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Review Article

Andrographolides as Antiviral Agents: Insights into Mechanisms, Modifications, and Delivery Innovations

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ABSTRACT

Viral infection remains a pressing global health challenge, underscoring the need for novel antiviral interventions. Recently, the potential of andrographolides as potent antiviral agents against major viral infections has garnered substantial attention. This comprehensive review highlights their antiviral activities, focusing on their inhibitory mechanisms and their effects on viral replication. This article also examines their semi-synthetic modifications, novel delivery systems, pharmacokinetic properties, and safety profiles. This review encompasses a wide range of viral infections, including influenza, HIV, HBV, HSV, etc. Key findings revealed the ability of andrographolides to impede viral entry, replication, and release, offering promising avenues for therapeutic intervention. Semi-synthetic modifications enhance antiviral efficacy and broaden the spectrum of action. Innovative delivery systems such as microspheres, liposomes, niosomes, and nanoparticles enhance bioavailability and target-specific delivery. Despite these efforts, research gaps persist, emphasizing the need for clinical validation, optimal dosing, and thorough safety assessment. Interdisciplinary efforts in molecular biology, pharmaceutical innovation, and clinical investigations will shape the future landscape of andrographolides as potent antiviral agents, bolstering global efforts against viral infections.

Keywords: *Andrographis paniculata*, andrographolides, antiviral activity, phytochemicals, viral infections

Introduction

The COVID-19 pandemic has shifted drug discovery from a "*one drug, one target*" approach to a "*one drug, multi-target*" strategy.[1] This transformation has led to the focus on active biological compounds, both synthetic and natural, with potential antiviral properties. Many of these compounds have been investigated for their effectiveness against SARS-CoV-2 infection. Among these approaches, repurposing natural phytochemicals from medicinal plants has emerged as a cost-effective and efficient method for identifying promising drug candidates.[2]

Andrographolide, an extract derived from various plants belonging to the *Andrographis* genus (family *Acanthaceae*), displays antimicrobial,[3] antiviral,[4] and anti-parasitic effects.[5] Notably, *Andrographis paniculata*, commonly referred as the '*King of Bitter*,' is the primary source of andrographolide and is found in regions including India, China, Sri Lanka, Malaysia, Japan, and Thailand at altitudes of up to 1000 m.[6] *A. paniculata*, an annual herb that grows between 30 to 110 cm in height, has white flowers with purple spots on the petals. The bitterness of bioactive components distinguishes this plant. Andrographolide can be extracted from *A. paniculata, A. lineata,* and *A. alata*, and it demonstrates solubility in organic solvents.[7]

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Andrographolides demonstrated potent antiviral activities (Table 1). For example, they offer protection against DNA and RNA viruses.[8] Computational analysis revealed that andrographolide could act as a potential inhibitor of SARS-CoV-2's main protease.[2] Another compound, 14-deoxy-11,12-dehydroandrographolide, effectively blocks the replication of influenza A virus, HIV, and HSV-1. Additionally, 14-deoxyandrographolide displays antiviral properties against HSV-1, HIV, and human papillomavirus (HPV).[9]

The antiviral potential of andrographolides and their analogs (Table 2) requires further study. The current knowledge regarding their modes of action and physicochemical attributes remains limited. Considerable research is required for the dosage forms of andrographolide and its derivatives in treatment design. Additionally, while research into drug delivery systems for these *A. paniculata* phytoconstituents has gained traction, there is still a need for heightened attention to enhance their bioavailability and bioactivity.[10]

To address these research gaps, this review aims to comprehensively examine the recent advances in the antiviral activities of andrographolides (and their derivatives) against major viral infections affecting global public health. Semi-synthetic modifications, delivery systems, pharmacokinetic properties, and safety profiles of andrographolides were also discussed. Three databases were used to search for relevant articles including PubMed, Google Scholar, and Scopus published from 2013-2023. The following keywords were used: "andrographolide antiviral", "*Andrographis* antiviral", and "andrographolide phytochemical".

Andrographolides: An Overview

The herb *Andrographis paniculata* has historical significance in traditional and clinical medicine across China, India, and Southeast Asian nations, such as Thailand and Vietnam.

Table 1: Mode of action of andrographolide and its derivatives on target viruses [6]

Name of viruses	In vitro/in vivo/in silico study	Mode of action
Herpes simplex virus	Vero cells	1. Reduces HSV-1-induced plaque formation
		2. Inhibits HSV-1 DNA replication and gp C and D expression
	Vero cells	Inhibits HSV-1 entry into the cell
	Vero cells	Andrographolide analogue, 3,19-isopropylidene andrographolide inhibits HSV-1 wild-type and drug resistant strains' DNA and protein synthesis
Human immunodeficiency virus	H9 cells	Increases CD4, T cell count
	MT2 cells	Reduces p24 antigen levels
Hepatitis B virus	HepG 2.2.15 cells	Inhibits HBV DNA replication
Hepatitis C virus	Huh 7 cells	Suppresses HCV genome replication by promoting IFNa response, viral HO-1 gene activity and inhibiting viral NS3/4A protease activity
	In silico study	Inhibits HCV NS3/4A protease and its drug-resistant mutants
Influenza A virus	BALB/c mice and MDCK cells	Inhibits H9N2, H5N1 and H1N1 virus both in vitro and in vivo
	DCs and macrophages	Inhibits the H1N1-induced RIG-1-like receptor signaling pathway
	MDCK cells	Inhibits H3N2 virus replication
Herpes simplex virus	Vero cells	1. Reduces HSV-1-induced plaque formation
		2. Inhibits HSV-1 DNA replication and gp C and D expression
	Vero cells	Inhibits HSV-1 entry into the cell
	Vero cells	Andrographolide analogue, 3,19-isopropylidene andrographolide inhibits HSV-1 wild-type and drug resistant strains' DNA and protein synthesis
Epstein Barr virus (EBV)	P3HR1 cells	Rta, Zta and EA-D is caused by the down regulation of the transcription of BRLF1 and BZLF1
Chikungunya virus (CHIKV)	HepG2 cells	Reduces viral RNA copy number and inhibits viral protein expression
Human Papillomavirus	CaSki cells	Inhibits E6 oncogenic envelope gp and restores tumor suppressor p53 protein
Zika virus	A549 cells	Down regulate HSPA1A, PGK1, TKT required for viral replication
SARS CoV2	In silico study	Main protease
	In silico study	3CLpro, PLpro, RdRp and spike protein
Dengue virus	HepG2 and HeLa	Reduces levels of cellular infection and virus output

Its application encompasses treating viral and bacterial infections, such as colds, cough, fever, sore throat, sores, and carbuncles.[11]

Since 1911, diverse compound categories, such as lactones, flavonoids, terpenoids, and diterpenoids, were reported across various components of plant species.[12] Labdane diterpenoid andrographolides, including 14-Deoxy-11,12-dehydroandrographolide and 14-deoxyandrographolide (Figure 1), have emerged as prominent bioactive compounds in this species.[13] Notably, andrographolide is primarily found in *A. paniculata* [1] and *A. lineata* leaves.[14] Nevertheless, *A. paniculata* remains the principal reservoir of this diterpene lactone, with functional bioactive properties.[15]

Antiviral Activities of Andrographolides

Influenza A Virus

Influenza A virus (IAV) affects both avian and human respiratory systems, fostering inflammation via cytokine elevation, especially in the alveolar compartment. This can lead to acute respiratory syndrome and mortality.[16] Viral transmission occurs through the air, with replication occurring in the respiratory tract epithelial cells.[17]

Investigations targeting *A. paniculata* and its principal compound, andrographolide, are known for their protection against influenza. Andrographolide inhibits gene replication and protein maturation phases of IAV (Figure 2), whereas the plant's second major constituent, 14-deoxy-11,12-didehydroandrographolide, inhibits viral entry. Moreover, both compounds curtail pro-inflammatory cytokine and chemokine expression caused by infection, thereby ameliorating IAV-induced lung pathology.[18]

IAV features two vital surface glycoproteins, neuraminidase (NA) and hemagglutinin (HA). NA facilitates receptor release via cleavage, whereas HA triggers infection by binding to sialic acid receptors on the host cells. Molecular docking revealed that andrographolide could bind to NA and HA, forming three and five hydrogen bonds, respectively. These interactions suggest that andrographolide interacts with viral infection-associated proteins. [19]

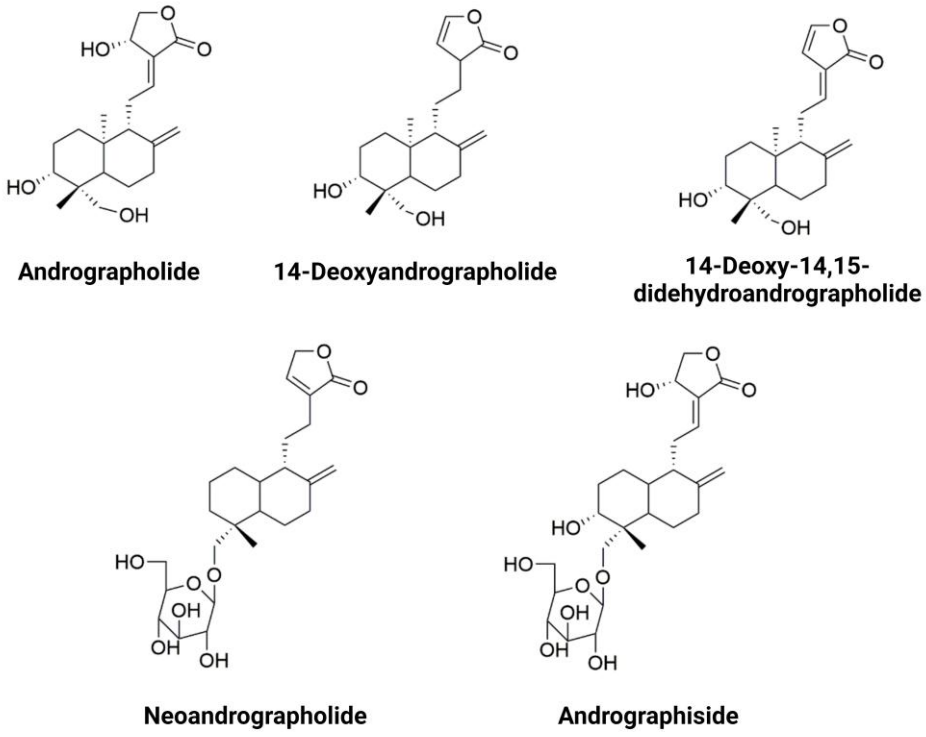

Figure 1: Structure of major andrographolides from *A. paniculata*

Concurrently, 14-deoxy-11,12-didehydroandrographolide demonstrated potent inhibition, effectively suppressing the replication of H5N1. Furthermore, this compound efficiently hinders the nuclear export process of viral ribonucleoprotein (vRNP) complexes during cellular replication. Consequently, this slowdown in cellular replication results in a significant decrease in the production of pro-inflammatory chemokines and cytokines.[20]

Furthermore, beyond their role in impeding viral production and replication, both andrographolide and 14-deoxy-11,12-didehydroandrographolide contribute to inflammation reduction by inhibiting cytokine and chemokine production triggered by IAV infection. Their actions involve various mechanisms, including inhibition of the NF-κB signaling pathway, which is pivotal for cytokine expression. Notably, both compounds downregulated JAK/STAT activation signals. The JAK/STAT pathway, including numerous interferon-stimulated genes (ISGs), is upregulated by IFNs and critically contributes to lung inflammation.[21]

Yu *et al.* [22] demonstrated that andrographolide displayed a significant viral inhibition activity of 43.90 ± 2.49% at a concentration of 250 µg/mL in HBE cell line. Moreover, 14-deoxy-11,12-didehydroandrographolide displayed IC_{50} values of 38 ± 1 and 5 ± 1 µg/mL for mitigating the cytopathic effect induced by IAV infection in MDCK and A549 cells, respectively.[22]

Human Immunodeficiency Virus

HIV, which belongs to the *Retroviridae* family, exhibits a 120 nm spherical morphology. HIV can lead to acquired immunodeficiency syndrome (AIDS) by infiltrating the immune system, which is characterized by the weakening of the immune system and susceptibility to rapid-onset diseases. Viral infection is initiated through the binding of the glycoprotein gp120, the "*hand*" of HIV, to the host cell's CD4 receptor. This process requires the participation of surface co-receptors on T cells, specifically CCR5 and CXCR4.[23]

Sixteen Chinese herbal medicines, including andrographolide, were analyzed both in vitro and in vivo to evaluate their potential to suppress CCR5 and CXCR4 coreceptors in T cells (Figure 2). Remarkably, the 70% ethanolic extract of *A. paniculata* demonstrated significant inhibition of the CCR5 and CXCR4 promoters. This extract also exhibited the ability to hinder decrease in p24 antigen levels and cell fusion, indicative of immunological failure. Human T cell-based clinical trials revealed a flow cytometry-observed reduction in CCR5 and CXCR4 levels, decreasing from 25% to 10% and 35% to 10%, respectively, upon *A. paniculata* extract administration.[24]

During the same time frame, andrographolide exhibited the capacity hindered gp120-mediated cell fusion. Molecular modeling indicated the binding of andrographolide to the V3 loop of gp120. This interaction suggests its potential use as an anti-HIV agent.[25] Moreover, both 14-deoxy-11,12-didehydroandrographolide and andrographolide display noteworthy anti-HIV activity.[26]

Herpes Simplex Virus

The herpes simplex virus is the most prevalent virus among humans within the *Herpesviridae* family. This virus targets both the skin and mucosal epithelial cells, leading to blister formation in areas such as the lips, mouth, throat, and genitals. In the ocular region, HSV infection can result in acute retinal necrosis, conjunctivitis, iridocyclitis, and keratitis. The conventional treatment for HSV involves acyclovir (ACV); however, resistance to ACV has emerged recently.[27] To combat this evolved virus, novel drug candidates are required for effective HSV treatment.

Andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide displayed virucidal effectiveness against HSV-1, without causing notable cytotoxic effects at inhibitory concentrations (Figure 2).[28] Moreover, both andrographolide and 14-deoxyandrographolide intervene in HSV's entry stage of HSV and curtail viral production during replication. These actions involve the disruption of early gene expression, modulated by glycoproteins C and D.[29]

SARS-CoV-2

The global impact of the SARS-CoV-2 pandemic has affected virtually every nation, primarily targeting the respiratory system and demonstrating rapid transmission. Given the likelihood of their ongoing presence, therapeutic interventions remain active. Various methods, including computational techniques and *in vitro*

investigations, are being explored to identify potential drugs against SARS-CoV-2.[2]

An effective approach to impeding SARS-CoV-2 involves targeting its key proteases: the main protease (Mpro), papain-like protease (PLpro), and 3C-like proteinases (3CLpro). These viral proteases play vital roles in generating functional polyproteins that are essential for viral replication. Moreover, the virus relies on ACE2 for entry into the host cell. Inhibition of non-structural protein formation in SARS-CoV-2 curtails viral replication, whereas ACE2 receptor blockade prevents viral entry into the host cell.[30]

Recent computational investigations have also pinpointed other essential components of SARS-CoV-2, including its main enzyme, spike glycoprotein, and cellular receptor, using *in silico* techniques (Figure 2). The spike glycoprotein, a viral antigen in SARS-CoV, assumes the role of binding to host receptors, facilitating viral internalization, and eliciting robust cellular and humoral immune responses during infection.[31] Notably, the spike glycoprotein and ACE2 receptor display Moldock scores of 98.80 and 99.354 kcal/mol, respectively.[32] Noteworthy molecular docking studies have revealed the interaction of andrographolide with the SARS-CoV-2 main protease.[33] Furthermore, four key constituents of *A. paniculata*, namely 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, neoandrographolide, and andrographolide, demonstrated the potential to counteract the viral spike protein, 3CLpro, PLpro, and RNA-dependent RNA polymerase (RdRp). These viral components govern host cell recognition, replication, and transcription.[34]

Recent experiments using Calu-3 cells infected with SARS-CoV-2 have revealed noteworthy findings. *A*ndrographolide significantly inhibits infectious virion production.[30] Furthermore, andrographolide's impact extends to suppressing the Mpro activity of both SARS-CoV-2 and SARS-CoV.[35]

Dengue Virus

The persistent threat of dengue virus (DENV) remains even after establishing endemicity in numerous countries over the years. It is transmitted through *Aedes albopictus* and *A. aegypti* mosquitoes.[36] The dengue virus can cause various symptoms, including dengue shock syndrome, dengue hemorrhagic fever, and self-limiting febrile illness.[37] Regrettably, therapeutic options for dengue are limited. Exploring drug possibilities derived from natural sources offers a promising avenue for the fight against this disease.

A. paniculata, a herbal plant with andrographolide as its key constituent, has been investigated for its potential anti-dengue activity (Figure 2).[38] During its inhibitory phase, andrographolide effectively dampens post-infection viral production, leading to reduced viral infections. However, at the virus entry stage, andrographolide administration demonstrated limited inhibition of HeLa and HepG2 cell lines, implying that andrographolide does not substantially prevent DENV from infecting cells.[39]

Moreover, in the course of its inhibitory mechanism, andrographolide triggers an increase in heme oxygenase-1 (HO-1) levels. HO-1, an inducible enzyme within the heme catabolic pathway, serves as a defence against oxidative stress induced by the generation of reactive oxygen species (ROS) during DENV infection. The induction of HO-1 also impedes RNA replication and protein synthesis across DENV serotypes 1–4.[40]

In the evaluation of andrographolide's efficacy as a potential anti-DENV agent across various cell lines, a minimal non-toxic dose of (15.62 µg/mL) exhibited a remarkable 97.23% inhibition against DENV2.[41] Furthermore, andrographolide has demonstrated substantial reduction in virus production and cell infection.[39,42]

Chikungunya Virus

Wintachai *et al.* [43] revealed that andrographolide effectively inhibited chikungunya viral genome replication and intervened in the post-viral entry phase (Figure 2). However, the precise molecular mechanism remains unclear. The impact of andrographolide encompasses the hindrance of genome replication and protein synthesis, potentially suggesting its involvement at an earlier replication stage.[43] Through Western blotting and confocal microscopy analyses, complete suppression of CHIKV E1:226AS protein expression and comprehensive inhibition of CHIKV E1:226VS protein expression were observed.

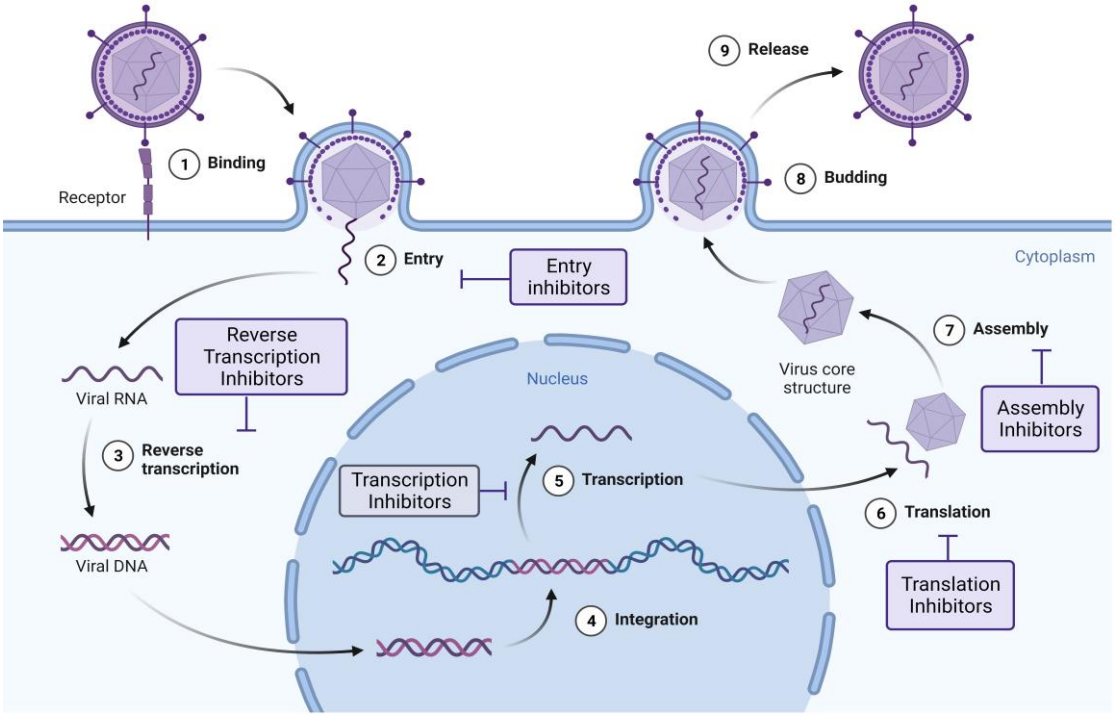

Figure 2: Inhibitory effects of andrographolide on the viral life cycle

In a study by Wintachai *et al.* [44], andrographolide displayed the ability to impede CHIKV infection, resulting in diminished viral titers while maintaining cell viability. This inhibitory effect on viral replication was consistent across the various cell types. Andrographolide's impact was most prominent when administered post-entry, showing its role in reducing protein synthesis and RNA genome levels.[44] Variations in inhibition were noted among the different strains, implying that andrographolide could directly target the CHIKV genome, proteins, or infection-induced proteins. These findings suggest that andrographolide acts synergistically through multiple pathways.

Andrographolide has been demonstrated to hinder the fusion of terminal autophagosomes with lysosomes, thus interfering with autophagic flux. This action influences CHIKV replication and contributes to the elimination of viral proteins.[43] Virus-induced autophagy, which is essential for viral replication, relies on interactions between viral and host cell proteins. The target of andrographolide might encompass the CHIKV genome or proteins, including those induced by infection.[45] These investigations indicate that andrographolide's multi-target approach can be used as a potent antiviral agent.

Zika Virus

The Zika virus (ZIKV), categorized within the *Flavivirus* genus, presents a significant health concern across tropical and subtropical regions. Li *et al.* [46] assessed the anti-ZIKV potential of 14-aryloxy analogs (ZAD-1 to ZAD-3) derived from andrographolide. Among these analogs, ZAD-1 exhibited superior activity against ZIKV than the parent compound (Figure 2). Exposure to ZAD-1 induced distinctive changes in the expression of key proteins, including GTP-binding nuclear protein Ran (Ran), phosphoglycerate kinase 1 (PGK1), transketolase (TKT), and heat shock 70 kDa protein 1 (HSPA1A). Notably, ZAD-1 treatment upregulated PGK1 and downregulated HSPA1A. These pathways have direct implications for viral replication.[46] Andrographolide regulates various cellular processes such as autophagy,[47] oxidative stress,[22] and mitochondrial function.[48]

Additional potential mechanisms of action of andrographolide derivatives involve interactions with glucose-regulated protein 78 (GRP78),[38] heme oxygenase I,[40] and NF-kB.[49] These findings suggest that the 14-aryloxy analogs of andrographolide exhibit promise for further applications as anti-ZIKV drugs.

Hepatitis B Virus

Hepatitis B virus (HBV) is a DNA virus belonging to the *Hepadnaviridae* family and *Orthohepadnavirus* genus that causes hepatitis B, a disease that affects hepatocytes and results in cirrhosis, fibrosis, and hepatocellular carcinomas. HBsAg can be present either as a viral particle-bound protein or as an independent noninfectious protein. Prenatal viral exposure can establish a symbiotic relationship between HBV and host immune cells, potentially contributing to its widespread prevalence in humans.[50]

HBV transmission occurs primarily via percutaneous, sexual, and perinatal routes, and via blood transfusion, although transmission patterns vary globally.[51] HBV infection triggers CD8 and CD4 T-cell and B-cell responses. Nevertheless, the existence of regulatory T cells (Tregs) obstructs efficient immune reactions, resulting in persistent infection.[52] The immune targets of HBV include Tregs, NK cells, DCs, and the IFN pathway.[53]

Numerous chemical and natural metabolites have been approved for treating HBV.[54] The emergence of side effects and viral resistance from existing approved drugs has prompted the exploration of novel anti-HBV agents.[55] Chen *et al.* [56] synthesized 48 derivatives of dehydroandrographolide and andrographolide and studied their impact on HBV. Their findings revealed that certain andrographolide derivatives also display anti-HBV effects through inhibition of HBV DNA replication.[56]

Hepatitis C Virus

Hepatitis C virus (HCV) is an RNA virus with a single-stranded, positive-sense genome, categorized within the *Flaviviridae* family and *the Hepacivirus* genus. Predominantly targeting hepatocytes, HCV has also been identified to infect peripheral mononuclear lymphocytes, particularly CD81-expressing B cells.[57] HCV infection leads to liver cirrhosis, hepatocellular carcinoma, chronic hepatitis, and extrahepatic infections linked to B-cell non-Hodgkin lymphoma.

Dynamic interactions between hosts and pathogens trigger immunity to combat viral infections. The production of IFNs is activated in response to HCV infection, which impedes viral proliferation. Nonetheless, HCV has evolved strategies to counteract the host antiviral immune defenses. For instance, HCV NS4B impedes stimulation of the IFN gene to reduce IFN production.[58] Immune cells also reduce the viral production in the host. Although CTLs can suppress HCV replication, they can also contribute to liver damage, fostering chronic HCV infection.[59] Therefore, dual-action antiviral drugs are required to suppress viral replication and stimulate host antiviral immune responses.

Numerous compounds targeting HCV have been examined for their ability to inhibit cyclophilin activity, NS3/4A protease, and viral polymerase (Figure 2).[60] The rapid development of HCV resistance has escalated the quest for novel antiviral agents. Lee *et al.* [61] conducted a study to combine andrographolide with IFN-α, telaprevir, and PSI-7977 to develop a potent antiviral drug.[61] Their findings revealed significant synergistic effects of andrographolide and these drugs. Andrographolide induction of HO-1 results in elevated liver biliverdin levels, intensifying the IFN response, and suppressing HCV replication.[61]

Chandramohan *et al.* [62] also identified andrographolide as an inhibitor of NS3/4A protease of HCV through molecular docking assessments.[62] The results showed the robust binding capacity of andrographolide to target proteins, minimally perturbing the protein backbone structure.[62] The multifaceted inhibitory mechanisms exhibited by andrographolide against HCV activity highlight its potential as a promising candidate for further exploration.

Epstein-Barr Virus

Epstein-Barr virus (EBV), classified within the *Herpesviridae* family and *Lymphocryptovirus* genus, exhibits distinctive characteristics. This linear double-stranded DNA virus, which measures 120–150 nm, generates icosahedral virions. With a diameter spanning 100-200 nm, the EBV nucleocapsid encapsulates a 172 kb genome. While predominantly targeting B cells and salivary gland epithelial cells, EBV can infect T cells, NK cells, and smooth muscle cells under certain conditions. It is transmitted through saliva, blood, and genital secretions, often causing mononucleosis or the "*kissing disease*" due to saliva-based transmission.[63] Notably, EBV is transmitted within epithelial cells via cell-in-cell structures that are formed upon contact. In epithelial cells, EBV follows a replication process culminating in the lytic cycle, eventually leading to viral transmission to B cells.[64]

EBV infection gives rise to a spectrum of symptoms including rash, fatigue, sore throat, weakness, and swollen glands. In addition to acute infectious mononucleosis, EBV plays a pivotal role in various malignancies such as nasopharyngeal carcinoma, Burkitt's lymphoma, gastric carcinoma, Hodgkin's lymphoma, lymphomatoid granulomatosis, and multiple sclerosis. This virus employs a dual approach to circumvent immune response. EBV triggers the activation and proliferation of B cells by infecting immune cells and utilizing latent membrane proteins. Simultaneously, these proteins curtail the efficacy of $CD8^+$ T cells in targeting EBV-infected cells.[65] Additionally, EBV orchestrates apoptosis induction in a caspase-dependent manner and impairs dendritic cell differentiation.[66]

During the immediate early phase of the lytic cycle, EBV employs two transcription factors, Rta and Zta, which activate early and late gene transcription, respectively, thus facilitating the progression of the lytic cycle (Figure 2). Inhibiting the expression of these proteins is vital for preventing EBV propagation. The efficacy of andrographolide against EBV was assessed under the influence of lytic cycle-inducing chemicals. Lin *et al.* [67] evaluated the impact of both *A. paniculata* ethanolic extract and andrographolide, revealing their capacity to hinder EBV lytic proteins, including Rta, Zta, and EA-D. Recent exploration also revealed that this inhibition stemmed from the suppressed transcription of two immediate-early genes, BRLF1 (encoding Rta) and BZLF1 (encoding Zta).[67]

Human Papilloma Virus

Human papillomavirus (HPV) is a compact, non-enveloped DNA virus with a diameter of approximately 55 nm. Classified within the *Papillomaviridae* family and *Alphapapillomavirus* genus, the HPV capsid is characterized by icosahedral symmetry.[68] HPV transmission primarily occurs through anal, oral, and vaginal sexual contact, with cellular entry being facilitated by clathrin-mediated endocytosis. HPV infects basal epithelial cells via abrasions or wounds, leading to the development of genital warts and subsequent cancers in regions such as the vaginal, cervical, vulvar, anal, penile, and oropharyngeal areas.[69]

To evade immune recognition, HPV employs strategies that disrupt immune cell differentiation, which results in immune tolerance. These modifications have various effects such as promoting tumor-associated macrophage differentiation, impairing cellular immune responses, causing an imbalance between Th1 and Th2 cells, facilitating Treg infiltration, and inhibiting the activation and maturation of dendritic cells (DCs).[68] However, innovative vaccine approaches, such as those centered on delivering the L1 gene of HPV (AcHERVHPV), have been assessed in cell lines and mouse models, revealing robust induction of both cellular and humoral immune responses.[69] Additionally, exploration of compounds capable of suppressing viral genes and rejuvenating tumor suppressor proteins offers a promising avenue for achieving potent antiviral effects against HPV.

The potential of andrographolide as an anti-HPV agent was also investigated (Figure 2). Ekalasananan *et al.* [9] explored the effects of andrographolide and its derivatives (14-DDA and IPAD) on HPV16 pseudovirus (HPV16PsVs), HPV E6 oncogene expression, and apoptosis.[9] Their findings revealed that andrographolide derivatives exhibit inhibitory effects on HPV16 infection, including the induction of apoptosis in cervical cells, restoration of the p53 tumor suppressor protein, and E6 oncogene suppression. Moreover, these compounds hindered the binding of HPV16PsVs to host cell receptors, with 14-DDA demonstrating the highest potency for post-attachment inhibition.[70]

Semi-Synthetic Modification of Andrographolides Towards Improved Antiviral Activity

The current focus on bioengineering active phytomolecules and their constituents underscores a rapidly increasing approach in the creation of antiviral drugs. Notably, this strategy involves purposeful modifications to the core structure of AGL, incorporating emerging trends such as novel structural attributes optimized for receptor interactions and the integration of metabolically active compounds such as lipoic acid to enhance analog reach and induce targeted biological actions, effectively functioning as prodrugs.[71] The resulting modified analogs and derivatives were subsequently subjected to *in vitro* assessments targeting a diverse array of viral infections, as detailed below.

Extensive research has demonstrated the robust antiviral capabilities of andrographolides and their constituents against a spectrum of disease-causing viruses, including notable pathogens such as SARS-CoV, MERS-CoV, and flaviviruses.[72] A key derivative, 14-DDA, derived from AGL, exhibited noteworthy activity against diverse viral infections.[73] Alterations at C-3, involving OH-to-NOH substitutions and amides, led to a superior therapeutic index (TI), displaying moderate efficacy compared to the parent compound. Notably, modifying 13,14-dihydroandrographolide through-lactone to N-methyl–lactam yielded remarkable HIV efficacy, achieving almost half the activity of andrographolides while doubling the TI.[74]

Numerous andrographolide derivatives have demonstrated antiviral efficacy and pre-infection-preventive potential.[75] Table 2 provides an overview of various andrographolide analogs and their derivatives, showing their activity levels (CC_{50}, EC_{50}, SI/TI) against target viruses in specific cell lines.

Various modified derivatives of andrographolides have shown positive antiviral effects against diverse viruses. For instance, 12-ester 12-hydroxy-14-deoxy-13,14-dehydroandrographolide and andrographolic acid amide derivatives exhibited efficacy against HIV.[76] Unique analogs, such as 14-a-Lipoylandrographolide, effectively targeted H9N2, H5N1, and H1N1 influenza strains by hindering viral adsorption and interfering with haemagglutinin interactions [56]. HBV was inhibited by 2-thiophenic and furoic acids or esterified 19-C derivative of 14-DDA with pyridinecarboxylic acid, leading to suppressed antigen expression and DNA replication.[75] In the case of H3N2 influenza, the benzyl amino derivative 19-dihydroxyl-17-(N-benzylamino)-7, 13-ent-labdadien-15, 16-olide displayed enhanced potency compared to andrographolides.[77] Conjugated double bonds, aromatic moieties, and free -OH groups were found to enhance the anti-HBV activity.[75] Notably, 3,19-isopropylidenyl andrographolide exhibited effects on viral replication-associated genes and proteins, possibly due to its structural resemblance to the anti-DNA replication compound.[78] Andrographolides and their derivatives are being extensively explored and are recognized as semi-synthetic antiviral agents with promising pharmaceutical applications.

Table 2: AGL analogs and derivatives against target viruses [71]

AGL Analogues $/ Derivatives #	**Virus**	**Targeted Cells**	**CC50 (µM)**	**EC50 (µM)**	**Cytotoxicity Assay**	**Selective Index (SI)/Therapeutic Index (TI)**
14-aryloxy analogues ZAD-1 $	ZIKV DENV	BHK-21	136.3 ± 6	27.9 ± 1.7	MTT Assay	9.8 (SI)
		Vero	510.3 ± 53			
		A549	272.9 ± 22	22.6 ± 1.8		6.6 (SI)
		HEK293T/17	148.8 ± 40			
14-aryloxy analogues ZAD-2 $		BHK-21	217.7 ± 16	-		-
		Vero	179.2 ± 13			
		A549	194.2 ± 17			
		HEK293T/17	196.8 ± 7			
14-aryloxy analogues ZAD-3 $		BHK-21	175.0 ± 7	-		-
		Vero	186.1 ± 25			

			190.1 ± 22			
			201.4 ± 25			23.74 (TI)
3-*O*-Nicotinoyl-19-*O*-(*n*-decanoyl)-dehydroandrographolide #			103.5 ± 128.73	82.89 ± 7.33		34.07 (TI)
3-*O*-Nicotinoyl-19-*O*-(1-naphthalene acetyl)-dehydroandrographolide #	Human immunodeficiency virus HIV-1		>200	5.87 ± 1.96		18.02 (TI)
3-*O*-Nicotinoyl-19-*O*-phenylacetyldehydroandrographolide		C8166	>200	11.34 ± 2.56	MTT Assay	16.8 (TI)
3-*O*-Nicotinoyl-19-*O*-(3,4-dimethoxyphenylacetyl) dehydroandrographolide			>200			
3-*O*-Nicotinoyl-19-*O*-(3,4-dimethoxyphenylacetyl) dehydroandrographolide			>200	11.76 ±3.66		
14-deoxyandrographolide (DAD) $			80			
3,19-isopropylideneandrographolide (IPAD) $	Herpes simplex virus type 1 (HSV 1)		40			
3,19-dipalmitoylandrographolide $			4.2	-		-
14-acetyl-3,19-isopropylideneandrographolide $		Vero	5.9		Anti-HSV-1 assay	
3,14,19-triacetylamdrographolide $			6.4			
14-dehydroxyandrographolide- 12-sulfonic acid sodium salt (DASS) #	H9N2 H5N1 H1N1	MDCK	3720 ± 725	142.2 ± 10.3 222.9 ± 14.9 171.1 ± 13.5	MTT Assay	26 (SI) 17 (SI) 22 (SI)
14-a-lipoyl andrographolide (AL-1) #			785 ± 330	8.4 ± 2.4 15.2 ± 4.09 7.2 ± 1.5		93(SI) 51(SI) 109(SI)
19-*O*-(3′, 4′, 5′-Trimethoxy) cinnamoyl dehydroandrographolide #			>1706			165.1 (SI)
19-*O*-(2′-Thenoyl)-14-deoxy-14,15-didehydroandrographolide #	Hepatitis B (HBV)	HepG2 C8166	2466		MTT Assay	104.9 (SI)
19-*O*-Nicotinoyl-14-deoxy-14,15-didehydroandrographolide #			2054	-		126.0 (SI)
19-*O*-Cinnamoyl dehydroandrographolide #			183			15.5 (SI)
3,19-(30-Nitrobenzylidene)-andrographolide #			745		MTT Assay	1460 (TI)
14-(20,60-Dichloronicotinoyl) ester of andrographolide #	Human immunodeficiency virus (HIV)	TZM-bl cells	10354	-		12,474 (TI)
(14α)-(Quinolyl-5′,7′-dichloro-8′-	ZIKV	SNB-19 Vero	88.7 ± 1.1	4.5 ± 0.2	ZIKV titer	19.7 (SI)

oxy)-19-acetoxyandrographolide #			85.0 ± 1.6		assay	18.9 (SI)
14β-(8′-quinolyloxy)-3,19-diol #			22.7 ± 1.1 20.8 ± 0.5	1.3 ± 0.1		>16
(14β)-(Quinolyl-5′,7′-dichloro-8′-oxy)andrographolide #			>100	13.3 ± 0.5		>7.5
(14α)-(Quinolyl-5′,7′-dichloro-8′-oxy)andrographolide #			85.2 ± 1 82.5 ± 2.2	7.8 ± 0.4		10.9 7.5
3,19-isopropylideneandrographolide (IPAD) $	HSV 2 HSV 1-KOS HSV 1-dxpIII	Vero	39.71	-	Anti-HSV-1 assay	2.20 (SI) 2.34 2.32

Delivery Systems for Andrographolides

However, the use of andrographolide as an antiviral agent remains limited. While *in vitro* studies have demonstrated its potential against diverse diseases,[18] this promise is curtailed by its limitations. Challenges arise when transitioning from *in vitro* to *in vivo* applications owing to the suboptimal bioavailability of andrographolide, hindering its efficacy.[79] Its absorption and blood concentration are often insufficient to achieve optimal treatment outcomes.

To address these issues, the implementation of delivery systems has sought to enhance the bioavailability of andrographolide and extend its half-life in the bloodstream.[79] Without a carrier, drug compounds exhibit brief half-lives as the body absorbs them based on their capacity upon entry, often leading to excretion.[80] Consequently, researchers have developed andrographolide delivery approaches. Various systems such as microspheres, microemulsions, liposomes, noisomes, and nanoparticles have been explored to enhance their delivery and biocompatibility.[80] For detailed examples, please refer to Table 3.

Microsphere

Microspheres are spherical microparticles spanning 1–1000 µm, which possess an elevated surface area and surface-to-volume ratio, rendering them efficient adsorbents and drug carriers.[81] The integration of andrographolide into microspheres, particularly using polylactic co-glycolic acid (PLGA) as a biodegradable polymer, offers a viable strategy for controlled delivery.[82]

The solvent emulsion evaporation technique is commonly employed to prepare andrographolide-loaded PLGA microspheres, leading to sustained-release profiles that enhance the bioavailability of andrographolide. *In vitro* assessments demonstrated sustained release of andrographolide over 9 days from PLGA microspheres with a diameter of 53.18 ± 2.11 µm. In contrast, pure andrographolide exhibits a rapid decline in plasma concentration shortly after administration.[82]

The extended-release kinetics of andrographolide from microspheres result from polymer diffusion and erosion of the polymer layer on the microspheres.[83] However, certain limitations persist with andrographolide-loaded microspheres, such as challenges in solvent removal during microsphere preparation, which, if inadequately executed, may lead to cellular toxicity.[82]

Microemulsion

Microemulsions are thermodynamically stable dispersions of water, oil, and surfactants with domain sizes of approximately 10–100 nm, offering a strategy to enhance oral drug bioavailability.[84] The incorporation of andrographolide into microemulsions is a promising approach to overcome its low oral bioavailability by exploiting its solubility in both polar (water) and nonpolar (oil) phases.[85]

Various microemulsion formulations have been investigated for encapsulation of andrographolide. Du *et al.* [86] employed alcohol, aqua bides, isopropyl myristate, and Tween 80,[86] Sermkaew *et al.* [87] utilized labrasol, cremphor, and capryol,[87] while Syukri *et al.* [88] adopted capryol, polyethylene glycol (PEG) 400, and Tween 20 as microemulsion components.[88] The solubility of andrographolide in these microemulsions displayed remarkable improvement, demonstrating stability across various conditions and enhanced bioavailability.

An *in vivo* assessment by Syukri *et al.* [88] demonstrated that andrographolide-loaded microemulsions had significantly enhanced bioavailability compared to free andrographolide.[88] Interestingly, the LD_{50} of the andrographolide-microemulsion was 138.36 mg/kg, approximately 266 times the daily oral dose for adult Kunming mice (0.52 mg/kg). Augmented solubility and bioavailability enabled dose reduction, mitigating the potential toxic effects associated with oral administration.[86]

Liposomes

Liposomes are artificial vesicles comprising a lipid bilayer membrane that offers an effective drug delivery approach to enhance cell membrane penetration and solubility.[89] Liposomes can be formulated with andrographolide using cholesterol, diacetyl phosphate (DCP), and phosphatidylethanolamine (PDEA) prepared via the thin-film hydration method.[89] Alternatively, soybean phosphatidylcholine (SPC), cholesterol, and DSPE-PEG2000-Mal followed by the addition of andrographolide and dissolution in ethanol (1:20 w/w), can be used to formulate liposomes.[90]

Andrographolide encapsulated within liposomes exhibits improved tumor tissue accumulation and deep intratumoral penetration.[91] Andrographolide-loaded liposomes demonstrated enhanced cytotoxicity compared with free andrographolide. In lung cancer cells, apoptosis rates were 39.9, 45.3, and 69% for free combination drugs (doxorubicin and andrographolide), DSPE-PEG2000-modified liposomes (Lipo-PEG), and cell-penetrating peptide-modified liposomes (Lipo-CPP), respectively.[90]

Table 3: Various delivery systems for andrographolides [10]

Type of Drug Delivery	Formulation	Method	Biocompatibility Aspects
Microsphere	PLGA (polylactic co-glycolic acid) and andrographolide	Emulsion solvent evaporation	Prolonged release (up to nine days) Increases the half-life of andrographolide
Microemulsion	Alcohol, Tween 80, isopropyl myristate, water, and andrographolide	Spheronization technique	Increases the solubility Stabilized over time, temperatures, and different gravity states Low acute oral toxicity
	Capryol, cremphor, labrasol, and *A. paniculata* extract	Extrusion/spheronization technique	Slow release of andrographolide Increases the oral absorption
	Capryol, Tween 20, PEG (polyethene glycol) 400, and andrographolide	Spheronization technique	Increases the stability, and improves the andrographolide bioavailability
Liposome	Phosphatidylethanolamine (PDEA), cholesterol, and dicetyl phosphate (DCP)	Thin-film hydration method	Higher cytotoxic effect Increases the accumulation in tumor tissue
	Soybean phosphatidylcholine (SPC), cholesterol, and DSPE-PEG2000-Mal		Increases the solubility of andrographolide
Niosome	Span 60 (50 mg), cholesterol (7.35 mg), and andrographolide (5 mg)	Film hydration/sonication method	Increases the andrographolide absorption Reduce toxicity
Nanoparticles	Compritol 888 ATO, Brij 78, and andrographolide	Emulsion/evaporation/solidifying	Expands the tissue distribution Excellent physical and chemical stability during storage Slow-release effect
	PLGA (poly(lactic-co-glycolic) acid) and andrographolide	Emulsion evaporation	Slow-release effect

Niosomes

Niosomes represent a novel drug delivery approach that offers tissue distribution and improved drug bioavailability.[92] These vesicles, formed from non-ionic surfactants with hydrophobic tails and hydrophilic heads, were employed to encapsulate drug compounds. Encapsulation of andrographolide within niosomes enhances tissue distribution and bioavailability. Niosomes are prepared using the film hydration/sonication method with cholesterol (7.35 mg), Span 60 (50 mg), and andrographolide (5 mg).[93] After hydration with 20% PEG and sonication, the particle size was reduced to 206 nm, which is suitable for liver targeting.[94]

Andrographolide niosomes achieved 72.36% encapsulation efficacy and a 5.90% drug-loading ratio. HPLC analysis revealed that the broad tissue distribution of andrographolide was facilitated by niosomes, owing to their bilayer membrane resembling cell membranes. Consequently, niosomes enhance cell penetration and are well-suited for intended applications.[93] Notably, encapsulation reduces drug toxicity [93]. Despite the drawback of a low drug-loading ratio requiring high doses, both andrographolide-loaded niosomes and free andrographolide show comparable activity against hepatocellular carcinoma (HCC).[93] Unlike liposomes, niosomes do not significantly amplify andrographolide activity, but increase the bioavailability of andrographolide.

Nanoparticles

To address the limitations of a low drug-loading ratio in niosomes, nanoparticles have emerged as an effective drug-delivery solution.[95] For andrographolide delivery, solid lipid nanoparticles (SLN) were synthesized using Brij 78 as the surfactant and Compritol 888 ATO as the solid lipid. This process involved solidification, evaporation, and emulsification.[96] Additionally, polymer-based nanoparticles using PLGA have been developed using the single emulsion evaporation method.[97]

SLN exhibited a remarkable encapsulation efficiency of 92% and particle sizes ranging from 262 to 278 nm. Nanoparticles enable broad tissue distribution, even crossing the blood-brain barrier.[96] The stability of andrographolide-loaded nanoparticles is robust; storage for 30 days as an aqueous dispersion or lyophilized powder at 25 °C and 4 °C showed negligible changes in zeta potential value, polydispersity index (PDI), and particle size.[96]

Moreover, nanoparticle-embedded andrographolide achieves controlled release owing to the dissolution system, which involves andrographolide dissolution and diffusion from the nanoparticle matrix. The release rate of andrographolide-loaded nanoparticles is intermediate, slower than that of the solution form, yet faster than the suspension form.[98] This nanoparticle approach overcomes the limitations of drug loading and offers enhanced stability and controlled release, rendering it a promising strategy for andrographolide delivery.

Pharmacokinetic Properties of Andrographolides

Investigations of andrographolide remain at an early stage, confronting challenges in optimizing therapeutic applications and enhancing bioavailability. Notably, its inadequate pharmacokinetic profile, which

is characterized by rapid absorption, metabolism, and elimination, contributes to its limited accessibility.[11] Addressing the low bioavailability of andrographolide has spurred the exploration of its derivatives and advanced methodologies.[99,100]

Drug efficacy can be influenced by pharmacokinetics, including drug passage through the body. Numerous investigations have examined the pharmacokinetic (PK) properties of andrographolide. In Wistar rats orally administered andrographolide at 100 mg/kg/day, it was noted that the peak andrographolide concentration in the kidney, followed by the spleen, liver, and brain, while similar levels were observed in the lungs and heart.[101] In a corresponding model with reduced oral andrographolide dosage (60 mg/kg/day), the apparent C_{max} value diminished to 11.52 µg/mL, T_{max} increased to 2.01 h, and clearance stood at 0.19 L/h/kg.[102]

The diminished oral bioavailability is likely attributable to the rapid metabolism of 14-deoxy-12-suloandrographolide within the duodenum, jejunum, and potentially the latter ileum or colon sections. The action of P-glycoproteins further impedes the bioavailability.[103] In a parallel research, the same group evaluated andrographolide at identical dosages in Sprague Dawley (SD) rats, yielding an AUC0-t of 67.19 h/µg/mL, t1/2 of 7.30, clearance of 0.03 L/h/kg, and C_{max} value of 9.73 µg/mL.[104] Intravenous injection in SD rats with an 80 mg/kg dose resulted in a clearance of 0.7 L/h/kg and t1/2 of 0.4 h.[105]

Using a beagle dog model, intravenous administration of andrographolide at 50 mg/kg resulted in a clearance of 1.02 L/h/kg and t1/2 of 0.828 h.[106] In terms of distribution, the interaction between andrographolide and human serum albumin was demonstrated by Godugu *et al.* [107] involving hydrogen bonds with Lys444, Trp214, and Agr218 residues.[107] Zhao *et al.* [108] identified eight phase I and five phase II metabolites arising from deoxygenation, dehydration, glucuronidation, and hydrogenation reactions.[108]

Recent investigations by Yu *et al.* [109] showed that CYP3A4 predominantly metabolizes the α-β-unsaturated lactone moiety, whereas uridine diphosphate glucuronyltransferases (UGTs) mediate conjugation reactions.[109] Preclinical pharmacokinetic analysis revealed a minor 7–9% urinary excretion of andrographolide, with the remainder eliminated via diverse routes.[110,111]

Zhang *et al.* [112] demonstrated increased warfarin systemic exposure from 60.58 to 118.92 µg h/mL and prolonged t1/2 from 14.27 to 22.73 h upon co-administration with andrographolide, revealing CYP3A4 and CYP2C9 inhibition by andrographolide.[112,113] Furthermore, in an HepG2 model, andrographolide modulated CYP2D6 expression, affecting 5-fluorouracil's pharmacokinetics.[114]

Clinical data were consistent with those of the animal experiments. Pholphana *et al.* [115] administered 97.92 an equivalent dose of andrographolide to volunteers for three days, revealing a T_{max} of 0.78 h. The metabolites exhibited greater C_{max} and AUC values than andrographolide, suggesting their potential contribution to the biological activity of diterpenes. The apparent clearance data demonstrated a sex parity.[115] These clinical insights corroborate those of animal studies, expanding our understanding of the pharmacokinetics and interactions of andrographolide.

Safety Profiles of Andrographolides

Nephrotoxicity has been documented as a notable adverse effect of andrographolide, regardless of the route of administration. Andrographolide induced apoptosis, increased malondialdehyde (MDA), inhibited human renal tubular epithelial (HK-2) cell proliferation, and reduced superoxide dismutase (SOD) expression. Endoplasmic reticulum damage was observed, marked by increased levels of caspase-4 and C/EBP homologous protein (CHOP).[116,117] Liang *et al.* [118] showed the impact of andrographolide on female rodents, reduced reproductive capacity, and inducing oocyte apoptosis.[118]

Clinical observations revealed mild adverse events, such as taste disturbance and rash, in a 140 mg twice-daily oral administration study.[119] Calabrese *et al.* [120] reported soreness, headache, taste alterations, rash, diarrhea, and itching anaphylactic reaction upon oral administration of andrographolide.[120] Toxicity assessment of the AG-2-HyP-β-CYD complex revealed an LD_{50} of > 2000 mg/kg and a NOAEL of 666 mg/kg via oral and inhalation routes.[121,122] Understanding andrographolide toxicity remains limited, as recent research has primarily concentrated on the molecule itself rather than on its metabolites or its synergistic effect with other drugs.[123]

Conclusion

In this comprehensive review, the multifaceted antiviral potential of andrographolides against prevalent viral infections that pose a significant threat to global public health was comprehensively examined. Our exploration encompassed both the intrinsic antiviral activities of andrographolides and their modified analogs, highlighting their inhibitory effects on viral entry, replication, and propagation.

Despite the remarkable progress, several research gaps remain. Mechanistic insights into andrographolide-mediated antiviral activities, such as precise interactions with viral components and host factors, warrant further elucidation. Future research should focus on establishing optimal dosing regimens, assessing drug-drug interactions, and investigating long-term safety profiles. Clinical trials of andrographolides in various viral infections are crucial to validate their therapeutic potential.

In conclusion, andrographolides have immense potential as a novel class of antiviral agents in the era of emerging viral threats. Unlocking their full therapeutic value requires interdisciplinary collaboration combining molecular insights, pharmaceutical innovations, and rigorous clinical assessments. This comprehensive approach is poised to transform andrographolides into effective tools for combating major viral infections and bolstering global public health preparedness.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

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References

1. Hossain S, Urbi Z, Karuniawati H, Mohiuddin RB, Moh Qrimida A, Allzrag AMM, Ming LC, Pagano E, Capasso R. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: An Updated Review of Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. Life Basel Switz. 2021;11(4):348.
2. Enmozhi SK, Raja K, Sebastine I, Joseph J. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: an in silico approach. J Biomol Struct Dyn. 2021;39(9):3092-3098.
3. Shao ZJ, Zheng XW, Feng T, Huang J, Chen J, Wu YY, Zhou LM, Tu WW, Li H. Andrographolide exerted its antimicrobial effects by upregulation of human β-defensin-2 induced through p38 MAPK and NF-κB pathway in human lung epithelial cells. Can J Physiol Pharmacol. 2012;90(5):647-653.
4. Arifullah M, Namsa ND, Mandal M, Chiruvella KK, Vikrama P, Gopal GR. Evaluation of anti-bacterial and anti-oxidant potential of andrographolide and echiodinin isolated from callus culture of *Andrographis paniculata* Nees. Asian Pac J Trop Biomed. 2013;3(8):604-610; discussion 609-610.

5. Zaid OI, Abd Majid R, Sabariah MN, Hasidah MS, Al-Zihiry K, Yam MF, Basir R. Andrographolide effect on both *Plasmodium falciparum* infected and non infected RBCs membranes. Asian Pac J Trop Med. 2015;8(7):507-512.
6. Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and Clinical Pharmacology of *Andrographis paniculata* and Its Major Bioactive Phytoconstituent Andrographolide. Evid-Based Complement Altern Med ECAM. 2013;9:846740.
7. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pacific J Trop Dis. 2014; 4 (3):213-222.
8. Gupta S, Mishra KP, Ganju L. Broad-spectrum antiviral properties of andrographolide. Arch Virol. 2017;162(3):611-623.
9. Ekalaksananan T, Sookmai W, Fangkham S, Pientong C, Aromdee C, Seubsasana S, Kongyingyoes B. Activity of Andrographolide and Its Derivatives on HPV16 Pseudovirus Infection and Viral Oncogene Expression in Cervical Carcinoma Cells. Nutr Cancer. 2015;67(4):687-696.
10. Adiguna SP, Panggabean JA, Atikana A, Untari F, Izzati F, Bayu A, Rosyidah A, Rahmawati SI, Putra MY. Antiviral Activities of Andrographolide and Its Derivatives: Mechanism of Action and Delivery System. Pharmaceuticals. 2021;14(11):1102.
11. Zhang H, Li S, Si Y, Xu H. Andrographolide and its derivatives: Current achievements and future perspectives. Eur J Med Chem. 2021;224:113710.
12. Kumar G, Singh D, Tali JA, Dheer D, Shankar R. Andrographolide: Chemical modification and its effect on biological activities. Bioorganic Chem. 2020;95:103511.
13. Rafi M, Karomah AH, Heryanto R, Septaningsih DA, Kusuma WA, Amran MB, Rohman A, Prajogo B. Metabolite profiling of *Andrographis paniculata* leaves and stem extract using UHPLC-Orbitrap-MS/MS. Nat Prod Res. 2022;36(2):625-629.
14. Bhat M, Murthy H, Murthy H, Murthy H. Isolation of Andrographolide from *Andrographis lineata* Wall. ex Nees var. lawii C.B. Clarke and its Anticancer Activity against Human Ovarian Teratocarcinoma. Pharmacogn J. 2021;13(3):660-668.
15. Tundis R, Patra JK, Bonesi M, Das S, Nath R, Das Talukdar A, Das G, Loizzo MR. Anti-Cancer Agent: The Labdane Diterpenoid-Andrographolide. Plants. 2023;12(10):1969.
16. Herold S, Becker C, Ridge KM, Budinger GRS. Influenza virus-induced lung injury: pathogenesis and implications for treatment. Eur Respir J. 2015;45(5):1463-1478.
17. Lindsley WG, Noti JD, Blachere FM, Thewlis RE, Martin SB, Othumpangat S, Noorbakhsh B, Goldsmith WT, Vishnu A, Palmer JE, Clark KE, Beezhold DH. Viable influenza A virus in airborne particles from human coughs. J Occup Environ Hyg. 2015;12(2):107-113.
18. Jiang M, Sheng F, Zhang Z, Ma X, Gao T, Fu C, Li P. *Andrographis paniculata* (Burm.f.) Nees and its major constituent andrographolide as potential antiviral agents. J Ethnopharmacol. 2021;272:113954.
19. Seniya C, Shrivastava S, Singh SK, Khan GJ. Analyzing the interaction of a herbal compound Andrographolide from *Andrographis paniculata* as a folklore against swine flu (H1N1). Asian Pac J Trop Dis. 2014;4:S624-S630.
20. Cai W, Li Y, Chen S, Wang M, Zhang A, Zhou H, Chen H, Jin M. 14-Deoxy-11,12-dehydroandrographolide exerts anti-influenza A virus activity and inhibits replication of H5N1 virus by restraining nuclear export of viral ribonucleoprotein complexes. Antiviral Res. 2015;118:82-92.
21. Ding Y, Chen L, Wu W, Yang J, Yang Z, Liu S. Andrographolide inhibits influenza A virus-induced inflammation in a murine model through NF-κB and JAK-STAT signaling pathway. Microbes Infect. 2017;19(12):605-615.
22. Yu B, Dai C qi, Jiang Z you, Li E qing, Chen C, Wu X lin, Chen J, Liu Q, Zhao C lin, He J xiong, Ju D hong, Chen X yin. Andrographolide as an anti-H1N1 drug and the mechanism related to retinoic acid-inducible gene-I-like receptors signaling pathway. Chin J Integr Med. 2014;20(7):540-545.
23. Kamp C. Understanding the HIV coreceptor switch from a dynamical perspective. BMC Evol Biol. 2009;9(1):274.
24. Feng L, Wang L, Ma Y yun, Li M, Zhao GQ. A Potential *In Vitro* and *In Vivo* anti-HIV Drug Screening System for Chinese Herbal Medicines. Phytother Res. 2012;26(6):899-907.
25. Uttekar MM, Das T, Pawar RS, Bhandari B, Menon V, Nutan, Gupta SK, Bhat SV. Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding. Eur J Med Chem. 2012;56:368-374. doi:10.1016/j.ejmech.2012.07.030
26. Reddy VLN, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV, Rao TP, Ram TS, Gonnade RG, Bhadbhade M, Venkateswarlu Y. A new bis-andrographolide ether from *Andrographis paniculata* nees and evaluation of anti-HIV activity. Nat Prod Res. 2005;19(3):223-230.
27. Jiang YC, Feng H, Lin YC, Guo XR. New strategies against drug resistance to herpes simplex virus. Int J Oral Sci. 2016;8(1):1-6.
28. Wiart C, Kumar K, Yusof MY, Hamimah H, Fauzi ZM, Sulaiman M. Antiviral properties of ent-labdene diterpenes of *Andrographis paniculata* nees, inhibitors of herpes simplex virus type 1. Phytother Res. 2005;19(12):1069-1070.
29. Priengprom T, Ekalaksananan T, Kongyingyoes B, Suebsasana S, Aromdee C, Pientong C. Synergistic effects of acyclovir and 3, 19- isopropylideneandrographolide on herpes simplex virus wild types and drug-resistant strains. BMC Complement Altern Med. 2015;15(1):56.
30. Sa-ngiamsuntorn K, Suksatu A, Pewkliang Y, Thongsri P, Kanjanasirirat P, Manopwisedjaroen S, Charoensutthivarakul S, Wongtrakoongate P, Pitiporn S, Chaopreecha J, Kongsomros S, Jearawuttanakul K, Wannalo W, Khemawoot P, Chutipongtanate S, Borwornpinyo S, Thitithanyanont A, Hongeng S. Anti-SARS-CoV-2 Activity of *Andrographis paniculata* Extract and Its Major Component Andrographolide in Human Lung Epithelial Cells and Cytotoxicity Evaluation in Major Organ Cell Representatives. J Nat Prod. 2021;84(4):1261-1270.
31. Kumar S, Maurya VK, Prasad AK, Bhatt MLB, Saxena SK. Structural, glycosylation and antigenic variation between 2019 novel coronavirus (2019-nCoV) and SARS coronavirus (SARS-CoV). VirusDisease. 2020;31(1):13-21.
32. Maurya VK, Kumar S, Prasad AK, Bhatt MLB, Saxena SK. Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. VirusDisease. 2020;31(2):179-193.
33. Rajagopal K, Varakumar P, Baliwada A, Byran G. Activity of phytochemical constituents of *Curcuma longa* (turmeric) and *Andrographis paniculata* against coronavirus (COVID-19): an in silico approach. Future J Pharm Sci. 2020;6(1):104.
34. Murugan NA, Pandian CJ, Jeyakanthan J. Computational investigation on *Andrographis paniculata* phytochemicals to evaluate their potency against SARS-CoV-2 in comparison to known antiviral compounds in drug trials. J Biomol Struct Dyn. 2021;39(12):4415-4426.
35. Shi TH, Huang YL, Chen CC, Pi WC, Hsu YL, Lo LC, Chen WY, Fu SL, Lin CH. Andrographolide and its fluorescent derivative inhibit the main proteases of 2019-

nCoV and SARS-CoV through covalent linkage. Biochem Biophys Res Commun. 2020;533(3):467-473.
36. Halstead SB. Dengue Virus–Mosquito Interactions. Annu Rev Entomol. 2008;53(1):273-291. doi:10.1146/annurev.ento.53.103106.093326
37. Tantawichien T. Dengue fever and dengue haemorrhagic fever in adolescents and adults. Paediatr Int Child Health. 2012;32(s1):22-27.
38. Paemanee A, Hitakarun A, Wintachai P, Roytrakul S, Smith DR. A proteomic analysis of the anti-dengue virus activity of andrographolide. Biomed Pharmacother. 2019;109:322-332.
39. Panraksa P, Ramphan S, Khongwichit S, Smith DR. Activity of andrographolide against dengue virus. Antiviral Res. 2017;139:69-78.
40. Tseng CK, Lin CK, Wu YH, Chen YH, Chen WC, Young KC, Lee JC. Human heme oxygenase 1 is a potential host cell factor against dengue virus replication. Sci Rep. 2016;6:32176.
41. Kaushik S, Dar L, Kaushik S, Yadav JP. Identification and characterization of new potent inhibitors of dengue virus NS5 proteinase from *Andrographis paniculata* supercritical extracts on in animal cell culture and in silico approaches. J Ethnopharmacol. 2021;267:113541.
42. Tang LI, Ling AP, Koh RY, Chye SM, Voon KG. Screening of anti-dengue activity in methanolic extracts of medicinal plants. BMC Complement Altern Med. 2012;12(1):3.
43. Wintachai P, Kaur P, Lee RCH, Ramphan S, Kuadkitkan A, Wikan N, Ubol S, Roytrakul S, Chu JJH, Smith DR. Activity of andrographolide against chikungunya virus infection. Sci Rep. 2015;5:14179.
44. Oliveira DM de, Santos I de A, Martins DOS, Gonçalves YG, Cardoso-Sousa L, Sabino-Silva R, Von Poelhsitz G, Franca E de F, Nicolau-Junior N, Pacca CC, Merits A, Harris M, Jardim ACG. Organometallic Complex Strongly Impairs Chikungunya Virus Entry to the Host Cells. Front Microbiol. 2020;11. Accessed August 21, 2023. https://www.frontiersin.org/articles/10.3389/fmicb.2020.608924
45. Judith D, Mostowy S, Bourai M, Gangneux N, Lelek M, Lucas-Hourani M, Cayet N, Jacob Y, Prévost MC, Pierre P, Tangy F, Zimmer C, Vidalain PO, Couderc T, Lecuit M. Species-specific impact of the autophagy machinery on Chikungunya virus infection. EMBO Rep. 2013;14(6):534-544.
46. Li F, Khanom W, Sun X, Paemanee A, Roytrakul S, Wang D, Smith DR, Zhou GC. Andrographolide and Its 14-Aryloxy Analogues Inhibit Zika and Dengue Virus Infection. Mol Basel Switz. 2020;25(21):5037.
47. Lu H, Zhang XY, Wang YQ, Zheng XL, Yin-Zhao null, Xing WM, Zhang Q. Andrographolide sodium bisulfate-induced apoptosis and autophagy in human proximal tubular endothelial cells is a ROS-mediated pathway. Environ Toxicol Pharmacol. 2014;37(2):718-728.
48. Xing WM, Yuan TJ, Xu JD, Gu LL, Liang P, Lu H. Proteomic identification of mitochondrial targets involved in andrographolide sodium bisulfite-induced nephrotoxicity in a rat model. Environ Toxicol Pharmacol. 2015;40(2):592-599.
49. Wang J, Tan XF, Nguyen VS, Yang P, Zhou J, Gao M, Li Z, Lim TK, He Y, Ong CS, Lay Y, Zhang J, Zhu G, Lai SL, Ghosh D, Mok YK, Shen HM, Lin Q. A quantitative chemical proteomics approach to profile the specific cellular targets of andrographolide, a promising anticancer agent that suppresses tumor metastasis. Mol Cell Proteomics MCP. 2014;13(3):876-886.
50. Hong M, Sandalova E, Low D, Gehring AJ, Fieni S, Amadei B, Urbani S, Chong YS, Guccione E, Bertoletti A. Trained immunity in newborn infants of HBV-infected mothers. Nat Commun. 2015;6(1):6588.
51. Sa-Nguanmoo P, Rianthavorn P, Amornsawadwattana S, Poovorawan Y. Hepatitis B virus infection in non-human primates. Acta Virol. 2009;53(2):73-82.
52. Stoop JN, van der Molen RG, Baan CC, van der Laan LJW, Kuipers EJ, Kusters JG, Janssen HLA. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatol Baltim Md. 2005;41(4):771-778.
53. Busca A, Kumar A. Innate immune responses in hepatitis B virus (HBV) infection. Virol J. 2014;11(1):22.
54. Chen Y, Zhu J. Anti-HBV effect of individual traditional Chinese herbal medicine *in vitro* and *in vivo*: an analytic review. J Viral Hepat. 2013;20(7):445-452.
55. Kim KH, Kim ND, Seong BL. Discovery and Development of Anti-HBV Agents and Their Resistance. Molecules. 2010;15(9):5878-5908.
56. Chen H, Ma YB, Huang XY, Geng CA, Zhao Y, Wang LJ, Guo RH, Liang WJ, Zhang XM, Chen JJ. Synthesis, structure-activity relationships and biological evaluation of dehydroandrographolide and andrographolide derivatives as novel anti-hepatitis B virus agents. Bioorg Med Chem Lett. 2014;24(10):2353-2359.
57. Ito M, Kusunoki H, Mizuochi T. Peripheral B Cells as Reservoirs for Persistent HCV Infection. Front Microbiol. 2011;2:177.
58. Yi G, Wen Y, Shu C, Han Q, Konan KV, Li P, Kao CC. Hepatitis C Virus NS4B Can Suppress STING Accumulation To Evade Innate Immune Responses. J Virol. 2016;90(1):254-265.
59. Freeman AJ, Marinos G, Ffrench RA, Lloyd AR. Immunopathogenesis of hepatitis C virus infection. Immunol Cell Biol. 2001;79(6):515-536.
60. Yu Y, Jing J feng, Tong X kun, He P lan, Li Y chao, Hu Y hong, Tang W, Zuo J ping. Discovering novel anti-HCV compounds with inhibitory activities toward HCV NS3/4A protease. Acta Pharmacol Sin. 2014;35(8):1074-1081.
61. Lee JC, Tseng CK, Young KC, Sun HY, Wang SW, Chen WC, Lin CK, Wu YH. Andrographolide exerts anti-hepatitis C virus activity by up-regulating haeme oxygenase-1 via the p38 MAPK/Nrf2 pathway in human hepatoma cells. Br J Pharmacol. 2014;171(1):237-252.
62. Chandramohan V, Kaphle A, Chekuri M, Gangarudraiah S, Bychapur Siddaiah G. Evaluating Andrographolide as a Potent Inhibitor of NS3-4A Protease and Its Drug-Resistant Mutants Using In Silico Approaches. Adv Virol. 2015;2015:972067.
63. Tsang CM, Deng W, Yip YL, Zeng MS, Lo KW, Tsao SW. Epstein-Barr virus infection and persistence in nasopharyngeal epithelial cells. Chin J Cancer. 2014;33(11):549-555.
64. Ni C, Chen Y, Zeng M, Pei R, Du Y, Tang L, Wang M, Hu Y, Zhu H, He M, Wei X, Wang S, Ning X, Wang M, Wang J, Ma L, Chen X, Sun Q, Tang H, Wang Y, Wang X. In-cell infection: a novel pathway for Epstein-Barr virus infection mediated by cell-in-cell structures. Cell Res. 2015;25(7):785-800.
65. Rancan C, Schirrmann L, Hüls C, Zeidler R, Moosmann A. Latent Membrane Protein LMP2A Impairs Recognition of EBV-Infected Cells by CD8+ T Cells. PLOS Pathog. 2015;11(6):e1004906.
66. Wang JJ, Li YF, Jin YY, Wang X, Chen TX. Effects of Epstein-Barr virus on the development of dendritic cells derived from cord blood monocytes: an essential role for apoptosis. Braz J Infect Dis Off Publ Braz Soc Infect Dis. 2012;16(1):19-26.
67. Malat P, Ekalaksananan T, Heawchaiyaphum C, Suebsasana S, Roytrakul S, Yingchutrakul Y, Pientong C. Andrographolide Inhibits Lytic Reactivation of Epstein-Barr Virus by Modulating Transcription Factors in Gastric Cancer. Microorganisms. 2021;9(12):2561.

68. Song D, Li H, Li H, Dai J. Effect of human papillomavirus infection on the immune system and its role in the course of cervical cancer. Oncol Lett. 2015;10(2):600-606.
69. Wierzbicka M, San Giorgi MRM, Dikkers FG. Transmission and clearance of human papillomavirus infection in the oral cavity and its role in oropharyngeal carcinoma – A review. Rev Med Virol. 2023;33(1):e2337.
70. Orosco FL. Recent advances in peptide-based nanovaccines for re-emerging and emerging infectious diseases. J Adv Biotechnol Exp Ther. 2024;7(1):106-117.
71. Mishra A, Shaik HA, Sinha RK, Shah BR. Andrographolide: A Herbal-Chemosynthetic Approach for Enhancing Immunity, Combating Viral Infections, and Its Implication on Human Health. Molecules. 2021;26(22):7036.
72. Khanal P, Dey YN, Patil R, Chikhale R, Wanjari MM, Gurav SS, Patil BM, Srivastava B, Gaidhani SN. Combination of system biology to probe the anti-viral activity of andrographolide and its derivative against COVID-19. RSC Adv. 2021;11(9):5065-5079.
73. Aromdee C. Modifications of andrographolide to increase some biological activities: a patent review (2006 – 2011). Expert Opin Ther Pat. 2012;22(2):169-180.
74. Aromdee C, Suebsasana S, Ekalaksananan T, Pientong C, Thongchai S. Stage of action of naturally occurring andrographolides and their semisynthetic analogues against herpes simplex virus type 1 in vitro. Planta Med. 2011;77(9):915-921.
75. Yuan L, Zhang C, Sun H, Liu Q, Huang J, Sheng L, Lin B, Wang J, Chen L. The semi-synthesis of novel andrographolide analogues and anti-influenza virus activity evaluation of their derivatives. Bioorg Med Chem Lett. 2016;26(3):769-773.
76. Chen JX, Xue HJ, Ye WC, Fang BH, Liu YH, Yuan SH, Yu P, Wang YQ. Activity of Andrographolide and Its Derivatives against Influenza Virus i*n Vivo* and *in Vitro*. Biol Pharm Bull. 2009;32(8):1385-1391.
77. Wang D, Guo H, Chang J, Wang D, Liu B, Gao P, Wei W. Andrographolide Prevents EV-D68 Replication by Inhibiting the Acidification of Virus-Containing Endocytic Vesicles. Front Microbiol. 2018;9. Accessed August 21, 2023. https://www.frontiersin.org/articles/10.3389/fmicb.2018.02407
78. Cai W, Wen H, Zhou Q, Wu L, Chen Y, Zhou H, Jin M. 14-Deoxy-11,12-didehydroandrographolide inhibits apoptosis in influenza A(H5N1) virus-infected human lung epithelial cells via the caspase-9-dependent intrinsic apoptotic pathway which contributes to its antiviral activity. Antiviral Res. 2020;181:104885.
79. Dasgupta Mandal D, Mandal T, Hazra M. Strategic approach in hepatic delivery of andrographolide: Key challenges and new insights. J Herb Med. 2020;24:100411.
80. Su C, Liu Y, Li R, Wu W, Fawcett JP, Gu J. Absorption, distribution, metabolism and excretion of the biomaterials used in Nanocarrier drug delivery systems. Adv Drug Deliv Rev. 2019;143:97-114.
81. Toyama T, Nitta N, Ohta S, Tanaka T, Nagatani Y, Takahashi M, Murata K, Shiomi H, Naka S, Kurumi Y, Tani T, Tabata Y. Clinical trial of cisplatin-conjugated gelatin microspheres for patients with hepatocellular carcinoma. Jpn J Radiol. 2012;30(1):62-68.
82. Jiang Y, Wang F, Xu H, Liu H, Meng Q, Liu W. Development of andrographolide loaded PLGA microspheres: optimization, characterization and in vitro-in vivo correlation. Int J Pharm. 2014;475(1-2):475-484.
83. Staff RH, Landfester K, Crespy D. Recent Advances in the Emulsion Solvent Evaporation Technique for the Preparation of Nanoparticles and Nanocapsules. In: Percec V, ed. Hierarchical Macromolecular Structures: 60 Years after the Staudinger Nobel Prize II. Advances in Polymer Science. Springer International Publishing; 2013:329-344.
84. Gibaud S, Attivi D. Microemulsions for oral administration and their therapeutic applications. Expert Opin Drug Deliv. 2012;9(8):937-951.
85. Spernath A, Aserin A. Microemulsions as carriers for drugs and nutraceuticals. Adv Colloid Interface Sci. 2006;128-130:47-64.
86. Du H, Yang X, Li H, Han L, Li X, Dong X, Zhu Q, Ye M, Feng Q, Niu X. Preparation and evaluation of andrographolide-loaded microemulsion. J Microencapsul. 2012;29(7):657-665.
87. Sermkaew N, Ketjinda W, Boonme P, Phadoongsombut N, Wiwattanapatapee R. Liquid and solid self-microemulsifying drug delivery systems for improving the oral bioavailability of andrographolide from a crude extract of Andrographis paniculata. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2013;50(3-4):459-466.
88. Syukri Y, Martien R, Lukitaningsih E, Nugroho AE. Novel Self-Nano Emulsifying Drug Delivery System (SNEDDS) of andrographolide isolated from Andrographis paniculata Nees: Characterization, in-vitro and in-vivo assessment. J Drug Deliv Sci Technol. 2018;47:514-520.
89. Jayanta Sinha SM Nirmalendu Das, Mukul Kumar Basu. Targeting of Liposomal Andrographolide to *L. donovani* -Infected Macrophages in Vivo. Drug Deliv. 2000;7(4):209-213.
90. Kang X, Zheng Z, Liu Z, Wang H, Zhao Y, Zhang W, Shi M, He Y, Cao Y, Xu Q, Peng C, Huang Y. Liposomal Codelivery of Doxorubicin and Andrographolide Inhibits Breast Cancer Growth and Metastasis. Mol Pharm. 2018;15(4):1618-1626.
91. Li M, Zhang T, Zhu L, Wang R, Jin Y. Liposomal andrographolide dry powder inhalers for treatment of bacterial pneumonia via anti-inflammatory pathway. Int J Pharm. 2017;528(1):163-171.
92. Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. J Control Release Off J Control Release Soc. 2014;185:22-36.
93. Tu YS, Sun DM, Zhang JJ, Jiang ZQ, Chen YX, Zeng XH, Huang DE, Yao N. Preparation and characterisation of andrographolide niosomes and its anti-hepatocellular carcinoma activity. J Microencapsul. 2014;31(4):307-316.
94. Maitani Y, Soeda H, Junping W, Takayama K. Modified ethanol injection method for liposomes containing β-sitosterol β-d-glucoside. J Liposome Res. 2001;11(1):115-125.
95. Parveen R, Ahmad FJ, Iqbal Z, Samim M, Ahmad S. Solid lipid nanoparticles of anticancer drug andrographolide: formulation, *in vitro* and *in vivo* studies. Drug Dev Ind Pharm. 2014;40(9):1206-1212.
96. Graverini G, Piazzini V, Landucci E, Pantano D, Nardiello P, Casamenti F, Pellegrini-Giampietro DE, Bilia AR, Bergonzi MC. Solid lipid nanoparticles for delivery of andrographolide across the blood-brain barrier: in vitro and in vivo evaluation. Colloids Surf B Biointerfaces. 2018;161:302-313.
97. Kulsirirat T, Sathirakul K, Kamei N, Takeda-Morishita M. The in vitro and in vivo study of novel formulation of andrographolide PLGA nanoparticle embedded into gelatin-based hydrogel to prolong delivery and extend residence time in joint. Int J Pharm. 2021;602:120618.
98. Yang T, Sheng HH, Feng NP, Wei H, Wang ZT, Wang CH. Preparation of Andrographolide-Loaded Solid Lipid Nanoparticles and Their *In Vitro* and *In Vivo* Evaluations: Characteristics, Release, Absorption, Transports, Pharmacokinetics, and Antihyperlipidemic Activity. J Pharm Sci. 2013;102(12):4414-4425.
99. Orosco FL. Current progress in diagnostics, therapeutics, and vaccines for African swine fever virus:

https://doi.org/10.12982/VIS.2023.054. Vet Integr Sci. 2023;21(3):751-781.
100. Wang Z, He R, Chen Y, Wu F. Regio-selective PEGylation of 14-deoxy-11,12-didehydroandrographolide and their biological evaluation. Tetrahedron. 2016;72(39):5909-5913.
101. Bera R, Ahmed SKM, Sarkar L, Sen T, Karmakar S. Pharmacokinetic analysis and tissue distribution of andrographolide in rat by a validated LC-MS/MS method. Pharm Biol. 2014;52(3):321-329.
102. Balap A, Lohidasan S, Sinnathambi A, Mahadik K. Pharmacokinetic and Pharmacodynamic Interaction of Andrographolide and Standardized Extract of *Andrographis paniculata* (Nees) with Nabumetone in Wistar Rats. Phytother Res PTR. 2017;31(1):75-80.
103. Ye L, Wang T, Tang L, Liu W, Yang Z, Zhou J, Zheng Z, Cai Z, Hu M, Liu Z. Poor oral bioavailability of a promising anticancer agent andrographolide is due to extensive metabolism and efflux by P-glycoprotein. J Pharm Sci. 2011;100(11):5007-5017.
104. Balap A, Lohidasan S, Sinnathambi A, Mahadik K. Herb-drug interaction of *Andrographis paniculata* (Nees) extract and andrographolide on pharmacokinetic and pharmacodynamic of naproxen in rats. J Ethnopharmacol. 2017;195:214-221.
105. Zhang SQ, Wang X, Zhang Y, Li X. Pharmacokinetics of Andrographolide Sodium Bisulphite and its Related Substance in Rats by Liquid Chromatography–Tandem Mass Spectrometry. J Anal Chem. 2020;75(8):1033-1037.
106. Zhang SQ, Fan YM. Determination of andrograpolide sodium bisulphite in Beagle dog plasma by LC–MS/MS and its application to pharmacokinetics. J Chromatogr B. 2012;907:173-177.
107. Godugu D, Rupula K, Sashidhar RB. Binding Studies of Andrographolide with Human Serum Albumin: Molecular Docking, Chromatographic and Spectroscopic Studies. Protein Pept Lett. 25(4):330-338.
108. Zhao HY, Hu H, Wang YT. Comparative metabolism and stability of andrographolide in liver microsomes from humans, dogs and rats using ultra-performance liquid chromatography coupled with triple-quadrupole and Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun Mass Spectrom. 2013;27(12):1385-1392.
109. Yu Z, Chen Z, Li Q, Yang K, Huang Z, Wang W, Zhao S, Hu H. What dominates the changeable pharmacokinetics of natural sesquiterpene lactones and diterpene lactones: a review focusing on absorption and metabolism. Drug Metab Rev. 2021;53(1):122-140.
110. Panossian A, Hovhannisyan A, Mamikonyan G, Abrahamian H, Hambardzumyan E, Gabrielian E, Goukasova G, Wikman G, Wagner H. Pharmacokinetic and oral bioavailability of andrographolide from *Andrographis paniculata* fixed combination Kan Jang in rats and human. Phytomedicine. 2000;7(5):351-364.
111. Qiu F, Cui L, Chen L, Sun J, Yao X. Two novel creatinine adducts of andrographolide in human urine. Xenobiotica Fate Foreign Compd Biol Syst. 2012;42(9):911-916.
112. Mahedi MRA, Rawat A, Rabbi F, Babu KS, Tasayco ES, Areche FO, Pacovilca-Alejo OV, Flores DDC, Aguilar SV, Orosco FL, Syrmos N, Mudhafar M, Afrin S, Rahman MM. Understanding the Global Transmission and Demographic Distribution of Nipah Virus (NiV). Res J Pharm Technol. 2023;16(8):3588-3594.
113. Zhang X, Zhang X, Wang X, Zhao M. Influence of andrographolide on the pharmacokinetics of warfarin in rats. Pharm Biol. 2018;56(1):351-356.
114. Suriyo T, Chotirat S, Rangkadilok N, Pholphana N, Satayavivad J. Interactive effects of *Andrographis paniculata* extracts and cancer chemotherapeutic 5-Fluorouracil on cytochrome P450s expression in human hepatocellular carcinoma HepG2 cells. J Herb Med. 2021;26:100421.
115. Pholphana N, Panomvana D, Rangkadilok N, Suriyo T, Puranajoti P, Ungtrakul T, Pongpun W, Thaeopattha S, Songvut P, Satayavivad J. Andrographis paniculata: Dissolution investigation and pharmacokinetic studies of four major active diterpenoids after multiple oral dose administration in healthy Thai volunteers. J Ethnopharmacol. 2016;194:513-521.
116. Gu LL, Zhang XY, Xing WM, Xu JD, Lu H. Andrographolide-induced apoptosis in human renal tubular epithelial cells: Roles of endoplasmic reticulum stress and inflammatory response. Environ Toxicol Pharmacol. 2016;45:257-264.
117. Orosco FL. Immune evasion mechanisms of porcine epidemic diarrhea virus: A comprehensive review: https://doi.org/10.12982/VIS.2024.014. Vet Integr Sci. 2024;22(1):171-192.
118. Liang H xing, Lu S sheng, Yan Z, Kuang Y ping, Zhu X xing, Yan Z guang, Du T, Chai W ran, Long H, Lyu Q feng, Liang H xing, Lu S sheng, Yan Z, Kuang Y ping, Zhu X xing, Yan Z guang, Du T, Chai W ran, Long H, Lyu Q feng. Andrographolide disrupts meiotic maturation by blocking cytoskeletal reorganisation and decreases the fertilisation potential of mouse oocytes. Reprod Fertil Dev. 2017;29(12):2336-2344.
119. Ciampi E, Uribe-San-Martin R, Cárcamo C, Cruz JP, Reyes A, Reyes D, Pinto C, Vásquez M, Burgos RA, Hancke J. Efficacy of andrographolide in not active progressive multiple sclerosis: a prospective exploratory double-blind, parallel-group, randomized, placebo-controlled trial. BMC Neurol. 2020;20(1):173.
120. Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, Wells K, Wenner CA, Standish LJ. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytother Res. 2000;14(5):333-338.
121. Chandrama Singh S, Choudhary M, Mourya A, Khatri DK, Singh PK, Madan J, Singh H. Acute and Subacute Toxicity Assessment of Andrographolide-2-hydroxypropyl-β-cyclodextrin Complex via Oral and Inhalation Route of Administration in Sprague-Dawley Rats. Sci World J. 2022;2022:e6224107.
122. Orosco F. Advancing the frontiers: Revolutionary control and prevention paradigms against Nipah virus. Open Veterinary Journal. 2023;13(9):1056–1056.
123. Sato H, Hiraki M, Namba T, Egawa N, Baba K, Tanaka T, Noshiro H. Andrographolide induces degradation of mutant p53 via activation of Hsp70. Int J Oncol. 2018;53(2):761-770.