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Review

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Andrographolide: A review on experimental Clinical Trials and Applications

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Keywords

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Abstract

Throughout human history, plants have been used for medicinal purposes. Plants can synthesise a vast range of chemical compounds that possess biological activities and can be employed as medicines. Andrographis paniculata is an Asian medicinal herb and a well-known Chinese herbal remedy. It is referred to as “king of bitters” in English, “Chiretteverte” and “Roi des amers” in French, “Kariyatu” in Gujarati, and “Kirayat” or “Kalpanath” in Hindi. In India, it is commonly known as "kalmegh". Traditionally, it has been used to treat lung infections, cold, fever, diarrhoea, flu, syphilis, rabies, upper respiratory infections, leptospirosis, leprosy, malaria, sinusitis, HIV/AIDS, and TB, among other conditions. A chemical constituent present in the leaves of Andrographis paniculate, a diterpenoid labdane also known as andrographolide, was identified as a key bioactive component of A. paniculata in 1951. It is believed to be responsible for the various biological applications like anti-oxidant, immunity enhancer, hepatoprotective, cardioprotective, anti-inflammatory, anti-cancer, and upper respiratory tract infection. Since 1984, andrographolide and its analogues have been studied for anti-inflammatory activities using contemporary drug development strategies. The anticancer properties of andrographolide have been investigated on several cancer models, yielding promising results. This manuscript primarily focuses on the clinical aspects of andrographolide. We aim to elucidate its applications, preclinical findings, and clinical studies.

1 Introduction

Andrographis paniculata, a plant steeped in ancient Asian traditional medicine, has earned its moniker as the "king of bitterness" in English, "Chiretteverte" and "Roi des amers" in French, "Kariyatu" in Gujarati, and "Kirayat" or "Kalpanath" in Hindi, with the colloquial name "kalmegh" in India [6][7]. This member of the Acanthaceae family thrives in various Asian regions, spanning tropical, subtropical, and southeastern locales, including India [8]. A. paniculata (AP) is predominantly used in the form of extracts and exhibits various pharmacological activities such as anti-bacterial [9], anti-viral [10], and immunosuppressant[11]. The main constituent of AP is andrographolide, which displays a wide range of biological activities including hepatoprotective, cardioprotective, antibacterial, antidiabetic, anti-inflammatory, antimalarial, and antitumor properties. In addition to these pharmacological benefits, AP has potential applications in treating rheumatoid arthritis. Over the last few decades, numerous andrographolide derivatives have emerged, and their pharmacological properties have been extensively studied [12]. Formulations based on nanotechnology also play a crucial role in biotechnology and phytochemical-based delivery. Burgos et al. conducted a study on the effectiveness of AP in relieving rheumatoid arthritis (RA) symptoms. They concluded that an AP formulation containing 30% andrographolide was effective against RA in clinical settings, although further studies are necessary [13]. Sandborn et al. examined the impact of AP extract on active ulcerative colitis. Their observations revealed that individuals with mild to moderately active ulcerative colitis who received AP extract (HMPL-004) at a daily dosage of 1,800 mg were more likely to achieve a clinical response compared to those who received a placebo [14]. Chiu et al. conducted a phase II clinical trial to assess the efficacy of AP water extract in palliative care for metastatic esophageal squamous cell cancer. They concluded that patients who completed AP therapy significantly lived longer survival periods and maintained their quality of life throughout the survival period compared to those who couldn't complete AP treatment [15]. We aimed to provide comprehensive information on the pharmacological activity of A. paniculata and its primary compound, andrographolide, in this work.

2 Chemistry of Andrographolide

Andrographolide is a bioactive compound found in the medicinal plant Andrographis paniculata is a diterpenoid lactone with a molecular formula C20H30O5 and a molar mass of approximately 350.45 g/mol. The IUPAC name of andrographolide is 3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8adi methyl-2-methylene-1-napthalenyl]ethylidene]dihydro -4-hydroxy-2(3H)-furanone [16].

It is a colorless, crystal-like, extremely bitter compound in taste. The bitterness is due to the presence of the lactone ring refs. The lactone ring is also responsible for the compound's biological activity refs. Andrographolide has been shown to inhibit the activity of several enzymes, including cyclooxygenase-2 (COX-2) refs, which is involved in inflammation. Andrographolide has also been shown to inhibit the growth of cancer cells [17]. The chemical structure of andrographolide consists of a labdane diterpene backbone, containing a carbocyclic ring, an α, β-unsaturated-γ-lactone, two olefin bonds Δ8 and Δ12, and three hydroxyls at C-3, C-14, and C-19 (Figure 1). It is sparingly soluble in water but more soluble in organic solvents such as ethanol, methanol, chloroform, and ethyl acetate [18]. Andrographolide is relatively stable under normal conditions but can degrade when exposed to high temperatures, light, and moisture [19].

The biosynthesis of andrographolide is a complex process that involves multiple pathways. The deoxy xylulose pathway (DXP) and mevalonate (MVA) pathway are the two main pathways involved in the production of this compound [20]. The DXP pathway begins with the condensation of two molecules of pyruvate to form methyl-D-erythritol-4-phosphate (MEP). MEP is then converted to several other compounds, including isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). IPP and DMAPP are enzymatically condensed to form geranyl

pyrophosphate (GPP), a C-10 isoprenoid compound refs. Then another condensation reaction involving GPP and IPP produces geranylgeranyl pyrophosphate (GGPP), a C20 isoprenoid compound. GGPP is then converted into various diterpene precursors through cyclization and rearrangement reactions. These precursors serve as the building blocks for the formation of different diterpenoid compounds in the plant refs. One of the diterpene precursors undergoes a cyclization reaction to form a labdane-type diterpene, specifically labdadienyl/copalyl diphosphate. This reaction is catalyzed by the enzyme ent-copalyl diphosphate synthase (CPS). The labdadienyl/copalyl diphosphate is further converted into andrographolide through a series of enzymatic reactions involving oxidation, rearrangement, and lactonization. Additionally, the MVA pathway begins with the condensation of three molecules of acetyl-CoA to form mevalonate. Mevalonate is then converted to IPP and DMAPP, which can then be used to synthesize a variety of diterpenes, including andrographolide refs.

The isolation of andrographolide from A. paniculata involves a series of steps [21]. Initially, the plant material is harvested and either dried or used fresh. The dried plant material, mainly leaf, is Grinded into a fine powder. The extraction of the leaf powder by cold maceration in a 1:1 mixture of dichloromethane and methanol is carried out and then andrographolide is directly isolated from the resulting extract by recrystallisation method [22]. Other solvents like ethanol, ethyl acetate, etc. can also be used for extraction purpose. Sometimes, the crude extract can also be purified using chromatography techniques. Finally, the presence and purity of andrographolide are confirmed through analytical techniques such as TLC, HPLC, NMR spectroscopy, and mass spectrometry [23].

Although, andrographolide has been recognized for its potential therapeutic properties, one of the significant challenges associated with its use as a therapeutic agent is its poor bioavailability. Suresh et al. have tried to solve this problem by developing a cocrystal for andrographolide [24]. The study revealed that andrographolide-salicylic acid cocrystal proved to be particularly promising as it completely inhibited the chemical transformation of andrographolide to its inactive sulfate metabolite. Additionally, the cocrystal showed a significantly faster dissolution rate and higher drug release compared to pure andrographolide. This discovery may offer a potential solution to enhance the efficacy and pharmaceutical properties of andrographolide as a herbal medicine [24].

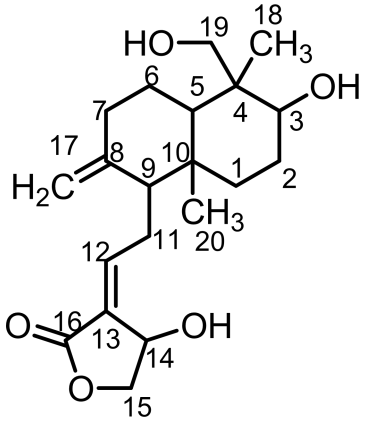

Figure 1: Structure of andrographolide

3 Applications of Andrographolide

The Acanthaceae family of plants includes A. paniculata [25]. This herb has been used for many years to treat several diseases conditions such as colds, laryngitis malaria, fever, diarrhea, hypertension, and diabetes [26]. Various pharmacological properties of this herb have been already documented such as antiviral, antibacterial, hypocholesterolemic, hypoglycaemic, and adaptogenic [27]. The diterpene lactone andrographolide was the most prominent biologically active chemical of the plant. It has been reported that andrographolide has a variety of pharmacological effects such as immunostimulatory,

anti-inflammatory, antidiarrheal, anti-cancer, anti-microbial, antihepatitic , cardioprotective, antihyperglycemic anti-microbial, antioxidant , and cardiovascular [28]. Currently, techniques for extracting and assessing andrographolide from a diverse range of herbal mixtures, complex compositions, and biological sources have been developed. These techniques include Electroanalytical methods, Chemiluminescence methods, Spectrophotometry, and Chromatography. These methodologies are utilized for the quality analysis of biological samples and pharmaceutical formulations [29].

3.1 Immunity enhancer

In various traditional medicine practices across the globe, A. paniculata, a plant of medicinal value, has been recognized and utilized as a powerful herbal medicine to treat a variety of health conditions [30]. Andrographolide, an active constituent of the plant has been shown to improve non-specific immunity by promoting lymphocyte phagocytosis and replication as well as specific immunity, such as antibody reactions and delayed-type hypersensitivity response [31]. The formulation of Andrographolide sulfonate has been reported to directly modulate the immune response of T-lymphocytes and neutrophils, thereby enhancing the efficiency of the host's immune response in treating various disease conditions [32]. Andrographolide is an immunostimulant agent that may influence immune function by triggering macrophages, natural killer cells, and cytokines [33]. By reducing T-cell and antibody responses to myelin antigens, andrographolide treatment markedly reduced experimental autoimmune encephalomyelitis symptoms in mice (Figure 2). In vivo experimental results showed that AGL can inhibit T-cell activation in vitro and may help to reduce negative T-cell reactions [34]. Andrographolide alters the mitogen-activated protein kinase (MAPK)-Nrf2-HO-1 signaling cascade in cerebral endothelial cells and provides a protective effect in ischemic stroke in rats [35].

3.2 Hepatoprotective

The primary active anti-hepatotoxic compound in A. paniculata is andrographolide [37]. Numerous biological activities of andrographolide have been related to reports of its therapeutic and preventative benefits on liver disorders. It effectively prevents liver injury induced by external factors, which may be due to oxidative stress and inflammatory reactions [38]. Tang et al. have synthesized derivatives of andrographolide with increased water solubility and

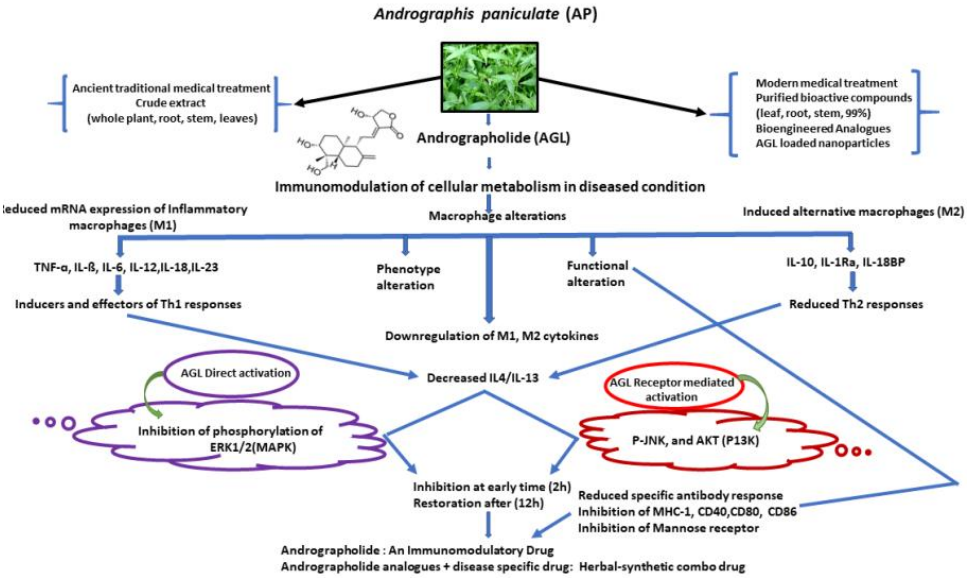

Figure 2: Diagram depicting the effects of andrographolide and a potential mechanism for regulation of the immune system. (This figure adopted CCBY4 from reference [36])

assessed for hepatoprotective potency. The outcomes of the experiment suggested that adequate aqueous solubility may enhance the drug absorption and bioavailability, which indirectly increases their hepatoprotective effect in mice [39]. Chen et al. have reported potential in vitro and in vivo activity of synthetic derivatives of andrographolide for the treatment of liver damage. These derivatives of andrographolide effectively prevent chemically induced acute liver injury by blocking STAT3 (Signal Transducer and Activator of Transcription 3) activity [40]. Additionally, it has been observed that nano-formulated andrographolide particles exhibit hepatoprotective activities [41]. Polylactide co-glycolide nano capsulated andrographolide was well reported with enhanced hepatoprotective potency compared to free andrographolide against liver damage inflicted by the poison arsenic [42].

3.3 Cardioprotective

Andrographolide reduces LPS-induced dysfunction of the heart in mice by inhibiting TNF-α, IL-1β, IκB phosphorylation, and NO (nitric oxide) production, myocardial apoptosis and hence offering a potential treatment for myocardial dy sfunction caused by sepsis [43]. Zhang et al. investigated the impact of andrographolide on Lewis's rat models of experimental autoimmune myocarditis. Their study revealed that andrographolide treatment led to reduced levels of TNF-α, IL-17, and myosin antibodies. Moreover, it inhibited the infiltration of CD3+ positive T cells and CD14+ positive monocytes in myocarditis rats. The protective effects of andrographolide proposed suppression of cardiac inflammation and blockade of the PI3K/Akt pathway [44]. Andrographolide was also reported to improve cardiac function in diabetic mice. The experimental result reported with andrographolide reduces diabetes-induced cardiac hypertrophy and blocks NF-κB activation, protein expression of IL-6, and adhesion molecules. As suppressing NF-κB-mediated inflammation and modulated NOXs/Nrf2-mediated oxidative stress andrographolide was found promising compound for the treatment of diabetic cardiomyopathy [45]. A 50 mg/kg dose of andrographolide protects against high-fat diet-induced cardiac damage in mice by inhibiting apoptosis and by efficiently improving the IGF-1R (Insulin like Grwth Factor – 1R) compensatory mechanism and significantly supporting the efficacy of andrographolide supplementation in managing the symptoms of cardiovascular disease in obese people [46].

In neonatal rat cardiomyocytes, Woo et al. observed the cardioprotective action of andrographolide against reoxygenation/hypoxic damage, upregulating antioxidant enzyme activities and lowering cellular glutathione levels [47]. By boosting NF-E2–related factor 2 expression both in vivo and in vitro, andrographolide plays a crucial role in heart protection by defending against oxidative stress upon myocardial infarction and subsequently enhanced cardiac performance [48]. The study by Shu et al. explored the favourable impact of andrographolide on a mouse model of coronary heart disease. This effect was achieved by inhibiting the expression of Peroxisome Proliferator-Activated Receptors (PPARs) and the NF-κB signaling pathways. [49]. Beneficial effect of andrographolide was also reported on adverse cardiac remodelling after myocardial infarction through reducing oxidative stress by enhancing the expression of Nrf2 [50].

3.4 Anti-inflammatory Properties of Andrographolide

Andrographolide's anti-inflammatory effects were attributed to its multifaceted mechanisms of action. A key player in inflammation regulation is the NF-κB signaling pathway, which andrographolide prominently inhibits by blocking its activation and translocation into the nucleus [51]. NF-κB regulates the expression of various pro-inflammatory genes, including cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as enzymes like cyclooxygenase-2 (COX-2) [52]. By impeding NF-κB, andrographolide leads to reduced expression of pro-inflammatory cytokines, contributing to the attenuation of the inflammatory

response at the molecular level.

Andrographolide's interference with the NF-κB pathway extends to COX-2, a crucial enzyme involved in prostaglandin synthesis. Prostaglandins are lipid mediators that contribute to inflammation, pain, and fever [54]. Andrographolide's ability to suppress COX-2 expression leads to diminished production of prostaglandins, further contributing to the attenuation of the inflammatory response at the molecular level (Figure 3). These coordinated actions of andrographolide result in the effective regulation of inflammation, modulating the inflammatory environment and preventing the overactivation of immune responses that can lead to tissue damage and chronic inflammation [55].

Moreover, andrographolide exerts its anti-inflammatory prowess by modulating the activity of immune cells. Macrophages play a central role in the initiation and resolution of inflammation [56]. Andrographolide promotes the polarization of macrophages toward an anti-inflammatory M2 phenotype, reducing their pro-inflammatory cytokine secretion while enhancing the secretion of anti-inflammatory mediators [57]. Similarly, Andrographolide affects T-cells, promoting the differentiation of regulatory T-cells (Tregs), which have anti-inflammatory properties and help regulate immune responses [55]. By modulating these immune cells, Andrographolide further contributes to its anti-inflammatory effects.

Andrographolide's potent antioxidant activity also plays a major role in its anti-inflammatory actions [58]. Inflammation often leads to the generation of reactive oxygen species (ROS) and oxidative stress, which can further exacerbate tissue damage and inflammation [59]. Andrographolide's potent antioxidant effects help neutralize these harmful ROS, thereby mitigating oxidative stress and its detrimental effects on cells and tissues

3.5 Anti-cancer Property of Andrographolide

Andrographolide's anti-cancer actions encompass various stages of cancer development, from halting uncontrolled cell proliferation to interfering with the formation of new blood vessels crucial for tumor growth and metastasis [60].

One of the central aspects of its anti-cancer activity lies in its ability to impede cancer cell proliferation. Andrographolide achieves this by targeting the cell cycle and disrupting the intricate regulatory checkpoints that control cell division. Through the downregulation of key cell cycle regulatory proteins such as cyclins and cyclin-dependent kinases (CDKs), Andrographolide induces cell cycle arrest at specific phases, such as the G0/G1, S, or G2/M phases [61]. This arrest prevents cancer cells from continuously dividing and accumulating, effectively inhibiting tumor growth. By interfering with the cell cycle progression, andrographolide effectively thwarts the uncontrolled proliferation characteristic of cancer cells, offering a potential means of curbing tumor progression [62].

Moreover, andrographolide's induction of apoptosis (programmed cell death) represents a pivotal mechanism in its anti-cancer effects [61], [63]. Cancer cells often evade apoptosis, allowing them to survive and multiply uncontrollably. However, andrographolide addresses this evasion by triggering a series of cellular events that promote apoptosis. It upregulates pro-apoptotic proteins like Bax and activates caspases, the enzymes responsible for carrying out the process of apoptosis. Concurrently, it downregulates anti-apoptotic proteins like Bcl-2, which counteract the pro-apoptotic signals. These coordinated actions tip the balance in favor of apoptosis, prompting cancer cells to undergo programmed self-destruction. This pro-apoptotic activity of Andrographolide contributes significantly to its anti-cancer efficacy and provides a means to selectively eliminate cancer cells [63], [64].

Angiogenesis, the process by which new blood vessels sprout from existing ones, is essential for tumor survival and growth. Tumors exploit this process to create a network of blood vessels that supply them with nutrients and oxygen [65]. However, andrographolide disrupts this advantageous network by suppressing the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases

(MMPs). VEGF is a potent pro-angiogenic factor that stimulates blood vessel formation, while MMPs play a role in breaking down the extracellular matrix, allowing new blood vessels to grow. By inhibiting VEGF and MMPs, andrographolide impedes the growth of new blood vessels, thereby depriving tumors of their essential lifeline for sustenance [66]. As a consequence, the tumor's ability to grow and metastasize is compromised.

Overall, andrographolide's capacity to modulate critical pathways involved in cancer progression makes it an attractive candidate for further exploration in cancer research and drug development. While preclinical studies and in vitro experiments have provided compelling evidence of its anti-cancer efficacy, additional investigations, including clinical trials, are essential to fully unlock its therapeutic potential and establish its safety and efficacy as a part of cancer treatment strategies.

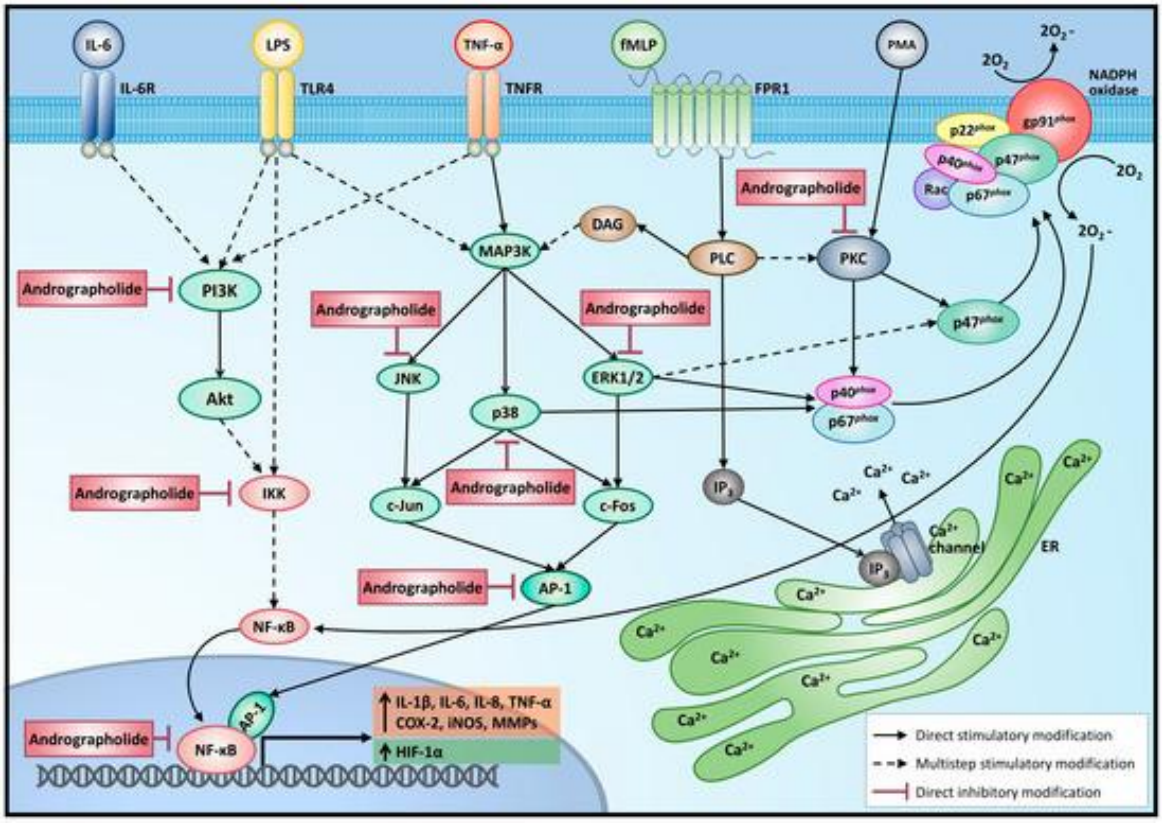

Figure 3: Anti-inflammatory effects of Andrographolide (Adopted under CC BY from [53])

3.6 Upper Respiratory Tract Infection:

Upper respiratory tract infections (URTIs) are widespread globally, leading to substantial morbidity and imposing a substantial economic burden. The escalating concern over antibiotic resistance has spurred interest in alternative therapies [67]. Andrographolide has emerged as a promising remedy for managing URTIs.

Andrographolide expertly influences the immune response by enhancing the body's defense mechanisms against both viral and bacterial infections. It stimulates the activity of vital immune cells, such as macrophages and natural killer (NK) cells, which serve as crucial sentinels in identifying and eliminating invading pathogens [57], [68].

Extensive studies have revealed that Andrographolide possesses potent antiviral activity, encompassing a wide spectrum of viruses, including respiratory viruses like influenza viruses and coronaviruses. It exhibits its antiviral prowess by disrupting viral replication and interfering with viral entry into host cells. Consequently, the viral load is significantly reduced, resulting in a milder and less severe manifestation of URTI symptoms[69].

URTIs are frequently accompanied by an inflammatory response in the upper respiratory tract,

culminating in distressing symptoms such as sore throat, nasal congestion, and cough [70]. Notably, Andrographolide's anti-inflammatory properties play a pivotal role in alleviating these discomforts. By downregulating the production of pro-inflammatory cytokines and mediators, Andrographolide mitigates the intensity of the inflammatory response. This dampening effect on inflammation significantly contributes to the relief of URTI-related symptoms [55].

Andrographolide exhibits several properties that make it a promising asset for the treatment of URTIs. This includes broad spectrum antiviral activity [71], Immune system modulation [68], Anti-inflammatory [55] and anti-bacterial activities [72].

4 Preclinical and clinical studies on Andrographoliden

Several clinical and preclinical trials on andrographolide have been completed, revealing significant results across various conditions and diseases, including cardiovascular issues, rheumatoid arthritis, cancer, upper respiratory tract infections, inflammation, and more. Andrographolide (ADP) has proven effective in boosting the immune system at specific concentrations. [12]. In the study conducted by Aldurrah et al. [73] the anti-depressant effects of andrographolide were evaluated using chronic unpredictable stress (CUS) zebrafish model. Zebrafishes were subjected to CUS, and then treated with CUS + Andrographolide (100 mg/L) and CUS + fluoxetine (0.01 mg/L). After inducing stress, zebrafishes' behaviours and potential toxicity effects were assessed within a 24-hour period. The study's findings revealed an increase in the total distance travelled, and importantly, andrographolide exhibited a significant reduction in freezing duration. Indirapriyadarshinni et al. [74] studied an effect of andrographolide in UV-B induced mouse skin. A group of mice were subjected to UVB radiation at the dose of 180mL/cm2 for 10 days. The drug andrographolide (3.6mg kg/b.Wt) was topically administered 1 hour before each UVB radiation. The pretreatment with the drug reduced lipid peroxidation and restored antioxidant status in mouse skin. Further, it slowed down the UVB-induced inflammatory cytokines such as CD34, iNOS, NF-Kb, COX-2, IL-6, 10 in the mice skin. Yang et al. [75] conducted a study demonstrating andrographolide supresses aerobic glycolysis and triggers cell death in cancer cells. It exhibited anticancer activity by inhibiting pyruvate dehydrogenase kinase 1 expression in lung cancer cells. This is achieved through cleavage of polymerase, activation of caspase 3, and damage to the mitochondria, resulting in increased reactive oxygen species. Further details of these clinical trials are provided in Table 1.

5 Side Effects and Toxicity of Andrographolide

The administration of andrographolide is linked to a spectrum of common side effects, encompassing various categories. Gastrointestinal disturbances stand out as the most prevalent, including discomforts like nausea, vomiting, diarrhea, and abdominal pain [81]. Another notable side effect is the occurrence of headaches [82]. Some individuals administered with andrographolide have reported skin rashes [81]. Additional side effects include dysgeusia[81] and alterations in libido [83]. In more rare circumstances, andrographolide has been associated with more serious side effects, such as anaphylactic reactions, which represent severe and potentially life-threatening allergic responses [81]. Furthermore, the administration of andrographolide has been linked to the possibility of causing renal damage in select individuals [84]. The presence of these rare but significant side effects emphasizes the importance of close monitoring and immediate medical attention. The wide range of potential side effects underscores the need for careful evaluation and personalized risk assessment when considering andrographolide as part of a therapeutic regimen.

Earlier experiments of andrographolide using animal models were reported without any serious toxicity. [85], [86] However recent reported studies have shown toxicity associated with andrographolide is not negligible. Huang et al. examined the effect of andrographolide on the embryonic stem cell test

model and reported the reproductive toxicity increasing ROS level, potential damage to the mitochondrial membrane, and interfering caspase-3 and nuclear factor 2 related factor 2 protein. [87] Male reproductive toxicity of andrographolide was examined using a male albino rat model and the result showed a disruption of somniferous epithelium and abnormalities in sperm count and motility. [88] Andrographolide is well reported with nephrotoxicity and case studies also support the nephrotoxicity with a reduction in urine output and acute tubular necrosis in patients. [89] Observations reported as andrographolide activates endoplasmic reticulum stress signaling by increasing the expressions of C/EBP homologous protein and Caspase-4. This endoplasmic reticulum stress and TNF-α, and IL-6-induced inflammations can be a key mechanism for the nephrotoxic effect of Andrographolide.3 Further studies identified the potential toxicity biomarkers such as 2-ketoadipate and 1,5-anhydroglucitol which conform metabolic changes due to Andrographolide induced nephrotoxicity. [90] Safety profile of andrographolide extract demonstrated with no cytotoxicity with 13.2-81.5 μM range of andrographolide content on human cell lines sampled from sensitive organs including liver, brain, lungs, and intestine. [91]

In a notable study conducted by Batran et al., it was observed that Andrographolide exhibits a remarkable level of safety, particularly in the context of toxicity, as evidenced by their research findings. In their comprehensive investigation, the researchers administered Andrographolide to rats at a significant dosage of 500 mg/kg/day. The outcomes of this study affirmatively indicate that Andrographolide is characterized by a lack of toxicity at this substantial dosage level [92].

However, it is imperative to note that certain populations warrant circumspect usage of Andrographolide. For instance, prudent caution is advised against its consumption by pregnant or breastfeeding women due to considerations of potential adverse effects [93]. Furthermore, a corpus of literature signifies that individuals grappling with hepatic or renal ailments ought to exercise restraint in its utilization. Similarly, individuals concurrently prescribed specific medications, such as anticoagulants or immunosuppressants, should approach its utilization judiciously [94]. This caveat is underscored by the intricate interplay between Andrographolide and specific medications, which necessitates a vigilant assessment of potential contraindications and interactions to ensure optimal therapeutic outcomes and safety considerations.

6 Conclusion and future prospects

Numerous researchers have been drawn to andrographolide due to its remarkable pharmacological properties. To enhance its biological activities, diverse derivatives of andrographolide have been synthesized. In this article, we aim to provide a comprehensive summary of various experimental and clinical trials exploring its applications, including as an antioxidant, immunity enhancer, hepatoprotective agent, cardioprotective agent, anti-inflammatory agent, anti-cancer compound, and treatment for upper respiratory tract infections. Given the increasing global prevalence of inflammation, there is a pressing need for the development of highly effective anti-inflammatory medications Andrographolide, a chemical constituent of Andrographis paniculata, holds promise in this regard. Research conducted across various systemic disorders has demonstrated andrographolide's anti-inflammatory effects. Its potential extends to respiratory, digestive, immune, cardiovascular, nervous, and skeletal system disorders, as well as tumors and other inflammatory conditions. While existing knowledge is promising, further studies are essential to delve into the details and gain a deeper understanding of the diverse applications of andrographolide.

Table 1: Summary of clinical trials of Andrographolide

NCT number	Other names	Phase	Study design	Conditions	Volunteer	Outcome measures
NCT02273635	14PIE-26946CORFO 14-391	Phase 1 Phase 2	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment	Primary Progressive Multiple Sclerosis Multiple Sclerosis, Secondary Progressive	68	• Retarding the progression of brain atrophy as measured by MRI (Magnetic resonance imaging). • The progression of multiple sclerosis was decreased. • Andrographolide reduced IL-2 production in T cells by interfering with Nuclear factor of activated T cells (NFKT) and mitogen activated protein kinase (MAPK) activation [76]. • Another study resulted that by interfering with T cell activation, it reduces autoimmune encephalomyelitis in the mice [77].
NCT04196075	CRE-2017.616	Phase 3	Allocation:N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Supportive Care	Squamous Cell Carcinoma of Esophagus	30	• It provided symptomatic relief of dysphagia. • It retarded the growth of tumor. • After 4 months of treatment, side effects of andrographolide observed. It included gastrointestinal upset, nausea and vomiting, allergy, diarrhoea, abdominal pain and dizziness.
NCT03262792	VL/170105/PA/OA	Not Applicable	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Investigator, Outcomes Assessor)	Knee Osteoarthritis	108	• Tablets made from extract A. paniculata (AP) (30% total andrographolide) were given to patient of rheumatoid arthritis 3 times a day for 14 weeks. • Primary outcomes were resulted that decrease pain. Moreover, it was associated to a reduction of rheumatoid factor, IgA, and C4 [13].

NCT number	Other names	Phase	Study design	Conditions	Volunteer	Outcome measures
			Primary Purpose: Treatment			
NCT03455049	IndonesiaU-02	Not Applicable	Allocation: Randomized Intervention Model: Crossover Assignment Masking: Double (Participant, Investigator) Primary Purpose: Treatment	• Increased Insulin	73	• Beta cell secretion decreased. • Insulin resistance observed • Also analysed beta cell function after 2h-OGTT • A crossover study designed to check effect of andrographolide on GLP-1 and DPP-4 concentrations in normal and predictable subjects. • Study resulted that, AP extract increased GLP-1 concentration (19.6 % compared to placebo) without inhibiting the DPP-4 enzyme [78].
NCT02280876	PaCRU-02/PCNS. EM/12PCNS-EM	Phase 1 Phase 2	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment	Multiple sclerosis, relapsing remitting	30	• There were no changes in clinical parameters had been observed. It significantly reduced fatigue in patients with relapsing-remitting MS (RRMS) receiving interferon beta in comparison to placebo. • Patients treated with AP showed a significant reduction in FSS score, a 44% reduction in 12 months. • AP significantly lessened fatigue in RRMS patients receiving interferon beta compared to placebo and solely interferon beta therapy [79].
NCT04463875	Corfu HC	-	Observational Model: Case-Only Time Perspective: Prospective	Migraine	113	• Migraine pain reduced after treatment of 3 months. • Change in acute migraine conditions was observed. • Change in headache impact was observed.
NCT03780621	EP-1004	Phase 1	Allocation: Randomized	Cognitive Impairment, Mild	16	• Responses of electric brain activity as spectral power in 17 different brain regions

NCT number	Other names	Phase	Study design	Conditions	Volunteer	Outcome measures
			Intervention Model: Crossover Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment			within 6 specially defined frequency ranges. • Overall cognitive function was impaired. • AP exhibited anxiolytic and calming effect without sedation and was associated with stress reducing activity [80].
NCT00749645	PCT06-AG-02 DO4I1240FONDEF	Phase 2	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment	Arthritis, Rheumatoid (AR)	60	• It was associated to a reduction of rheumatoid factor, IgA, and C4. • These findings suggested that AP could be a useful "natural complement" in the treatment of AR
NCT04955327	HP/200802/PARACTIN/CC	Phase 3	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Triple (Participant, Care Provider, Investigator) Primary Purpose: Treatment	Upper Respiratory Tract Infection	225	• The severity score decreased after treatment for 7 days • Fever, common cold, burning eyes were decreased • In COVID-19 patients, symptoms were relieved after 8 to 15 days.

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Conflict of Interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization, Divya Teli and Dixa A. Vaghela; Data curation,Amit Chaudhri; Formal analysis, Hetvi K. Solanki; Methodology, Keshav Jetha; Writing-Original draft, Vivek P. Chavda and Divya Teli; Writing-review and editing, Dixa A. Vaghela and Amit Chaudhri; All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Medical Ethics Committee, and the patients were informed and consented.

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary Material

Not applicable

Reference

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