Andrographolide as a Potent and Promising Antiviral Agent

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[ABSTRACT] Andrographolide is a labdane diterpenoid extracted and purified from the aerial parts of plants belonging to genus Andrographis (Acanthaceae). The research has shown the plant based compound is low cytotoxic having antimicrobial, anti-cancer, antiviral and anti-parasitic effects. Andrographolide both prevent spread as well as transmission of virus to neighboring cells by interfering with different cell signaling pathways. In addition to its medicinal value, plant has been found having nutritional value. Therefore being cost effective, easy availability and having nutritional value as a natural supplement, can be used to improve the quality of life in countries having low standard of living. Due to the limited number of effective vaccines, the plant-based antiviral drugs have provided considerable hope for fighting against the viral infections. The plant-derived compound when produced in large quantities is cost effective with low cytotoxic effects. However, much deep insight research at the molecular level is needed to develop the molecules against the viral infection. This paper aims to highlight the antiviral role of Andrographolide that can made significant contributions towards the improvement of human health and will also summarize the current status and future strategies concerning the therapeutic applications of Andrographolide to combat different viral disease in humans.

[KEY WORDS] Andrographolide; vaccines; antiviral drugs; antimicrobial; life cycle; viral infection

Introduction

Andrographis is an annual herb widely cultivated in southern Asia, China, India and select European countries. The secondary metabolite in the plant has been disclosed to possess biological activities in both in-vivo as well as in-vitro studies like anti-bacterial, anti-viral, anti-inflammatory, immunomodulatory/immunostimulatory, anti-inflammation and anticancer. Andrographolide penetulica has been traditionally used for centuries against different diseases. The major constituent are diterpenoids, flavonoids and polyphenols. However Andrographolide (C_{20}H_{20}O_{3}) is the principle and major diterpenoid lactone compound which makes up about 4%, 0.8–1.2% and 0.5–6% in dried whole plant, stem and leaf extracts, respectively. The compound is mainly concentrated in leaves and can be easily isolated from the crude leaf extracts, respectively. The compound has been elucidated by X-Ray crystallographic analysis and the molecular stereochemistry, bond distances, bond angles, and so forth all were determined. The multiple modes of action of Andrographolide shows that this compound is a promising candidate for advanced research. In addition to medicinal use, recently the plant has been found having nutrition value as a natural supplement in diet mixed with different foods to prevent disease and improve quality of life in countries having low standards of living. At present, the plant has found an important place as a medicine in the form of Andrographolide extract which is available as both in injection as well as tablet form in Asia. In China, in tablet form, it has different names, Kan Jang, Chuanxinlian, Chuan Xin Lian antiphlogistic pills, and Yamdeping and Chuan Xin Lian Ruangas (injection form). In India, 26 ayurvedic formulations of Andrographolide are used. The countless benefits of Andrographolide both nutritionally as well as medicine for different human disease is available as both in injection as well as tablet form in Asia. In China, in tablet form, it has different names, Kan Jang, Chuanxinlian, Chuan Xin Lian antiphlogistic pills, and Yamdeping and Chuan Xin Lian Ruangas (injection form). In India, 26 ayurvedic formulations of Andrographolide are used. The countless benefits of Andrographolide both nutritionally as well as medicine for different human disease is available as both in injection as well as tablet form in Asia. In China, in tablet form, it has different names, Kan Jang, Chuanxinlian, Chuan Xin Lian antiphlogistic pills, and Yamdeping and Chuan Xin Lian Ruangas (injection form). In India, 26 ayurvedic formulations of Andrographolide are used. The countless benefits of Andrographolide both nutritionally as well as medicine for different human disease is available as both in injection as well as tablet form in Asia. In China, in tablet form, it has different names, Kan Jang, Chuanxinlian, Chuan Xin Lian antiphlogistic pills, and Yamdeping and Chuan Xin Lian Ruangas (injection form). In India, 26 ayurvedic formulations of Andrographolide are used. The countless benefits of Andrographolide both nutritionally as well as medicine for different human disease is available as both in injection as well as tablet form in Asia. In China, in tablet form, it has different names, Kan Jang, Chuanxinlian, Chuan Xin Lian antiphlogistic pills, and Yamdeping and Chuan Xin Lian Ruangas (injection form). In India, 26 ayurvedic formulations of Andrographolide are used.
Viral infections generally, start with local invasion and result in the infection of the target organ. Host, in turn, use different mechanisms like the process of apoptosis to eliminate the infected cells [18-21]. However, taking the lead in this disease cycle depends on the host immune system or the virus. The host can cause self-destruction of infected cells by the process of apoptosis or the virus after taking control of the whole cell, uses the process of apoptosis either to produce a new progeny of viruses or directly release the new viral progeny to the adjacent cells to aid in the spread of viral infection. The prevention and treatment of viral infections are particularly challenging because viruses use the host cell machinery to replicate and to interrupt viral replication without damaging host cell structures. Following their entry into the host cell, viruses face a variety of hurdles that constitute the cellular host’s immune defense system, which is designed to protect the organism and abolish the infecting agent. It has been shown that Andrographolide modulates the host immune responses by interfering with different cellular signaling molecules/pathways to aid in fighting different diseases.

Substantial antiviral activity of Andrographis paniculata against Enterovirus-D68 infection.

The largest outbreak and rapid spread of enteroviruses infection in 2014 have raised a serious public health concern. Till now there are no effective vaccines or antiviral agents available for the treatment of disease but mostly in Asian countries Andrographolide has been traditionally used to treat various diseases showing symptoms like diarrhea, fever and respiratory infection, [22, 23]. The crystal structure of EV-D68 shows similarity to that of human rhinovirus [24]. EV-D68 is a small, single stranded RNA virus encoding only single open reading frame (ORF) that encodes viral polyprotein, with icosahedral symmetry [25] Previous studies have suggested that cell surface sialic acid is required for host cell infection by EV-D68 [26, 27]. This studies recently identified...
neuro-specific intercellular adhesion molecule 5 (ICAM-5/telencephalon) as a functional entry receptor for sialic acid-dependent and -independent EV-D68 viruses.

In this study the treatment of RD cells infected with EV-D68 with A. paniculata showed significant antiviral effect against EV-D68 with a significant decreases in viral RNA replication and protein synthesis without producing any significant toxicity. Later further studies have showed that Andrographolide treatment has not any direct effect on viral attachment, viral endocytosis processes, or innate immune activation in infected cells but rather showed played a role interfering with viral cytoplasmic traffic by preventing vesicle acidification as shown by authors in pH-sensitive fluorescent dye conjugate experiment to track virus endocytosis in live cells. Previous study carried by Wei et al has shown that ICAM-5 as a functional receptor for both sialic acid sensitive and insensitive EV-D68 strains. In this study, Andrographolide had not only blocked the virus (EV-D68) entry into RD cells but also interfered with the cytoplasmic trafficking of the virus inside the cell. Therefore by this new findings shows that acidification of the virus-containing endocytic vesicle was a critical step inhibited by Andrographolide which ultimately inhibiting EV-D68 infection.

Andrographolide exerts its anti-viral effect through modulation of the inflammasome in Dengue Virus (DENV)

The studies carried against two serotypes of dengue virus (DENV) serotype 2 and DENV serotype 4 in different cell lines have shown that Andrographolide had significant anti-DENV activity in both cell lines, reducing both the levels of cellular infection and virus output, with 50% effective concentrations (EC50) for DENV 2 of 21.304 mM and 22.739 mM for HepG2 and HeLa respectively. Here in this studies time course treatment of Andrographolide has shown that reductions in protein expression were generally only seen with post-treatment up to 3 to 6 h post infection, in comparison, significant reductions in protein expression up to 12 h for CHIKV infection. As discussed above that Andrographolide has a role to interfere with NF-kB binding/translocation to DNA/nucleus respectively. Previous studies has shown the role of NF-kB and actin in viral infection and Yang et al. has shown the DENV Protein E interacts with Actin and it is possible that Andrographolide directly disrupts this interaction. It is also possible that Andrographolide exerts its anti-viral effect through modulation of the inflammasome which has been shown to be activated in DENV infection as shown by Wu et al. More than that Several other studies has shown that autophagy induction upon DENV infection and Andrographolide has been proposed and shown to play a role in inhibiting the terminal stage of autophagy.

Andrographolide promote antiviral effect through the stimulation or inhibition of numerous targets in CHIKV virus

CHIKV belongs family Togaviridae, a positive sense, single stranded RNA virus with genome size of appo.11.8kb. The patients infected with CHIKV shows symptoms like that of DENV virus but currently there is no vaccine or any antiviral drug specific for the treatment of CHIKV fever. Recent studies carried by Phitchayapak et al has shown that Andrographolide a traditional herb not only inhibits the viral genome replication but also acts at post entry step. However the complete mechanism of action is still not completely understood. The previous studied carried in influenza virus and hepatitis C virus has shown that Andrographolide targets the RIG receptor and involve MAPK/Nrf 2 pathway (Fig 4). Some studies carried on Andrographolide has

### Table 1: Mechanism of action of Andrographolide against different viruses

<table>
<thead>
<tr>
<th>Virus Name</th>
<th>Cellular target.</th>
<th>Dosage Concentration</th>
<th>Effects</th>
<th>Mechanism of action of Andrographolide</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>Hepatocytes, DCs, T cells and NK cells</td>
<td>54.1 TO 200 uM</td>
<td>Liver cirrhosis, fibrosis and hepatocellular carcinomas</td>
<td>Inhibit HBV DNA replication</td>
<td>[72-75]</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>1. Epithelial cells of the upper and lower respiratory tract.2. MDCK cells.3. DCs and macrophages</td>
<td>1.400-125 mg/kg/d2. 200 to 3277.4 ug/ml3.250 ug/ml</td>
<td>Respiratory infection including pneumonia, sinus infections, asthma etc.</td>
<td>Inhibits H9N2, H5N1 and H1N1 virus both in vitro and in vivo.Inhibits H3N2 virus replicationInhibits the H1N1-induced RIG-1-like receptor signaling pathway</td>
<td>[76-80]</td>
</tr>
<tr>
<td>HIV</td>
<td>Macrophages, monocytes, DCs and CD4+ T cells</td>
<td>5-100 mg/ml and 50-200 ug/ml respectively</td>
<td>AIDS and opportunistic infections</td>
<td>Reduces p24 antigen level and increases CD4+ T cell count</td>
<td>[64-67]</td>
</tr>
<tr>
<td>CHIKV</td>
<td>Epithelial, endothelial, fibroblasts, monocytes and macrophage cells</td>
<td>1-100 uM</td>
<td>Fever, headache, rashes, polyarthalgia.</td>
<td>Reduces viral RNA copy number and inhibits viral protein expression.</td>
<td>[54, 56]</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Hepatocytes, PBMCs, especially B cells</td>
<td>1-10 uM</td>
<td>Liver cirrhosis, hepatocellular carcinoma and extrahepatic infection, chronic hepatitis.</td>
<td>Inhibits HCV NS3/4A protease and its drug resistant mutantsSuppress HCV genome replication by promoting IFN alpha response,</td>
<td>[70, 71]</td>
</tr>
<tr>
<td>HPV</td>
<td>Basal epithelial cells</td>
<td>9-15.34 uM</td>
<td>Vaginal, penile, anal and oropharynx geal region, genital warts</td>
<td>Restores P53 protein and inhibits E6 oncogenic envelop gp protein</td>
<td>[68, 69]</td>
</tr>
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</table>
shown that it exerts anti-cancer activity by targeting TLR4/NF-Kb, where NF-kB acts as a specific target of Andrographolide. Previous studies have shown that CHIKV Protein E interacts with actin but here in this studies shows no any change in the expression of actin but however the significant inhibition of protein synthesis and virus genome replication, suggesting that Andrographolide may work at an earlier stage of replication. Further analysis by western blotting and confocal microscopy had shown complete abolished detectable protein expression of CHIKV E1: 226AS, and greatly inhibited expression of CHIKV E1:226VS. Therefore this study has shown that Andrographolide may not only promote the inhibition of viral proteins but also help in clearance of viral proteins.

**Autophagy and Andrographolide in viral infection**

Several studies has shown Andrographolide induces autophagy. In CHIKV infection, the induction of autophagy by virus is used to help in viral replication but the process need the interaction of viral protein with host cell proteins and it is possible that Andrographolide interferes with the autophagic process, allowing the increased activation of autophagy to promote the clearance of viral proteins rather than promoting replication. On the other hand Andrographolide has also been shown to play a role in inhibiting terminal auto phagosome-lysosome fusion and therefore the interference in autophagic flux may significantly affect CHIKV replication, as well as aid in clearance of proteins. These results would suggest that Andrographolide is either targeting the CHIKV genome or proteins directly, or a protein or proteins induced by the infection. Therefore the ability of Andrographolide to act on multiple target sites/pathways at the same time could promote it as an effective antiviral agent.

**Antiviral role of Andrographolide upon Human immunodeficiency virus (HIV)**

The growing number of HIV patients day by day and the development of viral resistance have shifted the focus towards developing new drugs against HIV. Research has been carried on the antiviral role of Andrographolide on the HIV virus. One in-vivo study has found that Andrographolide in HIV infected patients showed a significant increase in CD4 lymphocytes not only that but the higher dose of Andrographolide resulted in a decrease in virus copy number. Reddy et al reported that Andrographolide acts at the molecular level by not blocking the viral-host interaction but disrupt the cell signaling pathways. The viral capsid protein p24 is highly expressed during the early stage of infection. However, the in-vitro study carried in the MT2 cell line exhibited a reduced level of p24 protein expression. Chang et al reported that Andrographolide and its related compounds to interfere with the binding of the new viral virus with the host cells by hindering a step in the viral replication cycle following virus-cell binding. Holt and co-workers found that Andrographolide can modify cellular signal transduction and stop the progress of the disease. Andrographolide inhibits C-mos enzyme for expression of C-mos involved in T-cell death and HIV propagation.

**Antiviral role of Andrographolide against Human papillomavirus (HPV) by affecting E6 oncogene**

HPV infection is usually associated by creating an imbalance in the host immune system. The misbalancing created in the immune system usually involves Th1-Th2, tumor-associated macrophages differentiation and down-regulation activation and maturation of DC’s. Studies have revealed the inhibitory effects of Andrographolide and its derivatives on HPV16 infection which include inhibition of the E6 oncogene, restoration of p53 tumor suppressor protein, and induction of cervical cell apoptosis. In addition, Andrographolide and its derivatives prevented the binding of HPV16PsVs to host-cell receptors, and 14-Deoxy-11, 12-didehydroandrographolide (14-DDA) showed the highest potency of post-attachment inhibition. These studies revealed that the Andrographolide has potential for anti-HPV activity by affect-
neuraminidase (NA) are responsible for host interaction and two surfaces encoded glycoproteins hemagglutinin (HA) and neuraminidase (NA) infecting a variety of animals including humans. The virion consists of outer lipoprotein and the hepatitis B surface antigen (HBsAg). Although the pattern of HBV transmission varies throughout the world, the immune cells and pathways targeted by HBV include NK cells, Tregs, interferon pathways and dendritic cells [50]. However, the presence of immunosuppressive regulatory T cells (Tregs) contribute to an inadequate immune response against HBV, causing chronic infections. The present vaccine available against HBV is HBsAg to generate the neutralizing antibodies to provide long time protection [51]. In addition, the docking study has shown that Andrographolide is effective in inhibiting H9N2, H5N1 and H1N1 strains of influenza virus [52].

**Andrographolide as a potent antiviral agent against Influenza B virus (HBV)**

HBV is a DNA virus belonging to family Hepeviridae. The virion consists of outer lipoprotein and the hepatitis B surface antigen (HBsAg). Although the pattern of HBV transmission varies throughout the world, the immune cells and pathways targeted by HBV include NK cells, Tregs, interferon pathways and dendritic cells [50]. However, the presence of immunosuppressive regulatory T cells (Tregs) contribute to an inadequate immune response against HBV, causing chronic infections. The present vaccine available against HBV is HBsAg to generate the neutralizing antibodies to provide long time protection [51]. Chen et al. synthesized 48 derivatives of dehydroandrographolide and Andrographolide and evaluated their anti-HBV activity. They reported that Andrographolide and some of its derivatives not only inhibited HBsAg and hepatitis B envelope antigen (HBeAg) secretion but also exhibited anti-HBV effects by inhibiting HBV DNA replication [52, 53]. They further investigated the relationship of the structure and its anti-HBV activity of Andrographolide and its derivatives [54].

**Andrographolide as a potent antiviral agent against Influenza A virus (IAV)**

Influenza A virus (IAV) belongs to family Orthomyxoviridae infecting a variety of animals including humans. The two surfaces encoded glycoproteins hemagglutinin (HA) and neuraminidase (NA) are responsible for host interaction and virus release, respectively [57]. Molecular docking study carried out by Raja and co-workers has shown that Andrographolide interacts with both HA and NA glycoproteins by forming 5 and 3 hydrogen bonds, respectively [58]. This study has shown that Andrographolide and its derivatives have the capability to interact with viral proteins used during infection for different biological functions in their respective hosts. In antiviral immunity including RIG-1 signaling pathway (Fig. 2) usually activated upon RNA viruses infections, Andrographolide has shown to modulate hosts antiviral defense system by inhibiting RIG-1 receptor signaling pathways in human bronchial epithelial cells [59, 60]. In another in-vitro, as well as in-vivo study carried by Chen et al. have shown Andrographolide is effective in inhibiting H9N2, H5N1 and H1N1 strains of influenza virus [52].

**Modulation of host immune response by Andrographolide**

Significant progress has been made in understanding the molecular mechanism underlying virus-host interactions, hosts both humeral as well as cellular adaptive immune response. Recent studies have shown that Andrographolide is able to down-modulate both cellular as well as humoral immune response. In in-vivo studies, the mice fed with Andrographolide showed that humoral (antibody) response to thymus-dependent antigen and delayed type hypersensitivity were both greatly reduced. In the case of in-vitro studies, Andrographolide interferes with the cytokine release and T-cell proliferation in response to allogeneic stimulation. This molecule was reliable both to hinder the maturation of dendritic cells and as well as their ability to present antigen to T-cells. This inhibition property AP towards the T-cell activation was further applied to EAE which is an inflammatory demyelination disease of the central nervous system [62]. The treatment with Andrographolide showed a significant decrease in reducing EAE symptoms by inhibiting T-cell and antibody responses directed to myelin antigens. The study revealed that Andrographolide effectively blocks the activation both in-vitro and in-vivo. Xu et al have shown that feeding the mice injected with killed Salmonella vaccine with different doses of Andrographolide resulted in significant increase of *S. Typhimurium* specific IgG antibodies [63]. On the other hand, Shan et al have shown that Andrographolide has the ability to inhibit both adhesions as well as the transmigration of neutrophil through suppression of Mac-1 upregulation. It was found that the inhibitory effect of Andrographolide on Mac-1 expression could be mediated by downregulation of ROS production by PKC dependent by calcium-independent mechanism [64, 65]. Therefore Andrographolide in limiting the early phase of neutrophil infiltration may be useful for the improvement of inflammatory disorders.

**Modulation of different Anti-inflammatory intracellular mediators by Andrographolide**

(A) Andrographolide block the binding of NF-κB to DNA by affecting IkB degradation (cysteine 62 of p50 subunit NF-κB)
There are a number of pro-inflammatory genes, such as iNOS, COX-2, TNF-alpha, IL-8 or IL-1, that are involved in the pathogenesis of large number of diseases either caused by viruses or bacterial infections. Here studied has shown that Andrographolide plays a major role by interfering with NF-kB pathways so as to block its interaction with cellular DNA or its directly its translocation into a nucleus results to cause further activation of any pro inflammatory disease. The interference in binding / translocation of NF-kB by Andrographolide has shown that this plant compound forms a covalent adduct with reduced cysteine 62 of p50 subunit NF-xB, which interferes in binding ability of NF-xB to DNA Fig 2 [69]. There are many other study carried in different cells involving in inflammatory processes such as bronchial epitheli al cells [67], endothelial cells [68] dendritic cells [69] and monocytes [70] where the treatment by different doses of Andrographolide disrupts the NF-kB pathway.

(b) Andrographolide to reduce the inflammatory process via AP-1 and/or STAT3 modulation.

The production of pro-inflammatory cytokines such as IL-1β, IL-6 and IL-10, plays a major role in a number of inflammatory diseases [71]. Here in this studies the treatment of macrophages with Andrographolide results in modulation of transcription factors AP-1 and STAT3. AP-1 and STAT3 which results in contributing inhibitory effect on iNOS and COX-2 expression in macrophages. It has been reported an overexpression of activated STAT3 and high DNA binding activity of AP-1 in synovial tissue from patients suffering with Rheumatoid Arthritis [72, 73]. In various studies Andrographolide has been found to decrease the STAT3 phosphorylation, which is crucial for nuclear translocation and DNA binding and also reduced the LPS-induced AP-1 DNA-binding activities Fig 2 [71]. Thus, Andrographolide may also be contributing to reduce the inflammatory process by interfering with AP-1 and/or STAT3 modulation.

(c) Andrographolide interferes with the activation of transcription factor Nuclear Factor of Activated T cells (NFAT)/p13/Akt and ERK1/2 pathways.

In another study it has been shown that Andrographolide interferes with the activation of transcription factor Nuclear Factor of Activated T cells (NFAT) in T cells. The interference of NFAT activation by Andrographolide is related to the increase of Andrographolide-induced JNK phosphorylation, which controls the export of NFAT from nucleus [74]. There are several cellular pathways, such as PI3K/Akt and ERK1/2 pathways, involved in the anti-inflammatory effect of Andrographolide Fig 2 [75, 76].

Cytoprotective and antioxidant role of Andrographolide against different oxidative stress

Oxidative stress is another dimension of virus-induced pathogenesis. The oxidative damage caused by the production of an increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), is a feature of many viral infections [77]. The excessively produced ROS and RNS deplete intracellular antioxidant compounds which consequently lead to cell death [78]. The role of oxidative stress in the pathogenicity of viruses is indicated by the finding that virus infections increase the levels of ROS [79, 80]. However, the role of ROS in viral diseases is more complex because it includes metabolic regulations for both host metabolisms and viral replication [78, 81]. Andrographolide has shown a neuroprotective effect in ischemic rats and against nicotine-induced oxidative stress in the brains of male waster rats [82].

Further studies demonstrate that Andrographolide prevents the production of ROS by modulation of protein kinase C (PKC)-dependent pathway., Thus down-regulates up-expression of Mac-1, an essential integrin for neutrophil adhesion and transmigration, the possible mechanism involved in its anti-inflammatory effect (Fig 3) [82, 83]. Nitric oxide (NO) is a critical mediator in inflammation. It is continuously produced by the inducible isofrom of NO synthases. (iNOS) in inflammatory macrophages under the stimulation of lipopolysaccharides (LPS) and cytokines [84]. It was observed that andrographolide and neandrographolide, obtained from Andrographis paniculata suppressed NO production, orally in a concentration-dependent manner in activated macrophages in-vitro and ex-vivo, which account for the anti-inflammatory activity [85].

Efficacy and safety regarding the clinical use of Andrographolide

A. paniculata is safe at its therapeutic dose and is neither associated with any side effects nor any serious toxicity. However when administered at higher dose it may cause vomiting, gastric discomfort, loss of appetite, etc. due to bitter taste. There are several clinical trials that demonstrate the positive effects of A. paniculata on infectious diseases, hypertriglyceridemia, and autoimmune disorders [86].

The study carried by Handa, 1990, Acute hepatitis was induced in rats by means of a single dose of galactosamine (800 mg/kg, ip)/paracetamol (3 g/kg, orally [po]) so that the hepatoprotective effects of andrographolide could be studied. Upon andrographolide treatment with dose concentration of (400 mg/kg ip or 800 mg/kg po) 48, 24, and 2 hours before galactosamine administration, or (200 mg/kg ip) 1, 4, and 7 hours after paracetamol, leads to complete normalization of toxin-induced increases in levels of hepatic enzymes and significantly reduced toxin-induced histopathologic liver changes in rats [87].

To check the possibility of (A. paniculata) standardized to ≥10% andrographolide, on male fertility. The study carried on albino Wistar rats. The treatment group were orally administered 0, 20, 200, and 1000 mg/kg of body weight per day, for 65 days prior to mating and 21 days during mating. The treatment groups when compared to control groups doesn’t showed any change in testosterone levels, body weight and feed consumption at any of the dose level. More than that the sperm count and morality were not affected. The testes did not show any histopathological changes. No observed adverse effect level of extract of A. paniculata (≥10%
andrographolide) was found to be more than 1000 mg/kg per day.\[84\]

In a study based on three in vitro testes: Ames test, clastogenicity tests and acute oral toxicity to check the genotoxicity of the standardized extract of A. paniculata (KalmCold™), Ames based on chromosome aberration (CA), and micronucleus (MN). Different concentration (doses) of (KalmCold™) used in Ames test: 5000 μg/ml, 1581 μg/ml, 500 μg/ml, 158 μg/ml, 50 μg/ml, 16 μg/ml, and in clastogenicity tests (KalmCold™) doses used: 80 μg/ml, 26.6 μg/ml, 8.8 μg/ml for short-term treatment without S9; 345 μg/ml, 115 μg/ml, 38.3 μg/ml for short-term treatment with S9; and 46 μg/ml, 15.3 μg/ml, 5.1 μg/ml for long-term without S9 using DMSO as a vehicle control. After treatment with different doses of KalmCold™, Ames test confirmed no mutations both in the presence as well as in absence of S9 in Salmonel typhimurium mutant strains TA98 and TAmix. In CA and MN, KalmCold™ did not induce clastogenicity in CHO-K1 cells in vitro. It is evident that KalmCold™ is genotoxically safe. More than that in clastogenicity tests, female rats were treated with 5000 mg/kg of KalmCold™ didn’t showed any for signs of toxicity for 14 days. Therefore the authors came to conclude that KalmCold™ did not produce any treatment-related toxic effects in rats \[89\]. However some adverse reactions are rare in adults, it has been associated with allergic reactions in children (15A, 16A). Yan Hu Ning injection contains Andrographolide and is commonly used in China to treat viral pneumonia and upper respiratory infections.

**Yan Hu Ning injection:**

A 2.5-year-old boy developed a red complexion, profuse sweating and shortness of breath about 35 minutes into an intravenous infusion of Yan Hu Ning injection 80 mg in 150 ml of 5% glucose in isotonic saline for suppurative tonsillitis. The infusion was stopped immediately and intravenous dexamethasone resulted in amelioration of the allergic reaction and in another case a 10-year-old girl after 24 minutes of an intravenous infusion of Yan Hu Ning injection: 120 ml in 150 ml of 5% dextrose for an upper respiratory infection, developed itching in the limbs, followed by a red complexion, red eyes, abdominal pain, a generalized rash with pruritus, chest distension, and shortness of breath. The infusion was stopped immediately and she was given dexamethasone and promethazine, which led to complete resolution \[90, 91\].

**Xiyanping injection**

The main ingredient of Immunologic Xiyanping injection is water-soluble andrographolide most commonly used for its anti-inflammatory, antipyretic and as well both as antiviral, antibacterial but it has been reported to cause anaphylactic shock. The intravenous infusion of Xiyanping injection 200 mg in 250 ml 5% dextrose to a 51-year-old woman diagnosed with cough and a fever (38 °C) and after20 minutes suddenly developed chest distension, palpitation, shivering, dysphoria, facial pallor, intolerance of cold, and a low blood pressure (80/50 mmHg). The infusion was stopped immediately and she made an uneventful recovery after receiving oxygen, adrenaline, dexamethasone, and diphenhydramine \[90\].

The underlying mechanism of Yan Hu Ning and Xiyanping injection induced allergic reactions are unknown, but it is probably attributable to impurities in the product.

**Conclusion**

Emerging of viral diseases have become a global threat. Therefore, additional intensive, detailed studies are required to elucidate the mechanisms of viral infections and provide new drug targets for the treatment of important virus infections \(\text{Fig. 1}\) \[90, 90\]. However, significant progress has been made in understanding the molecular mechanisms underlying virus-host interactions, host innate antiviral immune responses \[90\]. This knowledge helps in designing new drugs and strategies to protect humans as well as animals against pathogenic viruses. Mostly present-day vaccines which stimulate innate immunity in host is short lived if the antigenicity of the virus changes. Recent trend of using traditional approaches to treat diseases has been revived all over the world \[13, 99\]. Plant based antiviral drugs have provided great hope for combating viral infection. As mentioned in the review, above Andrographolide has shown promise against viral disease. Andrographolide prevented transmission of the virus to other cells and stopped the progress of the disease by modifying cellular signal transduction \(\text{Fig. 2}\). Different research groups have shown the main target of Andrographolide involves virus replication machinery \[96, 97\], fusion and adsorption of virus to the host cell \[98-106\] viral receptor and co-receptor binding \[101, 102\], Post-translational modifications \[103, 104\], reverse transcription and integration \[105, 106\] and viral protein translation \(\text{Fig. 1}\) \[107\]. Plant derived antiviral drugs fulfill this challenge by being cost-effective, easily available, low in cytotoxicity, and therapeutically effective against viral infections. Andrographolide, a plant-derived compound is widely distributed, having low cytotoxicity \[108-112\]. However, still much research is needed to identify target molecules in the viral life cycle \(\text{Fig. 1 and 2}\).


[40] Thon-Hoang VI, Denizot M, Li-Pat-Yuen G, Giyr C, Jaffar-


