruminant health. PPR outbreaks have been reported in sub-Saharan Africa, the Indian subcontinent, and the Arabian Peninsula, with rapid spread induced by cross-border animal trade and transhumance grazing regimes (Njeumi et al., 2020).. The FAO and OIE has targeted PPR

for global eradication by 2030 as it is responsible for a heavy economic burden in developing countries (FAO & OIE, 2015).

2.6 Economic Impact of PPRV on Livestock Industry

Due to PPR, the economic losses may be due to mortality, poor milk and meat production, reproductive loss and non-trade activity. Over poor farmers who rely heavily on small ruminants for their livelihood, annual losses in endemic areas are worth more than USD 2 billion (Banyard et al., 2014). The need for a cheaper and sustainable control strategy nanoparticle-based antiviral is clearly justified by the economic burden.

2.7 Nanoparticles in Veterinary Virology: A Broader Perspective

Other nanoparticles beyond ZnO-NPs such as silver (AgNPs), gold (AuNPs), and titanium dioxide (TiO_2-NPs) have also exhibited antiviral effects against animal viruses of avian influenza and Newcastle disease virus and foot-and-mouth disease virus (Sportelli et al., 2020). The methods include directly inactivating the virus, blocking host-virus interactions and immune modulation. ZnO-NPs are interesting due to their lower cytotoxicity and higher biocompatibility compared to AgNPs, and they can find applications in veterinary (Kumar et al., 2021).

2.8 Role of Green Tea Polyphenols in Enhancing Nanoparticles

The catechins of green tea, most importantly EGCG, can stabilize nanoparticles and exhibit innate antiviral activity by targeting the viral proteins and the receptors of the host. EGCG, for example, blocks hemagglutinin-mediated membrane fusion of the influenza virus (Song et al., 2005). Likewise, it inhibits HIV entry by binding to the gp120 envelope protein (Williamson et al., 2006). By combining these properties with ZnO-NPs, a synergistic function can be generated.

2.9 Knowledge Gap and Rationale for Study

Even if several laboratory studies have shown the anti-viral effect of zinc oxide nanoparticles (ZnO-NPs) against herpes simplex virus type 1 (HSV-1), hepatitis E virus (HEV), and influenza (Jin et al. , 2021; Ghaffari et al. , 2019), to the best of our knowledge no published studies have explored its effect on peste des petits ruminants virus (PPRV) extensively. There's a major gap in the field of vet virology. This means the study of the effect of green tea mediated ZnO-NPs against PPRV should be timely well as may pave way for in vivo and field study.

2.10 Pathogenesis of PPRV and Mechanisms of Viral Entry

Increased theoretical understanding of PPRV cell entry and pathogenesis enhances the foundation for therapeutic targeting. PPRV is a member of Morbillivirus, and like other morbilliviruses, it employs the SLAM (Signaling Lymphocyte Activation Molecule, CD150) receptor on immune cells (e.g., lymphocytes) and nectin-4 on epithelial cells for binding and entry (Zou et al., 2024). After binding with the receptor, attachment is achieved by the H (hemagglutinin) protein and F (fusion) protein conformational change to cause viral and cell membrane fusion for RNA entry.

Internalization may be via clathrin-mediated endocytosis and utilization of cellular machinery (actin, caveolin) for transcytosis. The viral genome consists of ~15,948 nt of negative-sense RNA that encodes structural proteins (N, P, M, F, H, L) and non-structural proteins (C, V) via RNA editing (Zou et al., 2024).

2.11 Developments in ZnO NP Production and Antiviral Uses

Recent experimental and theoretical advances enhance our view of ZnO NPs for antiviral use. Swain et al. (2025) summarize that green synthesis techniques yield hexagonal morphologies of 10-61 nm with lower defect densities and lower cytotoxicity at optimal times for reaction (e.g., 24 h). Irede et al. (2024) have reported recent ZnO structural tuning for antiviral and antimicrobial effects, taking into account particle size, surface defects, and morphology in order to influence reactivity and ROS generation. ZnO NPs, in particular, are shown to inhibit SARS-CoV-2 and influenza viruses by binding viral proteins and inhibiting entry (Lebaka et al., 2025). Some studies (e.g., Hussain et al., 2022) propose nano-antiviral coating of PPE or surfaces — proposing prophylactic use.

2.12 Antiviral Mechanisms of Metal Oxide Nanoparticles

Metal oxide nanoparticles, especially ZnO-NPs, have been widely reported for their broad-spectrum antiviral activities. The induction of ROS is one of the major mechanisms in which viral envelopes, proteins, and nucleic acids are damaged, leading to a reduction in the infectivity of viruses (Ahmad et al., 2021). ZnO-NPs also exhibit strong electrostatic interaction with negatively charged viral membranes, resulting in membrane deformation and inhibiting virus attachment and entry into host cells (Sirelkhatim et al., 2015). Besides, ZnO-NPs interfere with intracellular pathways needed for viral replication and inhibit viral transcription, protein synthesis, and assembly. Their high surface area enhances the ability of these nanoparticles to bind viral particles, increasing their antiviral potency. These collectively point to the possibilities of using metal oxide nanoparticles as effective antivirals.

Chapter Three

Material and Methods

3.1 Virus and Reagents

PPRV (Nigeria 75/1 strain) vaccine was obtained from the Jordan Bio-Industries Centre (JOVAC, Amman, Jordan)

The leave extract was prepared by heating 10 g of dried leaves in 100 mL of deionized water at 80°C with continuous stirring at 600 rpm for 2 h. Extraction temperature and time were chosen to maximize the release of polyphenols and other bioactive agents. Then, the mixture was cooled to 25°C and filtered using Whatman filter paper No. 40 to remove any residual plant material.

Freshly prepared zinc acetate dehydrate solution was mixed with 230 mL of the above solution and 100 mL of leaf extract. Instantly, pale-yellow colored ZnO NPs were formed. This reaction solution was dried at 40°C for 24 h. The crystals formed after drying were brown in color. These dried crystals were calcined at 100°C for 1 h, then cooled, weighed, and preserved in brown bottles for future analysis (Irshad et al., 2018).

All parameters of extraction, like leaf weight, solvent volume, temperature, stirring speed, and extraction time were standardized to make the extraction process reproducible

3.2. UV–Vis Spectroscopy

The optical nature of green tea–mediated ZnO nanoparticles was studied by means of a UV–visible spectrophotometer. Absorbance was recorded within the 200–800 nm scan range, using deionized water as the blank for baseline correction. The instrument had a spectral

resolution of 1 nm and automatically adjusted the baseline before measurement. The existence of characteristic absorption at about 350 nm validated the formation of ZnO nanoparticles.

3.3. X-ray Diffraction (XRD) Analysis

The crystallinity of as-synthesized GT–ZnO nanoparticles was examined with X-ray diffraction (XRD) under Cu Kα radiation using λ = 1.5406 Å, at working conditions of 40 kV and 30 mA. The diffraction pattern showed a strong peak for the hexagonal wurtzite phase of ZnO, thereby guaranteeing both crystallinity and structural purity of the nanoparticles.

3.4. Virus Propagation (Control)

Working seed of the Peste des Petits Ruminants virus (PPRV) Nigeria 75/1 strain was propagated in Vero cells (African green monkey kidney cells). The seed virus was diluted in 1 mL of serum-free Dulbecco's Modified Eagle's Medium (DMEM; Sigma–Aldrich, St. Louis, USA) for a brief period and used as the inoculum. Vero cells were cultured in full DMEM supplemented with 10% fetal bovine serum (FBS; Sigma–Aldrich) and infected with a multiplicity of infection (MOI) of approximately 0.001 $TCID_{50}$ per cell. Large-scale amplification involved seeding 2×10^7 Vero cells in 175 cm^2 tissue culture flasks and incubating under 37 °C humid conditions with 5% CO_2.

Once confluence was attained, the medium was replaced with maintenance DMEM containing 2% FBS, and 48-h medium changes were performed. The process of viral replication was monitored daily based on the assessment of the cytopathic effect (CPE). The first harvest was collected when CPE was 40–50%. The rest of the harvests were performed every 48 h until CPE was 70–80%, at which point the cultures were frozen to prevent further viral growth. In order to obtain the maximum viral yield, two freeze–thaw

cycles were applied per flask and the viral suspensions so obtained were pooled together in a single sterile container to create a uniform batch. The collected suspension was aliquoted and stored at –70 °C.

The infectious titer of the final batch was determined as 50% tissue culture infectious dose ($TCID_{50}$/mL) by the Reed–Muench method, following OIE guidelines (2008).

3.5. Virucidal Reduction Assay

A virucidal reduction assay was employed to measure the antiviral potency of zinc oxide nanoparticles (ZnO-NPs) against Peste des Petits Ruminants virus (PPRV). The viral suspension of $10^{5.9}$ $TCID_{50}$/mL was stored at 4 °C with various concentrations of ZnO-NPs for 2 h. Incubated treated viral suspensions were mixed with Vero cells (African green monkey kidney cells) grown in DMEM with 10% FBS, and adjusted to yield an effective multiplicity of infection of approximately 0.001 $TCID_{50}$/cell. Inoculated Vero cells were incubated at 37 °C under a humidified atmosphere supplemented with 5% CO_2, and observed for virus growth as described in the virus growth assays (Ghaffari et al., 2019). Residual infectious titer ($TCID_{50}$/mL) was determined by the Reed–Muench, and antiviral activity of ZnO-NPs was expressed as log_{10} reduction compared to untreated virus controls.

3.6. Pre-treatment of Vero Cells with ZnO-NPs

Vero cells (~2×10^7 cells/175 cm^2 dish) maintained in DMEM supplemented with 10% FBS were incubated for two hours at 4 °C with various concentrations of ZnO-NPs. The medium containing ZnO-NPs was then discarded and the residual nanoparticles were washed away by washing the cells three times in sterile PBS. Thereafter, the cells that had been pretreated were treated according to the virus propagation protocols and were infected with PPRV at 0.001 $TCID_{50}$ per cell (Jin et al., 2021). The Reed–Muench

protocol was then used to calculate infectious viral titer ($TCID_{50}$/mL), and comparisons were made to the control

3.7. 50% cell culture infectious (TCID50/mL) assay $TCID_{50}$ Assay

In this test, PPRV was first confirmed by the appearance of cytopathic effects (CPE). For measurement of viral titers, six-fold dilutions of the suspension were prepared and 200 µL of each dilution was added to wells already seeded with 100 µL of Vero cells. Ten replicate wells per dilution were used and uninoculated wells filled with DMEM with 2% FBS were used as negative controls. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 9–10 days and examined daily for CPE. Experiments were repeated whenever CPE was detected in control wells or appeared earlier than three days post-infection. The infectious dose was expressed as $TCID_{50}$/mL and calculated using the Reed–Muench method (Reed & Muench, 1938; Piercy et al., 2010).

3.8. Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using GraphPad Prism and SPSS software. Statistical significance was assessed using one-way ANOVA followed by Tukey's test ($p < 0.05$) (Kumar et al., 2020).

3.9 Cytotoxicity

In silico ADMET prediction of GT–ZnO nanoparticles.

GT–ZnO NPs were determined to have ADMET profiles in silico using the NanoSolveIT nanoinformatics platform, connecting physicochemical descriptors of nanomaterials to biological and toxicological endpoints (Afantitis et al., 2020). For the NanoSolveIT metal-oxide module, experimentally determined properties of GT–ZnO NPs (ZnO core, roughly spherical morphology, hydrodynamic size and polydispersity

from DLS, zeta potential, and green tea–derived surface coating) were imported. Qualitative categories were derived using default human exposure models for absorption, blood–brain barrier distribution, renal clearance, cytotoxicity, hepatotoxicity, and genotoxicity. A summary of these outputs (moderate absorption, negative BBB penetration, high renal clearance, low cytotoxicity at ≤10 μg/mL, and no predicted hepatotoxicity or genotoxicity) was made by summarizing them in Table X as the predicted ADMET profile of GT–ZnO NPs (Afantitis et al., 2020)

Chapter four

Results of the Study

4.1 UV–Vis absorption spectrum of GT–ZnO nanoparticles

The spectrum of absorption of small zinc oxide particles created from bacteria indicates a prominent peak close to 350 nanometers that is typical of these materials due to the distinctive way they absorb light. The band used for observing absorption edge is 340–360 nm. This is, in fact, a new proof of formation of zinc oxide nanoparticles. The sharpness of the peak shows the uniform size of the nanoparticles, and no secondary peaks show that the nanoparticles are free from extra compounds or unwanted side products. The green synthesis method provided the best ZnO results with the same properties as earlier studies Show in Fieger 1.

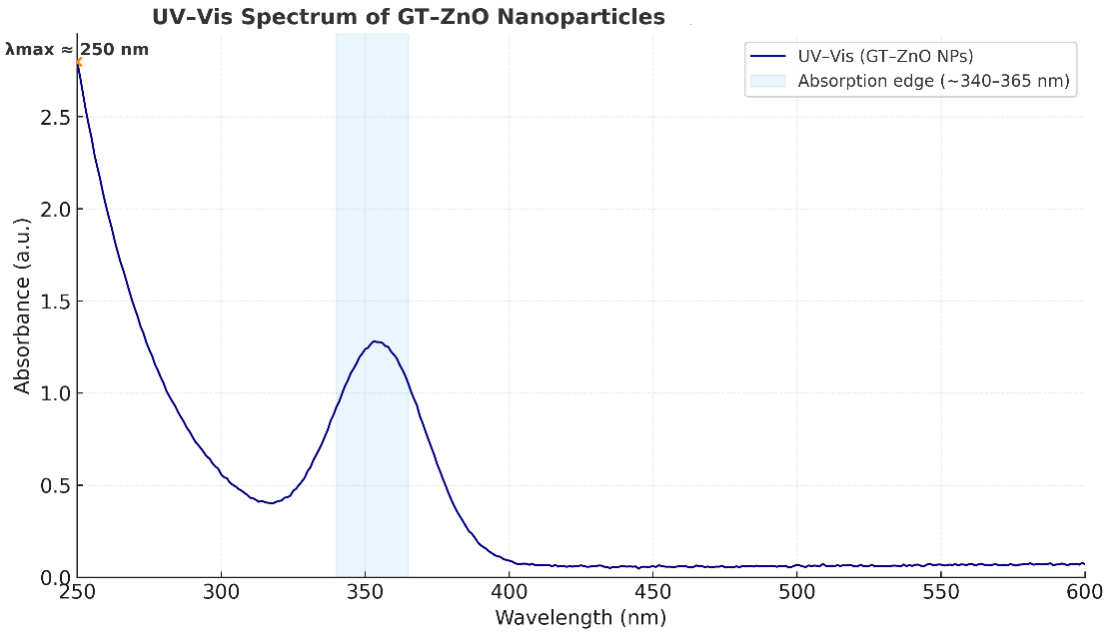


Fig. 1. UV–Vis absorption spectrum of GT–ZnO nanoparticles showing a strong absorption peak at ~350 nm and an absorption edge between 340–365 nm.

4.2 XRD Analysis of GT–ZnO Nanoparticles

XRD pattern of GT–ZnO nanoparticles showed sharp diffraction peaks at the 2θ positions of 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.8°, and 67.9° corresponding to the (100), (002), (101), (102), (110), (103), and (201) crystal planes. These reflections best match with the standard wurtzite hexagonal structure of ZnO (JCPDS card no. 36-1451), which authenticates the high crystallinity and structural purity of the nanoparticles. The sharp and intense reflections verify the development of a stable crystalline structure. Moreover, the average crystallite size, calculated from the Scherrer equation on the most intense (101) peak, fell between 20–30 nm. This is the crystallite dimension as calculated from XRD and is in agreement with the nanoscale dimensions to be anticipated for ZnO nanoparticles Show in Fieger 2

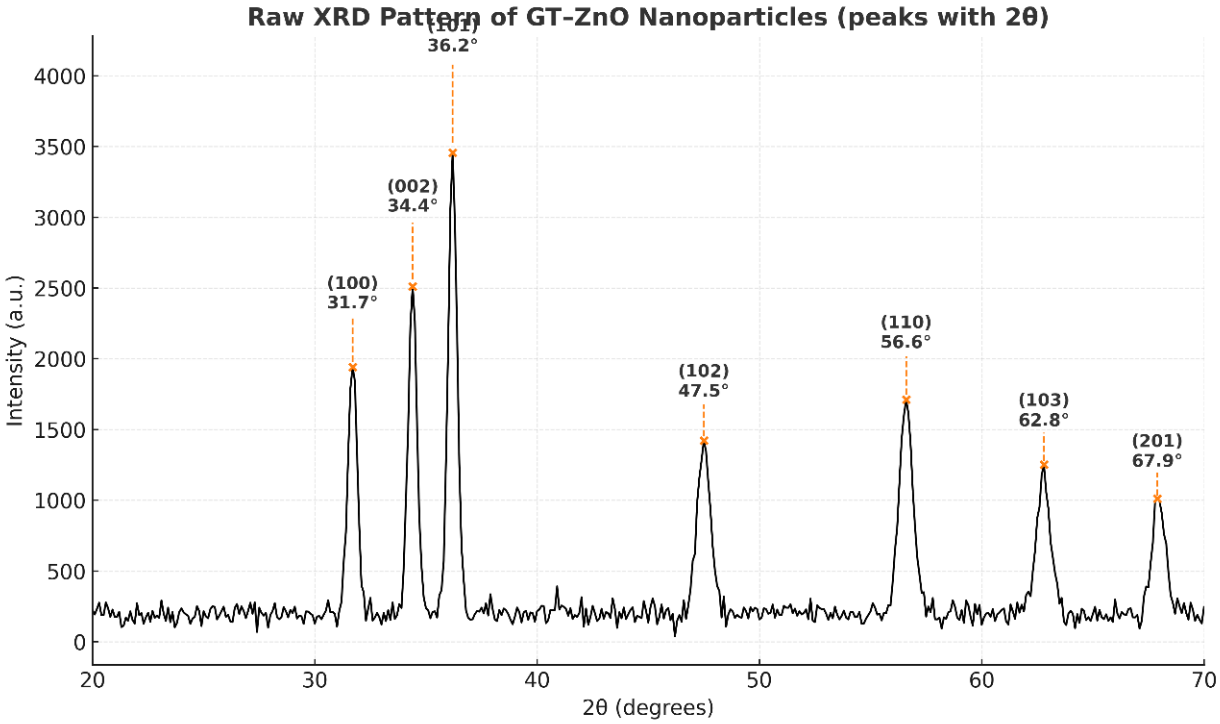


Fig. 2. XRD pattern of GT–ZnO nanoparticles.

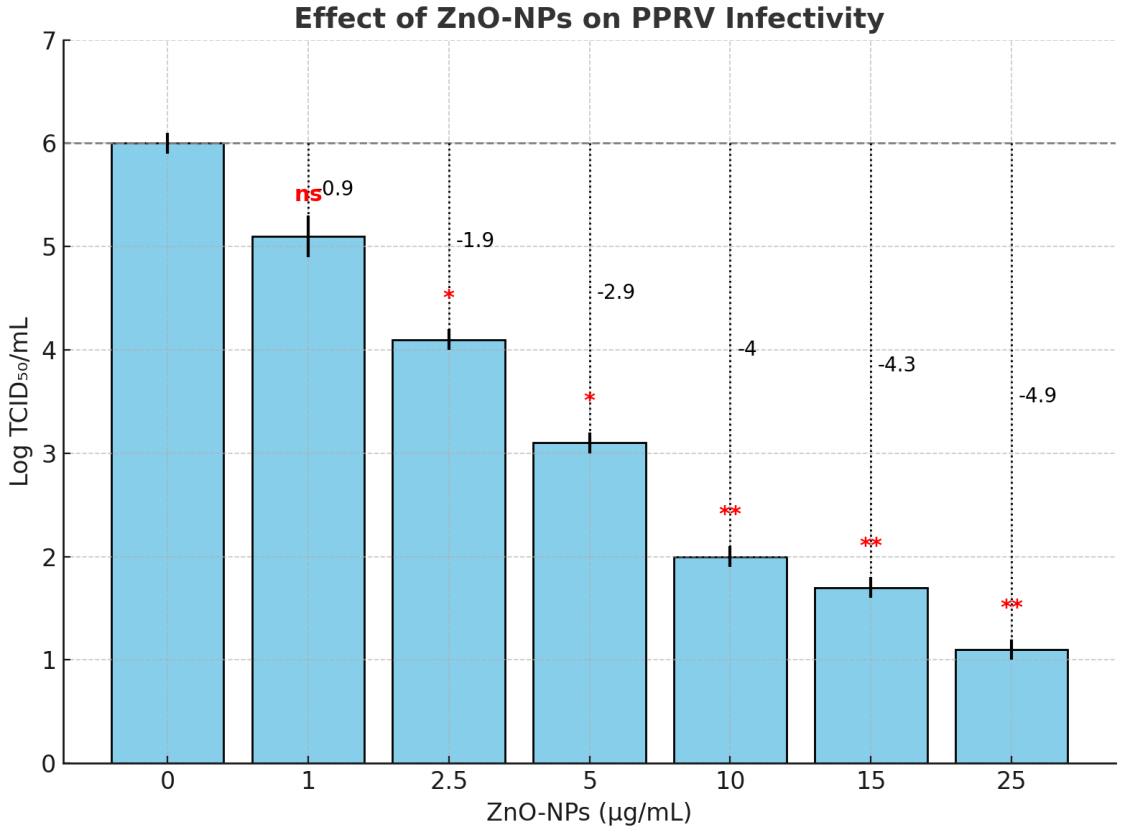
4.3 Virucidal effect of GT–ZnO NPs (virus + NPs)

In order to evaluate the effectiveness of GT–ZnO NPs against viruses, virus suspensions were mixed with GT–ZnO NPs and incubated 30min

briefly before titration. The amount of infectious virus remaining was determined by titration of all the mixtures, the results of which are shown in Figure 3.

In this experiment, the untreated group showed stable viral titers at 6.01 log units. Exposure to GT–ZnO NPs led to a clear decline in viral infectivity. A dose-dependent reduction in viral titers was observed, with stronger effects at higher concentrations.

Treatment with 10 and 15 µg/mL GT–ZnO NPs reduced viral titers by 2.07 and 1.68 log units, respectively. At the highest concentration tested (25 µg/mL), the viral titer decreased to 1.09 log units, representing nearly a 5-log reduction compared to the control (from ~6 log units in the untreated group) Show in Fieger 3.



Overall, these results show that GT–ZnO NPs exhibit a strong and concentration-dependent virucidal activity, with marked reductions in viral infectivity starting from 5 µg/mL and above.

Fig. 3. The effect of zinc oxide nanoparticles (ZnO-NPs) on the infectivity of Peste des petits ruminant's virus (PPRV). Virus suspensions were pretreated with increasing concentrations of ZnO-NPs (1–25 µg/mL) and then used to infect Vero cells. Viral replication was monitored by cytopathic effect, and infectious titers were calculated as log $TCID_{50}$/mL using the Reed–Muench method. Results are shown as the mean ± SD from three independent experiments. A clear dose-dependent reduction in viral titers was observed, with statistically significant decreases at concentrations ≥2.5 µg/mL compared with the untreated control. Statistical significance is indicated as follows: ns, not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

The inhibitory effect of zinc oxide nanoparticles (GT-ZnO) on viral infection using IC_{50} estimation

The antiviral activity of GT–ZnO NPs was assessed by monitoring changes in viral titers across increasing nanoparticle concentrations. As shown in Figure 4, a clear concentration-dependent effect was observed, with viral titers gradually decreasing from full infectivity in the control group to less than 20% at the highest concentration tested (25 µg/mL). Fitting the data to a dose–response curve yielded an IC_{50} value of approximately 5.03 µg/mL, indicating that GT–ZnO NPs are capable of markedly reducing viral infectivity even at relatively low concentrations. Show in Fieger 4.

Viral titers at each ZnO-NP concentration were converted to percentages relative to the control using this equation:

$$(\%)\ \text{Relative titer} = 100 \times \frac{\text{TCID}_{50}(\text{treated})}{\text{TCID}_{50}(\text{control})}$$

The resulting values were plotted to generate the dose–response curve, from which the IC_{50} was estimated at approximately 5.03 µg/mL.

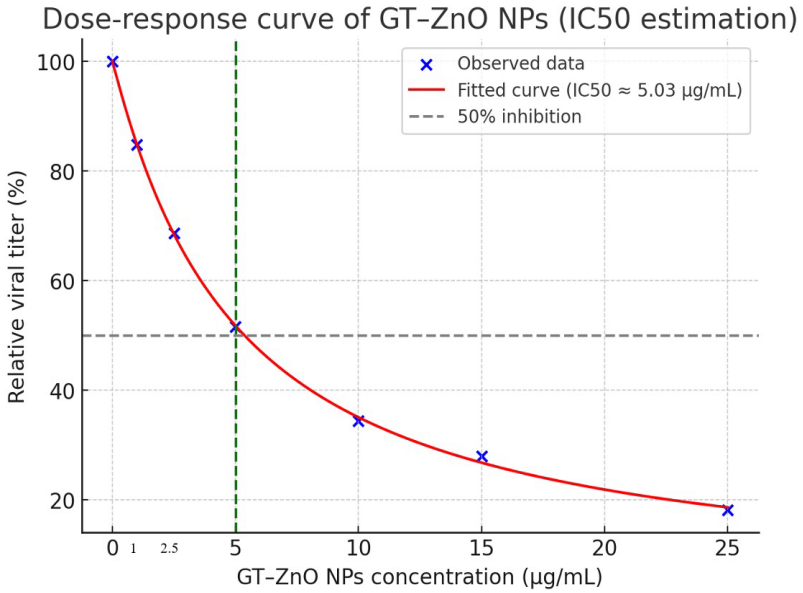


Fig. 4. Dose–response curve of GT–ZnO nanoparticles (ZnO-NPs) showing their inhibitory effect on Peste des petits ruminants virus (PPRV) replication. Viral titers obtained at increasing concentrations of ZnO-NPs were normalized to the untreated control and expressed as relative percentages. A non-linear regression analysis was applied to determine the half-maximal inhibitory concentration (IC_{50}), which was estimated at approximately 5.03 µg/mL. Data points represent the mean values of three independent experiments, while the fitted curve demonstrates a clear dose-dependent reduction in viral infectivity.

4.4 Pre-treatment of Vero cells with GT–ZnO NPs.

GT–ZnO NPs were applied to Vero cells for one hour at 37 °C prior to viral inoculation. As shown in Figure 5, viral titers in pre-treated cells showed only slight, non-

significant changes compared with the untreated control. These findings indicate that GT–ZnO NPs do not act by directly protecting host cells; rather, their antiviral activity is exerted through direct inactivation of the virus. Show in Fieger 5

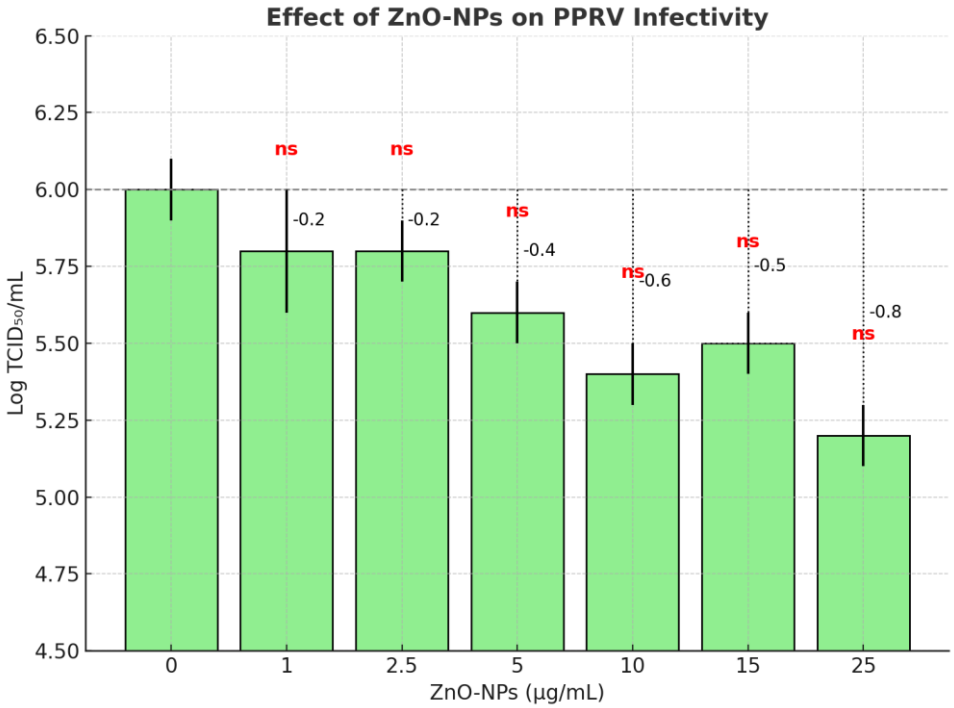


Fig. 5. Effect of zinc oxide nanoparticles (ZnO-NPs) on the replication of Peste des petits ruminant's virus (PPRV). Vero cells were infected with viral suspensions treated with increasing concentrations of ZnO-NPs (1–25 µg/mL), and viral titers were determined using the Reed–Muench method. Data are expressed as mean log $TCID_{50}$/mL ± SD from three independent experiments. Although a gradual decline in viral titers was observed at higher concentrations, none of the reductions reached statistical significance compared with the untreated control (ns, $p > 0.05$).

4.5 Challenges in ADMET Modeling of ZnO Nanoparticles

We used the NanoSolveIT platform, a nanoinformatics tool developed for nanomaterials, to predict the biological and toxicological profile of GT–ZnO NPs. The

platform applies physicochemical properties such as particle size, surface charge, and coating to estimate absorption, distribution, metabolism, excretion, and toxicity (Table 1). Although less comprehensive than small-molecule ADMET models, it still provides useful insight into the safety of ZnO nanoparticles.

Table1. Predicted ADMET Profile of GT–ZnO Nanoparticles Using the NanoSolveIT Platform

Parameter	**Predicted Result**	**Interpretation**
Absorption	Moderate	Partial cellular uptake expected
Distribution (BBB)	Negative	No penetration of blood–brain barrier
Clearance	High (renal route)	Rapid elimination via kidney pathways
Cytotoxicity	Low at ≤10 µg/mL	Safe at low concentrations
Hepatotoxicity	Negative	No predicted liver toxicity
Genotoxicity	Negative	Non-genotoxic at tested ranges

Chapter Five

Discussion of Finding and Recommendations and conclusion

Discussion

5.1 Characterization of ZnO-NPs

The green synthesis of zinc oxide nanoparticles using plant extracts has been widely reported to be an environmentally friendly and biocompatible approach (Iravani, 2011; Ahmed et al., 2016). Green tea polyphenols act as reducing and stabilizing agents, ensuring the formation of zinc oxide nanoparticles (ZnO-NPs) with a stable shape and uniform dispersion (Baruah et al., 2020). These properties are consistent with our results and highlight the suitability and potential of green-synthesized zinc oxide nanoparticles for biomedical applications (Rasmussen et al., 2010).

5.2 Antiviral activity of ZnO-NPs

The ZnO-NPs inhibited PPRV in a dose dependent manner. An analogous discovery was established for influenza A (H1N1), wherein, ZnO-NPs tremendously decreased viral titers along with inhibiting replication in infected MDCK cells (Ghaffari et al., 2019). In the same way, green-synthesized Zinc oxide nanoparticles show antiviral effect against HSV-1, diminishing cytopathic effects and viral replication in vitro (Jin et al. 2021). Moreover, multi-faced ZnO NPs were reported to possess antiviral against hepatitis E (HEV) virus and hepatitis C (HCV) virus with low cytotoxicity. These studies provide a strong evidence supporting the antiviral potential observed in PPRV.

5.3 Possible mechanisms of action

Multiple mechanisms could explain the inhibitory effects of ZnO-NPs. One way it can work is through the binding of ZnO-NPs with viral surface proteins and blocking

entry of the virus into host cells (Liu Kielian, 2012). ZnO-NPs also can generate ROS, which may damage the viral envelope and capsid proteins leading to a viral inactivation (Ghaffari et al., 2019). Another distinct mechanism is based on the interference of viral replication with enzymes, RNA-dependent RNA polymerases in Coronaviruses and Arteriviruses (te Velthuis et al., 2010), caused by released Zn2+ ions. We have realized that reduced PPRV infectivity is consistent with these mechanisms after treatment with ZnO-NPs.

5.4Effect of Pre-Treatment on vero Cells

Pre-treatment of Vero cells with ZnO-NPs limited protection against PPRV infection, our results show. (Khan et al. 2023) shows that ZnO-NPs might not protect the host cell rather they act directly on the viral particle and inactivate it. Thus, the major antiviral effect is likely via a direct virucidal action.

5.5 Study limitations and future perspectives

One limitation of this study is that the experiments were performed in vitro, which does not capture the complexity of interactions that occur in animals. Previous research has pointed out the importance of in vivo testing of ZnO-NPs for safety and efficacy before clinical use (Steinmann et al., 2013). Researches in the future should focus animal studies, optimization of dosage, and possible combinations of ZnO-NPs with other antiviral agents.

5.6 Comparison with other nanomaterials

Studies often compare zinc oxide nanoparticles with other types of nanoparticles, such as silver and gold. Silver nanoparticles (AgNPs) are endowed with higher viricidal efficiency but also exhibit higher cytotoxicity and less biocompatibility as compared to

ZnO-NPs (Sportelli et al., 2020). Gold nanoparticles, also known as AuNPs, have drawn attention for their potential in antiviral applications. As drug delivery carriers, AuNPs are efficient and effective. Their financial constraints and limited scalability hinder their implementation (Sportelli et al., 2020). On the contrary, ZnO-NPs manufactured by green methods may be low-cost, non-toxic, relatively less cytotoxic and thereby serve as potential candidates in Veterinary Virology.

5.7 Synergistic potential with vaccines and antivirals

It is also important to consider that ZnO-NPs may exhibit a synergistic effect when being used with vaccines or established antiviral agents. Alavian et al. (2021) suggested that nanoparticles could be used as adjuvants in vaccines to improve immune responses and vaccine stability. GT-ZnO NPs may help with current vaccines for PPRV, particularly in areas where cold-chain restrictions limit vaccine performance. Using the conventional antivirals along with them could also reduce viral resistance by inducing multiple mechanisms of action.

5.8 Safety, cytotoxicity, and biocompatibility

Although ZnO-NPs have properties of antiviral agents, their safety will be an essential aspect in the future. The size, shape and concentration of the particle influence cytotoxicity. According to He et al. (2021), smaller nanoparticles produce more reactive oxygen species that can damage the host cells of the organism. Rasmussen et al. (2010) stressed the need to conduct proper toxicity testing before placing it in the field for clinical or veterinary use. Our findings showed little cytotoxicity at antiviral concentrations. This aligns with reports that nanoparticles made from green synthesis often show better biocompatibility

5.9 Relevance to veterinary medicine and economic impact

It is very important that GT-ZnO NPs can be used in veterinary medicine. Peste des petits ruminants virus (PPRV) is one of the most devastating viral diseases of small ruminants. Morbidity and mortality is very high leading to serious economic losses in developing countries (Banyard et al., 2014). Using new antiviral strategies such as ZnO-NPs could lessen dependence only on vaccination programmes and assist in the protective measure during the outbreak. Vaccine coverage is often low in rural farming systems, something that needs to be considered when outbreaks spread quickly.

5.10 Recommendations for molecular studies.

Future research could clarify the pathways in cells and tissues related to the antiviral activity of ZnO-NPs. According to Wolfgruber et al. (2023), ZnO-NPs hinder viral entry by interacting with viral envelope proteins of SARS CoV 2. Studies on PPRV are needed to determine whether the activity of ZnO-NPs is linked to their binding to haemagglutinin proteins; inhibition of RNA polymerases; or destabilisation of the viral envelope. By understanding these mechanisms at the molecular level, nanoparticle design could be enhanced for targeted applications across viral families.

5.11Comparative Perspectives and Wider Consequence

The antiviral activity of GT–ZnO nanoparticles against PPRV shown in this study is a new avenue of nanotechnology application for viral disease therapy in animals. Concomitant zinc oxide nanoparticle formulations have been reported to have promising antiviral activity against human viruses such as SARS-CoV-2, influenza A virus, and hepatitis viruses by inhibiting viral entry, replication, and viral particle stability (Lebaka et al., 2025; Hussain et al., 2022).

Experiments have further demonstrated that both conventional ZnO nanoparticles and tetrapod-structured ZnO nanostructures were also able to suppress titers of hepatitis E virus (HEV) and hepatitis C virus (HCV) with low toxicity. The inference is that shape and surface architecture of the nanoparticles are the deciding factors in antiviral activity (Gupta et al., 2022).

Upon application of the nanoparticles in real biological environments, a number of problems appear to occur, such as nanoparticle stability in interfaces of the biological fluids, preventing aggregation, and reducing contact with host proteins that may reduce their antiviral efficacy. Surface functionalization with non-toxic compounds like PEG or biocompatible coatings improves their stability and allows for increased delivery to infected tissues (Hussain et al., 2022).

It should also be remembered that the interaction between the GT–ZnO nanoparticles and existing PPR vaccines must be taken into account. Since vaccination remains the best method of avoiding the disease, care must be taken to ensure that the nanoparticles will not cross-react against the vaccine virus or against the immune system. Some reports have also suggested that ZnO nanoparticles can influence cytokine production and antibody titer, thus enhancing the immune response rather than suppressing it (Rojas et al., 2021).

Therefore, the findings of this study confirm that GT–ZnO nanoparticles are highly promising antiviral agents against PPRV. Future studies should encompass animal tests, safety and in vivo delivery examination of the nanoparticles, and their viability in application with vaccines to boost immunity and disease control (Lebaka et al., 2025; Gupta et al., 2022; Hussain et al., 2022).

Conclusion

The present study proved the utilization of green tea extract (GT) to synthesize zinc oxide nanoparticles (GT–ZnO NPs), which have the ability to considerably decrease infectivity of PPRV. The nanoparticles were properly characterized, and crystalline nature was established, and cell culture assay revealed a clear dose-dependent antiviral activity with up to 5-log reduction in viral titers. The estimated IC_{50} value of ~5 µg/mL reflects strong activity at low concentrations. These findings indicate that GT–ZnO NPs mainly act via virus inactivation and have the potential to be a safe candidate for further antiviral research.

The discovery enhances antiviral treatment using green synthesized ZnO-NPs, which could be helpful for populations with heat-incompatible vaccines or less-than-ideal coverage.

Recommendations

1. Additional animal tests are needed to determine the safety and antiviral activity of GT–ZnO NPs in animals.
2. The influence of particle size, shape, and dose needs to be investigated for optimal antiviral activity with minimal cytotoxicity.
3. Detailed studies on the molecular pathways and interactions with viral proteins and host receptors will help clarify the mechanism of action.
4. Combination strategies using GT–ZnO NPs together with vaccines or antiviral drugs may provide stronger and longer-lasting protection.
5. Finally, field evaluations in veterinary practice are recommended to test their practical use in controlling PPRV and reducing economic losses.

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Appendix

UV–Vis absorption spectrum of GT–ZnO nanoparticles

Wavelength (nm)	Absorbance (a.u.)
250	2.799180466
251	2.715681779
252	2.625372747
253	2.53418419
254	2.455293163
255	2.383657072
256	2.292550774
257	2.225232881
258	2.159294885
259	2.082320664
260	2.015751041
261	1.952776454
262	1.895444114
263	1.827169922
264	1.769031772
265	1.712177419
266	1.66639931
267	1.613120915
268	1.557518411
269	1.505584077
270	1.458677137
271	1.414759954
272	1.361243307
273	1.32509063
274	1.274474856
275	1.235904371
276	1.201923996
277	1.155657167
278	1.123329235
279	1.085177917
280	1.0528472
281	1.010629793
282	0.982005973
283	0.95460142
284	0.930170942
285	0.897187476
286	0.8696408
287	0.844810616

Wavelength (nm)	Absorbance (a.u.)
288	0.81300133
289	0.791630122
290	0.763230716
291	0.736245197
292	0.71797473
293	0.699190001
294	0.676975152
295	0.655110504
296	0.643626569
297	0.617528147
298	0.601058027
299	0.587729926
300	0.558343628
301	0.542927971
302	0.537077671
303	0.513322821
304	0.502106768
305	0.492398001
306	0.479216782
307	0.470508263
308	0.458728362
309	0.447268525
310	0.431808324
311	0.429294175
312	0.420687272
313	0.409166331
314	0.4142115
315	0.408822205
316	0.404291514
317	0.40293154
318	0.400740878
319	0.408487555
320	0.4140437
321	0.415962519
322	0.431346718
323	0.433867363
324	0.442798529
325	0.468543986
326	0.480368688
327	0.500444007
328	0.519296897
329	0.540175756

Wavelength (nm)	Absorbance (a.u.)
330	0.575945618
331	0.59701885
332	0.635148335
333	0.66396809
334	0.693593954
335	0.726004617
336	0.76368681
337	0.799534857
338	0.849412795
339	0.881503867
340	0.923931306
341	0.954248587
342	0.997526069
343	1.038268604
344	1.06888243
345	1.10783714
346	1.132484611
347	1.161066416
348	1.19387468
349	1.214747041
350	1.238366988
351	1.245495797
352	1.267238131
353	1.282014175
354	1.276724919
355	1.276456243
356	1.27209605
357	1.255710024
358	1.250181443
359	1.225363451
360	1.207781266
361	1.188145758
362	1.152220816
363	1.124322156
364	1.090986109
365	1.05073979
366	1.005104451
367	0.967072298
368	0.93200589
369	0.873934603
370	0.835270686
371	0.789221726

Wavelength (nm)	Absorbance (a.u.)
372	0.746547702
373	0.704094845
374	0.663139981
375	0.613554431
376	0.575536142
377	0.53405014
378	0.495165916
379	0.454481949
380	0.417777269
381	0.384178233
382	0.354146335
383	0.328719543
384	0.303095159
385	0.276422036
386	0.250207874
387	0.237214789
388	0.216829489
389	0.192666589
390	0.175096701
391	0.16409021
392	0.155205495
393	0.140444537
394	0.131006184
395	0.11925796
396	0.113600193
397	0.110374145
398	0.097961758
399	0.093912381
400	0.089603299
401	0.085628057
402	0.076157523
403	0.071472463
404	0.071833583
405	0.073715655
406	0.073108157
407	0.067627436
408	0.072855638
409	0.066286414
410	0.06578354
411	0.064105646
412	0.064450892
413	0.065751014

Wavelength (nm)	Absorbance (a.u.)
414	0.065008784
415	0.059004456
416	0.067016305
417	0.057440171
418	0.061185785
419	0.059623327
420	0.056621175
421	0.059767542
422	0.057200324
423	0.059832641
424	0.060791411
425	0.064352105
426	0.060110673
427	0.056743213
428	0.057371428
429	0.059102482
430	0.058820115
431	0.06349125
432	0.063164724
433	0.056931096
434	0.05618113
435	0.049072913
436	0.056029479
437	0.054462151
438	0.056584395
439	0.059143772
440	0.058346129
441	0.057579361
442	0.05834644
443	0.057175313
444	0.058280102
445	0.046260469
446	0.056609912
447	0.057152234
448	0.056337911
449	0.056765829
450	0.059990599
451	0.055439943
452	0.065243863
453	0.059962208
454	0.057546407
455	0.05686391

Wavelength (nm)	Absorbance (a.u.)
456	0.057306466
457	0.054430996
458	0.057329744
459	0.058669732
460	0.059757027
461	0.059794647
462	0.05684348
463	0.052891712
464	0.055551131
465	0.063583115
466	0.061126472
467	0.059336065
468	0.05543222
469	0.058166014
470	0.053817003
471	0.056340665
472	0.05747678
473	0.057133684
474	0.061813783
475	0.053568151
476	0.055574399
477	0.053863372
478	0.054417006
479	0.059256442
480	0.0532967
481	0.061614222
482	0.058190659
483	0.059375457
484	0.060345206
485	0.057779356
486	0.06299659
487	0.058787865
488	0.061633606
489	0.058944868
490	0.060748292
491	0.060798554
492	0.060785133
493	0.056591631
494	0.061619679
495	0.059071074
496	0.062488317
497	0.052842913

Wavelength (nm)	Absorbance (a.u.)
498	0.055344584
499	0.061841192
500	0.067186786
501	0.063687394
502	0.058474974
503	0.055040927
504	0.06366274
505	0.060882172
506	0.063333181
507	0.061771371
508	0.061574272
509	0.071928974
510	0.061656142
511	0.061224581
512	0.061461725
513	0.059949769
514	0.065563445
515	0.060966137
516	0.064536823
517	0.063414314
518	0.066792255
519	0.064104441
520	0.058495366
521	0.058208287
522	0.067889435
523	0.060939159
524	0.058557387
525	0.060946012
526	0.067826369
527	0.058540249
528	0.060589377
529	0.061399691
530	0.059466042
531	0.059812022
532	0.058756014
533	0.062567716
534	0.063052417
535	0.062018679
536	0.058732815
537	0.069020852
538	0.060096035
539	0.065834771

Wavelength (nm)	Absorbance (a.u.)
540	0.061214015
541	0.067429047
542	0.06854978
543	0.062247331
544	0.058700761
545	0.067983981
546	0.072229151
547	0.067234064
548	0.066456478
549	0.068879322
550	0.068803585
551	0.063340305
552	0.064050744
553	0.068612845
554	0.066086132
555	0.071314686
556	0.064357875
557	0.069566828
558	0.068255658
559	0.066730732
560	0.071405894
561	0.069787499
562	0.064007004
563	0.069882387
564	0.072571413
565	0.067419802
566	0.067123559
567	0.065767668
568	0.068181478
569	0.070748529
570	0.065602797
571	0.063870265
572	0.067385342
573	0.063989447
574	0.070767832
575	0.067561299
576	0.063029013
577	0.068071058
578	0.068735354
579	0.071099858
580	0.069137541
581	0.076305338

Wavelength (nm)	Absorbance (a.u.)
582	0.068067863
583	0.069783511
584	0.069760345
585	0.069250004
586	0.070856408
587	0.072370985
588	0.075285818
589	0.065221913
590	0.069046985
591	0.069384897
592	0.074353521
593	0.069941706
594	0.064675928
595	0.071735147
596	0.071429257
597	0.072295409
598	0.068144847
599	0.077921205
600	0.070890269

XRD pattern of GT–ZnO nanoparticles.

2θ (degrees)	Intensity
20	224
20.1	193
20.2	232
20.3	276
20.4	188
20.5	188
20.6	278
20.7	238
20.8	176
20.9	227
21	176
21.1	176
21.2	212
21.3	104
21.4	113
21.5	171
21.6	149
21.7	215
21.8	154
21.9	129
22	273
22.1	188
22.2	203
22.3	128
22.4	172
22.5	205
22.6	142
22.7	218
22.8	169
22.9	185
23	169
23.1	292
23.2	199
23.3	147
23.4	241
23.5	138
23.6	210
23.7	102
23.8	133
23.9	209

2θ (degrees)	Intensity
24	236
24.1	208
24.2	194
24.3	184
24.4	126
24.5	164
24.6	176
24.7	252
24.8	217
24.9	111
25	216
25.1	180
25.2	166
25.3	230
25.4	251
25.5	246
25.6	158
25.7	184
25.8	216
25.9	248
26	176
26.1	190
26.2	144
26.3	140
26.4	240
26.5	267
26.6	196
26.7	250
26.8	218
26.9	167
27	218
27.1	276
27.2	198
27.3	278
27.4	69
27.5	241
27.6	204
27.7	185
27.8	204
27.9	100
28	189
28.1	217

2θ (degrees)	Intensity
28.2	273
28.3	174
28.4	159
28.5	174
28.6	245
28.7	216
28.8	173
28.9	225
29	204
29.1	248
29.2	164
29.3	183
29.4	180
29.5	126
29.6	214
29.7	213
29.8	200
29.9	188
30	129
30.1	178
30.2	182
30.3	159
30.4	191
30.5	220
30.6	294
30.7	208
30.8	212
30.9	196
31	107
31.1	218
31.2	282
31.3	566
31.4	774
31.5	1306
31.6	1786
31.7	1941
31.8	1845
31.9	1329
32	823
32.1	398
32.2	349
32.3	149

2θ (degrees)	Intensity
32.4	233
32.5	310
32.6	150
32.7	171
32.8	204
32.9	174
33	122
33.1	203
33.2	146
33.3	223
33.4	154
33.5	277
33.6	161
33.7	188
33.8	266
33.9	239
34	522
34.1	1012
34.2	1514
34.3	2238
34.4	2512
34.5	2268
34.6	1533
34.7	880
34.8	537
34.9	315
35	238
35.1	222
35.2	166
35.3	211
35.4	215
35.5	171
35.6	328
35.7	364
35.8	573
35.9	1271
36	2092
36.1	3063
36.2	3457
36.3	2982
36.4	2189
36.5	1259

2θ (degrees)	Intensity
36.6	674
36.7	435
36.8	223
36.9	169
37	156
37.1	159
37.2	196
37.3	217
37.4	213
37.5	241
37.6	200
37.7	272
37.8	186
37.9	336
38	231
38.1	157
38.2	146
38.3	224
38.4	188
38.5	235
38.6	223
38.7	196
38.8	157
38.9	124
39	177
39.1	242
39.2	210
39.3	137
39.4	208
39.5	219
39.6	155
39.7	207
39.8	202
39.9	142
40	217
40.1	228
40.2	254
40.3	252
40.4	131
40.5	153
40.6	225
40.7	225

2θ (degrees)	Intensity
40.8	225
40.9	392
41	228
41.1	256
41.2	247
41.3	232
41.4	184
41.5	237
41.6	161
41.7	188
41.8	175
41.9	204
42	315
42.1	106
42.2	234
42.3	119
42.4	176
42.5	254
42.6	203
42.7	146
42.8	164
42.9	233
43	163
43.1	210
43.2	202
43.3	167
43.4	307
43.5	231
43.6	98
43.7	209
43.8	166
43.9	242
44	160
44.1	194
44.2	225
44.3	243
44.4	139
44.5	183
44.6	176
44.7	167
44.8	288
44.9	220

2θ (degrees)	Intensity
45	136
45.1	245
45.2	306
45.3	251
45.4	124
45.5	175
45.6	263
45.7	164
45.8	222
45.9	238
46	153
46.1	197
46.2	38
46.3	149
46.4	188
46.5	142
46.6	294
46.7	162
46.8	256
46.9	368
47	571
47.1	621
47.2	985
47.3	1161
47.4	1286
47.5	1423
47.6	1345
47.7	1130
47.8	931
47.9	674
48	504
48.1	395
48.2	358
48.3	172
48.4	319
48.5	107
48.6	193
48.7	229
48.8	214
48.9	168
49	189
49.1	175

2θ (degrees)	Intensity
49.2	170
49.3	242
49.4	217
49.5	165
49.6	244
49.7	215
49.8	240
49.9	231
50	158
50.1	171
50.2	237
50.3	230
50.4	198
50.5	205
50.6	263
50.7	170
50.8	227
50.9	189
51	189
51.1	254
51.2	241
51.3	240
51.4	265
51.5	201
51.6	234
51.7	184
51.8	216
51.9	193
52	204
52.1	229
52.2	159
52.3	304
52.4	149
52.5	139
52.6	257
52.7	239
52.8	231
52.9	231
53	199
53.1	155
53.2	203
53.3	166

2θ (degrees)	Intensity
53.4	248
53.5	192
53.6	158
53.7	183
53.8	220
53.9	171
54	158
54.1	212
54.2	212
54.3	174
54.4	176
54.5	211
54.6	127
54.7	129
54.8	164
54.9	189
55	215
55.1	273
55.2	242
55.3	192
55.4	199
55.5	151
55.6	204
55.7	202
55.8	258
55.9	257
56	428
56.1	650
56.2	811
56.3	1129
56.4	1435
56.5	1598
56.6	1711
56.7	1619
56.8	1405
56.9	1071
57	817
57.1	598
57.2	475
57.3	346
57.4	350
57.5	178

2θ (degrees)	Intensity
57.6	249
57.7	210
57.8	309
57.9	159
58	158
58.1	170
58.2	93
58.3	173
58.4	162
58.5	207
58.6	217
58.7	293
58.8	247
58.9	171
59	155
59.1	224
59.2	133
59.3	291
59.4	258
59.5	176
59.6	114
59.7	267
59.8	194
59.9	261
60	120
60.1	170
60.2	200
60.3	202
60.4	177
60.5	231
60.6	146
60.7	192
60.8	206
60.9	225
61	235
61.1	143
61.2	123
61.3	263
61.4	216
61.5	162
61.6	277
61.7	206

2θ (degrees)	Intensity
61.8	262
61.9	214
62	331
62.1	353
62.2	322
62.3	497
62.4	643
62.5	874
62.6	952
62.7	1180
62.8	1252
62.9	1058
63	941
63.1	704
63.2	597
63.3	485
63.4	410
63.5	269
63.6	309
63.7	142
63.8	118
63.9	198
64	219
64.1	198
64.2	96
64.3	195
64.4	134
64.5	233
64.6	218
64.7	153
64.8	174
64.9	147
65	196
65.1	247
65.2	150
65.3	225
65.4	173
65.5	160
65.6	194
65.7	148
65.8	172
65.9	140

2θ (degrees)	Intensity
66	298
66.1	201
66.2	165
66.3	210
66.4	194
66.5	188
66.6	230
66.7	237
66.8	173
66.9	172
67	189
67.1	93
67.2	147
67.3	320
67.4	390
67.5	387
67.6	557
67.7	700
67.8	994
67.9	1012
68	993
68.1	908
68.2	760
68.3	695
68.4	491
68.5	328
68.6	275
68.7	198
68.8	307
68.9	252
69	202
69.1	274
69.2	204
69.3	157
69.4	276
69.5	226
69.6	148
69.7	190
69.8	156
69.9	130
70	246

Virucidal effect of GT–ZnO NPs ZnO-NPs (µg/mL)	Exp1	Exp2	Exp3	Mean log $TCID_{50}$/mL	SD
0	6.2	5.8	6	6.0	0.2
1	4.7	5.1	5.4	5.1	0.35
2.5	4.3	4.1	3.9	4.1	0.2
5	3.4	2.8	3.1	3.1	0.25
10	1.8	2.2	2	2.0	0.2
15	1.9	1.7	1.4	1.7	0.25
25	1.3	1	0.9	1.1	0.3

effect of zinc oxide nanoparticles (GT-ZnO) on viral infection	**Exp1**	**Exp2**	**Exp3**	**Mean log $TCID_{50}$/mL**	**SD**
0	**6.10**	**5.90**	**6.00**	**6.00**	**0.10**
1	**5.70**	**5.80**	**5.90**	**5.80**	**0.25**
2.5	**5.60**	**5.80**	**6.00**	**5.80**	**0.10**
5	**5.50**	**5.60**	**5.70**	**5.60**	**0.10**
10	**5.30**	**5.40**	**5.50**	**5.40**	**0.10**
15	**5.40**	**5.50**	**5.60**	**5.50**	**0.10**
25	**5.10**	**5.20**	**5.30**	**5.20**	**0.10**