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The Role of Complementary and Alternative Medicine in Regenerative Medicine

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Experimental and Clinical Pharmacology of *Andrographis paniculata* and Its Major Bioactive Phytoconstituent Andrographolide

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Abstract

Andrographis paniculata (Burm. F) Nees, generally known as "king of bitters," is an herbaceous plant in the family Acanthaceae. In China, India, Thailand, and Malaysia, this plant has been widely used for treating sore throat, flu, and upper respiratory tract infections. Andrographolide, a major bioactive chemical constituent of the plant, has shown anticancer potential in various investigations. Andrographolide and its derivatives have anti-inflammatory effects in experimental models asthma, stroke, and arthritis. In recent years, pharmaceutical chemists have synthesized numerous andrographolide derivatives, which exhibit essential pharmacological activities such as those that are anti-inflammatory, antibacterial, antitumor, antidiabetic, anti-HIV, antifeedant, and antiviral. However, what is noteworthy about this paper is summarizing the effects of andrographolide against cardiovascular disease, platelet activation, infertility, and NF-κB activation. Therefore, this paper is intended to provide evidence reported in relevant literature on qualitative research to assist scientists in isolating and characterizing bioactive compounds.

propose obtaining various leads by structurally modifying andrographolide. In recent decades, numerous andrographolide derivatives have emerged and their pharmacological activities have also been evaluated. However, studies that have comprehensively summarized or analyzed *A. paniculata* and its derivatives have been minimal. Therefore, to contribute to the advanced trends of research on andrographolide, this paper provides thorough information regarding the pharmacological activities of *A. paniculata* and its major compound andrographolide.

1.1. Chemical Structure

Andrographolide is a major bioactive phytoconstituent found in various parts of *A. paniculata* (Figure 1), but particularly in the leaves. The chemical name of andrographolide is 3α, 14, 15, 18-tetrahydroxy-5β, 9βH, 10α-labda-8, 12-dien-16-oic acid γ-lactone (Figure 2), and its molecular formula and weight are $C_{20}H_{30}O_5$ and 350.4 (C 68.54%, H 8.63%, and O 22.83%), respectively. The structure of andrographolide has been analyzed by using X-ray, 1H,13 C-NMR, and ESI-MS [6–10]. Although andrographolide is not very soluble in water, it is soluble in acetone, chloroform, ether, and hot ethanol. Crystalline andrographolide was reported to be highly stable, over a period of three months [11]. Rajani et al. [8] reported a simple and rapid method for isolating andrographolide from the leaf of *A. paniculata.* They extracted it using a 1 : 1 mixture of dichloromethane and methanol and then isolated the andrographolide directly from the extract by performing recrystallization. The purity of the compound has been evaluated with thin-layer chromatography (TLC), UV absorption spectrum, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LCMS), and differential scanning calorimetry (DSC), which revealed the melting point of andrographolide to be 235.3°C [8, 9].

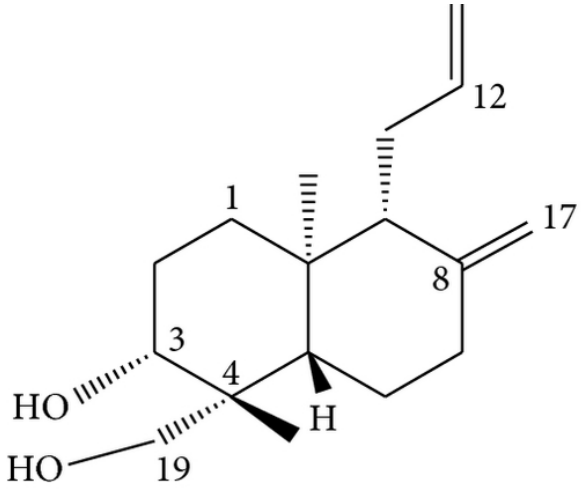

Figure 2

Chemical structure of andrographolide.

1.2. Biological Activities of Andrographolide

Andrographolide has been reported to have a wide range of biological activities, such as those that are anti-inflammatory [12], antiallergic [13], antiplatelet aggregation [14, 15], hepatoprotective [16], and anti-HIV [17]. In addition to these activities, the ability of ethanol or an aqueous extract of *A. paniculata* to decrease blood glucose levels in normal rats or streptozotocin diabetic rats has been documented [18]. In biological systems, andrographolide can interact with many inter- and intracellular constituents as a bipolar compound, thus ensuing in many biological responses. A recent study demonstrated that *A. paniculata* polysaccharides combined with andrographolide can ease the recovery of diabetic nephropathy [18].

2. Experimental Studies

2.1. Effects on Antioxidant Defense

Antioxidant defense systems may only partially prevent oxidative damage [19]. Hence, there is interest in using dietary supplements containing antioxidants to protect the components of the human body from oxidative damage. Currently, the most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and *tert*-butylhydroquinone. However, BHA and BHT have restricted use in foods because they are suspected to be carcinogenic and to cause liver damage [20]. Therefore, there is growing interest in using natural additives as potential antioxidants [21, 22].

Andrographolide has been reported to significantly reduce the inflammation caused by histamine, dimethyl benzene, and adrenaline [27]. Overproduction of NO and prostaglandin E2 (PGE2), because of the expression of inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), plays a significant role in the inflammatory processes of activated macrophages. The secretion of proinflammatory cytokines from macrophages stimulated and promoted by lipopolysaccharide, which causes induction of iNOS, results in increased production of NO. The methanol extract of *A. paniculata* and andrographolide incubated with macrophages have been reported to inhibit LPS-stimulated NO production in a concentration-dependent manner [28, 29]. Chiou et al. [30] observed that andrographolide inhibits lipopolysaccharide-induced nitric oxide (NO) production and inducible NO synthase (iNOS) expression in the murine macrophage-like cell line RAW 264.7. Administering andrographolide to rats fully restored the maximal contractile response of the thoracic aorta to phenylephrine after incubation with LPS and alleviated the decrease in the mean arterial blood pressure of anesthetized rats. Andrographolide has also been reported to suppress IL-2 production and T-cell proliferation in a mixed lymphocyte reaction and to inhibit dendritic cell maturation and antigen presentation [31].

2.3. Anticancer Activity

Natural products are recognized as sources for drugs used to treat several human ailments including cancers. Vincristine, irinotecan, etoposide, and paclitaxel are examples of many natural pharmaceuticals derived from plants [32]. Despite the discovery of numerous drugs of natural origin, searching for new anticancer agents is still necessary to provide drugs that are less toxic and more effective and to increase their variety and availability. Samples with pharmacological usage should be accounted for when selecting plants to treat cancer because several ailments reflect disease states bearing relevance to cancer or cancer-like symptoms [33]. Andrographolide exhibited potent cytotoxic activity against KB (human epidermoid leukemia) and P388 (lymphocytic leukemia) cells [34]. Among the diterpenoid lactones isolated from the ethyl acetate fraction of *A. paniculata*, andrographolide had strong anticancer activity by inducing cell differentiation in mouse myeloid leukemia cells [35]. Andrographolide was found to inhibit the proliferation of various cell lines including leukemia, breast cancer, lung cancer, and melanoma cells [2, 36]. Furthermore, this compound has strong anticancer activity against human colorectal carcinoma LoVo cells by inhibiting cell cycle progression [37]. A potent growth inhibitory effect of andrographolide has been demonstrated in acute promyelocytic leukemic cells (HL-60 and NB4) that are mediated by inducing cell differentiation and apoptosis [38, 39]. Andrographolide was also reported to suppress the adhesion of gastric cancer cells which express high-level sialyl Lewis X to human vascular endothelial cells by blocking E-selectin expression and, thus, may represent a candidate therapeutic agent for cancer [40]. Lim et al. [41] demonstrated that the anticancer mechanisms for andrographolide include the inhibition of Janus tyrosine kinases-signal transducers and activators of transcription, phosphatidylinositol 3-kinase and NF-κB signalling pathways, suppression of heat shock protein 90, cyclins and cyclin-dependent kinases, metalloproteinases and growth factors, and the induction of tumour suppressor proteins p53 and p21, leading to the inhibition of cancer cell proliferation, survival, metastasis, and angiogenesis.

In vivo models of the anticancer activity of andrographolide have been used against MCF-7 and HT-29 tumor xenografts and B16F0 melanoma [38]. In a radiation therapy study, andrographolide was found to sensitize Ras-transformed cells and significantly delay tumor growth [42]. Sheeja and Kuttan [43] demonstrated that *A. paniculata* extract or andrographolide alone could stimulate cytotoxic T lymphocyte production through the enhanced secretion of IL-2 and

Purified andrographolide (1 mg/kg body weight) or intragastric administration of ethanol extracts of the stems and leaves (25 mg/kg body weight) to mice stimulate antibody production and the delayed-type hypersensitivity response to sheep red blood cells [45]. The extract and purified andrographolide were also reported to stimulate an innate immune response in mice, which was measured according to the macrophage migration index, phagocytosis of leucine-labelled *Escherichia coli*, and proliferation of splenic lymphocytes stimulated by *A. paniculata* extract [45]. The immunomodulatory property of a diterpene lactone andrographolide was reported to be associated with the enhancement of the proliferation of human peripheral blood lymphocytes, as well as the production of key cytokines and the expression of Y Xu 21 immune activation markers in whole blood cells in culture *in vitro* [46]. Rajagopal et al. [2] and Kumar et al. [1] have reported the immunostimulatory activity of andrographolide *in vitro* in PHA-stimulated human peripheral blood lymphocytes (HPBLs) by increased proliferation of lymphocytes and production of IL-2. *In vivo* immune responses, such as an antibody response to a thymus-dependent antigen and delayed-type hypersensitivity, were considerably lessened in mice treated with andrographolide. In addition, Iruretagoyena et al. [47] reported that andrographolide enhanced the tolerogenic properties of immature dendritic cells (DCs) in experimental autoimmune encephalomyelitis (EAE) by inhibiting NF-kappa B activation in murine DCs. Andrographolide was also reported to reduce IFN-γ and IL-2 production in murine T cells stimulated with concanavalin A (Con A) *in vitro* [48]. Moreover, andrographolide was reported to inhibit the production of TNF-α and IL-12 in macrophages stimulated by lipopolysaccharide [49].

2.5. Hepatoprotective Activity

Liver diseases of various origins remain a serious health problem and a major cause of mortality. In the absence of reliable hepatoprotective drugs in modern medicine, herbs and plants play a vital role in managing several liver disorders [50, 51]. Extensive literature related to the hepatoprotective activity of molecules from herbal sources shows that there is a vast array of molecules exhibiting potent hepatoprotective efficacy. The Indian systems of medicine have long used *A. paniculata* as a hepatostimulant and hepatoprotective agent [16]. *A. paniculata* is also an ingredient in several polyherbal preparations used as hepatoprotectants [52], one of which has been reported to be efficacious in chronic hepatitis B viral infection [53]. A recent study showed that andrographolide attenuated concanavalin A-induced liver injury and inhibited hepatocyte apoptosis [54]. Shukla et al. [55] reported observing choleretic effects of andrographolide in conscious rats and anesthetized guinea pigs. The effect of andrographolide was found to be more potent than silymarin against acetaminophen-induced reduction of the volume and contents of bile. Andrographolide was also shown to protect against ethanol-induced hepatotoxicity in mice with an equivalent efficacy of silymarin [56]. Oral pre- and posttreatments of adult rats with an extract of *A. paniculata* were protective against an ethanol-induced increase in serum transaminases. A protective effect of a single oral dose each of the extract and of andrographolide has been studied in carbon tetrachloride-(CCl_4-) induced hepatic microsomal lipid peroxidation. Rana and Avadhoot [57] reported the hepatoprotective effects of the crude alcohol extract of leaves against CCl_4-induced liver damage; these effects have had also been established against paracetamol-induced toxicity in an *ex vivo* rat model of isolated hepatocytes [58]. Plant extracts of *A. paniculata* showed hepatoprotective characters consistent with the folk use and pharmacology [59].

2.6. Antimicrobial Effects

aqueous extract of leaves that exhibit significant antimicrobial activity against gram-positive *S. aureus*, methicillin-resistant *S. aureus*, and gram-negative *Pseudomonas aeruginosa* [60]. Significant activity against enterohemorrhagic strains of *E. coli* was found in the ethanol extract of *A. paniculata* [61]. The virucidal activity of andrographolide has been reported against herpes simplex virus 1 (HSV-1) without having any significant cytotoxicity [62]. At a concentration of 0.05 mg/mL of a chloroform extract of *A. paniculata,* the plant completely inhibits malarial parasitic growth within 24 h of incubation; and the same inhibition has been noted within 48 h with methanol extract concentration of 2.5 mg/mL [63]. A methanol extract was found to inhibit *Plasmodium falciparum* substantially at a 50% inhibitory concentration (IC50) of 7.2 μg/mL [64]. The ethanolic extract of *A. paniculata* was effective against upper respiratory tract infection [65]. The antimicrobial activity of *A. paniculata* against nine bacterial strains, *Salmonella typhimurium, E. coli, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Streptococcus pyogenes, Legionella pneumophila,* and *Bordetella pertussis,* has also been reported [66].

2.7. Antiviral Effects

The antiviral activities of plant extracts have been renewed and have been the topic of passionate scientific investigation. Several medicinal plant extracts have shown antiviral activities against some RNA and DNA viruses. Among these plants is *A. paniculata* which exhibits a neutralizing activity against the human immunodeficiency virus (HIV) [67]. Andrographolide was investigated for antiviral activity against herpes simplex virus (HSV) [62, 68], HIV [3], flaviviruses, and pestiviruses [69]. Lin et al. [70] demonstrated that 25 μg/mL of ethanolic extract of *A. paniculata* and 5 μg/mL of andrographolide effectively inhibit the expression of Epstein-Barr virus (EBV) lytic proteins, Rta, Zta, and EA-D, during the viral lytic cycle in P3HR1 cells. A recent study has demonstrated that *A. paniculata* has the most antiviral inhibitory effects among six medicinal plants tested against DENV1-infected Vero E6 cells [71].

2.8. Antipyretic and Analgesic Effects

In Asian countries, *A. paniculata* has been widely used for its antipyretic, analgesic, protozoacidal, antihepatotoxic, anti-HIV, immunostimulant, anticancer effects [36]. It had been reported that andrographolide, with oral doses of 100 and 300 mg/kg, produced a significant antipyretic effect after 3 h administration of brewer's yeast-induced fever in rats [72]. In addition, doses of 180 or 360 mg/kg of andrographolide were also found to relieve fever in humans by the third day after administration [73]. Madav et al. [72] have also reported that 300 mg/kg of andrographolide, administered orally, had significant analgesic activity on acetic-induced writhing in mice and on the Randall-Selitto test in rats, but without any effect on the hot plate test in mice. These authors have also reported that intraperitoneal administration of 4 mg/kg of andrographolide exhibited an analgesic effect, whereas the former study, 300 mg/kg administered orally did not. The different routes of administration between these experiments could contribute to this discrepancy [72].

2.9. Antimalarial Effects

In vitro and *in vivo* studies performed by Rahman et al. [63] showed that *A. paniculata* produced significant antimalarial effects. Chloroform extract of this plant shows better effect than the methanol extract because it showed complete parasite growth inhibition as low as 0.05 mg/mL drug dose within 24 h incubation period as compared to methanol

treatment of different products of *A. paniculata* greatly affected the larval growth of *Anopheles stephensi* and caused malformation and mortality in a dose-dependent manner [81]. An ethanolic extract of *A. paniculata* caused moderate ovicidal activity against various age groups of *Aedes stephensi*, but it inflicted delayed effects such as high larval, pupal, and adult mortality, thereby suppressing the vector population and adversely influencing transmission of the disease pathogen [82]. The leaf extract of *A. paniculata* with different solvents of benzene, hexane, ethyl acetate, methanol, and chloroform exhibited larvicidal and ovicidal activities against *Culex quinquefasciatus* Say and *Aedes aegypti* L., whereas ethyl acetate and methanol extracts of the plant showed only ovicidal activity against *Culex quinquefasciatus* and *Aedes aegypti* [83]. They have also found 100% mortality against two mosquito species exerted by ethyl acetate and methanol extracts of the plant. A recent study performed by Sheeja et al., 2012 suggest that the leaf extracts of *A. paniculata* may have the potential to be used as an ideal eco-friendly approach for the control of the filarial vector *Culex quinquefasciatus* [76].

2.11. Renoprotective Effects

The recurrence of urolithiasis is critical; thus, preventing and treating stone formation are highly recommended. The most recent data suggest that 27 million people have chronic kidney disease, representing nearly one in seven adults and a 30% increase over the past decade [84]. In the Unites States, more than 200 thousand people suffer from kidney failure. A similar increase in the incidence of end-stage renal failure caused by an increasing incidence of the risk factors for renal disease has occurred in many Asian countries [85]. A study found that the aqueous extract of *A. paniculata* could considerably alleviate the nephrotoxic action of gentamicin in male albino rats, thus exhibiting marked renoprotective activity [86].

2.12. Antifertility Effects

Efforts are underway to develop antifertility products from plants. Many plants are reported to have fertility-regulating properties in ancient Indian literature [87]. Numerous plants have been tested for their antifertility activities in laboratory animals [88, 89] and several animal studies have reported an effect of *A. paniculata* on male and female reproduction. Early reports of oral administration of the powdered stem of *A. paniculata* have shown an antifertility effect on male Wistar mice, but no impact on fertility in female mice [90]. It has also been reported that administering *A. paniculata* results in abortion in pregnant rabbits. Moreover, the herb is reported to suppress the growth of human placental chorionic trophoblastic cells in vitro [91]. Zoha et al. [92] reported feeding sun-dried *Andrographis* powder to female mice at a dose of 2 g/kg bw/day for 6 weeks and then mated them with untreated males of proven fertility, thus inhibiting pregnancy in 100% of the tested animals. Oral administration of *Andrographis paniculata* extract during the first 19 days of pregnancy in doses of 200, 600, and 2000 mg/kg did not exhibit any effect on the elevated level of progesterone in the blood plasma of rats [46]. Animal studies have also shown that *A. paniculata* may have contraceptive or antifertility effects following long-term treatment at high doses (20 mg/rat) [93]. However, there was a large degree of discrepancy in the results, with some studies demonstrating no untoward effects even at the 1000 mg/kg dose [48]. Administering dry leaf powder to male albino rats (20 mg daily for 60 days) has been shown to inhibit spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells, and regressive or degenerative changes in the epididymis, seminal vesicle, ventral prostate, and coagulating glands [94]. Andrographolide also produced similar results

nephropathy. This has prompted considerable interest in using traditional medicines to treat this condition. Orally administered glucose-induced hyperglycemia in nondiabetic rabbits was reported to be prevented by the extract of *A. paniculata.* Six weeks of chronic administration of the extract showed no effect on fasting blood glucose levels [98]. The ethanolic extract of *A. paniculata* at a dose of 400 mg/kg body weight twice daily for 2 weeks to diabetic rats was shown to produce a 49.8% reduction in fasting serum triglyceride levels. This was reported to be greater than the 27.7% decline that was achieved with 500 mg/kg body weight twice daily for 14 days [24]. An aqueous extract (50 mg/kg body weight) administered to streptozotocin-diabetic rats resulted in a 52.9% reduction in blood glucose levels. Dry powder of the plant material significantly decreased blood glucose levels by 61.8% at a lower dose of 6.25 mg/kg body weight [99]. Comparable results were observed by Dandu and Inamdar [100] with oral administration of an aqueous extract of *A. paniculata* leaves. A dose of 400 mg/kg was found to lower the blood glucose levels of streptozotocin-induced animals and increased the activity of superoxide dismutase and catalase. Oral administration of the decoction also significantly reduced blood glucose levels in alloxan-induced diabetic rats and reduced food and water intake when compared to vehicle-treated diabetic controls [100]. Extended mean estrous cycles were reduced from 8 to 5 days in treated diabetic rats [101]. Andrographolide appears to reduce plasma glucose concentration dose-dependently in streptozotocin-induced diabetic and normal rats, with the potential effect observed in normal rats rather than in diabetic rats [102]. This is a significant difference from the water extract, which did not show a glucose-lowering effect in a study on normoglycemic rats [100].

Andrographolide also attenuates the increase in plasma glucose in response to an intravenous glucose challenge in normal rats and enhances the uptake of radioactive glucose by isolating the soleus muscle of streptozotocin-diabetic rats in a concentration-dependent manner. Repeated intravenous administration of andrographolide in diabetic rats for three days resulted in an increase in mRNA and protein levels of glucose transporter in the soleus muscle, indicating that the glucose-lowering effect of andrographolide could be caused by more effective glucose use of the skeletal muscle [102]. However, an *in vitro* experiment concluded that the hypoglycemic effect of *A. paniculata* is caused by insulin release from pancreatic cells through ATP-sensitive potassium channels, an effect that is similar to that of other insulinotropic antidiabetic agents [103]. Subramanian et al. [104] conducted *in vitro* experiments and suggested that the inhibition of alpha-glucosidase and alpha-amylase enzyme could be the mechanism by which the ethanol extract of *A. paniculata* and andrographolide produce hypoglycemic effects. Water extract seems to be a more suitable candidate for further study because it does not affect the fasting blood glucose levels of nondiabetic animals. Therefore, identifying blood glucose-lowering constituents in both water and ethanol extracts may be of value.

2.14. Hypolipidemic Effects

Hyperlipidemia is a crucial factor, particularly in patients with high cholesterol levels and abnormal lipoprotein metabolisms, and has a direct relationship with cardiovascular diseases [105, 106]. Hence, the research and development of new functional foods and medicines for preventing coronary heart disease are crucial. Cholesterol and other fatty substances combine in the bloodstream and are deposited in the blood vessels to form a material called plaque [107]. The increase in lipids can cause plaque to grow over time and lead to obstructions in blood flow. If an obstruction occurs in the coronary arteries, it could result in a heart attack. Furthermore, an obstruction occurring in the arteries of the brain could lead to a stroke [108]. Hence, it is critical to actively decrease blood lipid counts to prevent and cure cardiovascular and cerebrovascular diseases. A recent study thoroughly demonstrated that andrographolide has potent

blood vessels and prevented the blood vessels from constricting and limiting blood flow to the brain, heart, and other organs [112]. A time-dependent protection of rat cardiomyocytes against hypoxia injury was reported to be caused by the pretreatment of andrographolide; this effect was reported to be associated with upregulation of cellular reduced glutathione (GSH) level and antioxidant enzyme activities [113]. Awang et al. [114] demonstrated that the dichloromethane extract of *A. paniculata* significantly reduced coronary perfusion pressure by up to 24.5 ± 3.0 mm Hg at a 3 mg dose and also reduced the heart rate by up to 49.5 ± 11.4 beats/min at this dose. The arterial constriction caused by high cholesterol in the diet and by injury to the inner lining of the blood vessel was also found to be diminished by *A. paniculata* [115]. It was reported that *A. paniculata* decreased the damage of the heart muscle, when it is administered to dogs one hour after the development of myocardial infarction [116]. These findings imply the promising use of *A. paniculata* as a favorable alternative for cardiovascular therapy.

2.16. Inhibitory Effects on Platelet Aggregation

An intravascular thrombosis is among the generators of a wide variety of cardiovascular diseases. Initiation of an intraluminal thrombosis is believed to involve platelet adherence and aggregation. Thus, platelet aggregation may play a crucial role in the atherothrombotic process [117]. Blood platelet activation and aggregation are common denominators in atherothrombotic events and various inflammatory diseases. Platelets have been viewed exclusively as mediators of thrombosis and hemostasis; their function has been extended to include prominent roles in inflammation and immunity [118]. Therefore, the use of antiplatelet agents, which can inhibit thromboembolic diseases (myocardial infarction, ischemic stroke, and vascular death) in the platelets, warrants investigation. Amroyan et al. [14] found that andrographolide inhibited PAF-induced human platelet aggregation. Moreover, Thisoda et al. [119] reported that the extract of *A. paniculata* (10–100 μg/mL) significantly inhibited platelet aggregation in washed rat platelets. Our recent study demonstrated for the first time that andrographolide exhibits potent antiplatelet activity through the activation of the eNOS-NO/cyclic GMP pathway and inhibition of both the PLCγ2-PKC and PI3 kinase/Akt-MAPK (i.e., p38 MAPK) cascades in washed human platelets (Figure 3) [120]. Our earlier study also showed that andrographolide may involve an increase in cyclic GMP/PKG, followed by inhibition of the p38 MAPK/$^{\bullet}$HO-NF-κB-ERK2 cascade in activated platelets. In that study, we also suggested that andrographolide may have a high therapeutic potential to treat thromboembolic disorders and may also be considered for treating various inflammatory diseases [15].

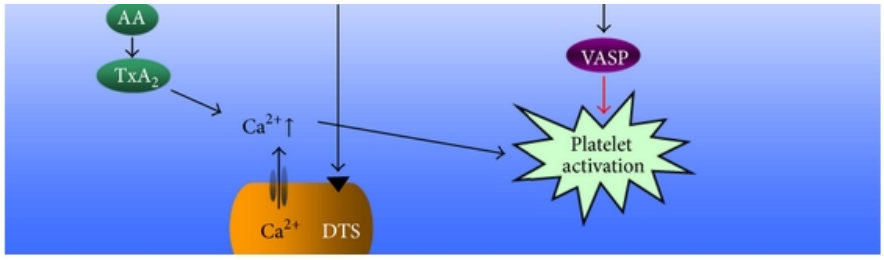

Figure 3

Hypothetical scheme shows the inhibitory signaling of andrographolide in platelet activation. Andrographolide can activate the endothelial nitric oxide synthase- (eNOS-) NO-cyclic GMP pathway, followed by the inhibition of both the PLCγ2-DAG-PKC and PI3 kinase/Akt cascades, and ultimately inhibits platelet aggregation [120].

Aqueous extract, andrographolide, and DDA inhibit thrombin-induced platelet aggregation in time- and concentration-dependent manners [119]. Andrographolide inhibits platelet-activating factor- (PAF-) induced platelet aggregation in a dose-dependent manner without affecting the biosynthesis of eicosanoids. An extract of *A. paniculata* significantly inhibited *ex vivo* ADP-induced platelet aggregation in 63 patients with cardiac and cerebral vascular diseases 3 h after administration. Thirty-three of these patients, who were observed for platelet aggregation after 1 week, experienced even more significant effects. Serotonin release from platelets was significantly reduced in 20 extract-treated volunteers, although the plasma serotonin levels remained unchanged [121].

2.17. Inhibitory Effects on NF-kappa B (NF- κB) Transcription Factors

NF-κB plays a pivotal role in the pathogenesis of inflammation, prompting various drugs designed to treat human inflammatory disease to be focused on inhibiting NF-κB activation [122]. Many natural compounds or herbal extracts reportedly exhibit anti-inflammatory activities that generally involve NF-κB activation [123, 124]. Phytochemicals, especially flavonoids, are currently of interest because of their essential biological and pharmacological properties, including the inhibition of NF-κB activation [125].

NF-kappa B comprises a family of inducible transcription factors that serve as crucial regulators of the host immune and inflammatory responses. The NF-kappa B transcription factor regulates the expression of various components of the immune system, including proinflammatory cytokines, chemokines, adhesion molecules, and inducible enzymes such as cyclooxygenase-2 and inducible nitric oxide synthase, as well as proteins that regulate the specific immune response, such as interleukin- (IL-) 2, IL-12, and interferon-γ that control lymphocyte proliferation and differentiation. Therefore, dysregulation of this transcription factor can lead to inflammatory and autoimmune diseases [126]. Andrographolide has been proven to attenuate inflammation by inhibiting NF-kappa B activation through the covalent modification of reduced Cys62 of p50. Mechanistically, andrographolide formed a covalent adduct with a reduced cysteine of p50, thus blocking the binding of NF-kappa B oligonucleotide to nuclear proteins. Andrographolide suppressed the activation of NF-kappa

inflammatory actions of andrographolide [128, 129]. Andrographolide inhibits nuclear factor kappa B (NF-κB) activation by blocking the binding of NF-κB oligonucleotides to nuclear proteins [30, 128]. Recently, we demonstrated that andrographolide enhances the NF-κB subunit p65 Ser536 dephosphorylation through the activation of protein phosphatase 2A in vascular smooth muscle cells [129]. We also demonstrated for the first time that andrographolide inhibited p65 Ser536 phosphorylation, reduced nuclear translocation of p65, and diminished p65 kB oligonucleotide binding in LPS/IFN-γ-stimulated rat VSMCs [129]. In addition, PP2A may contribute to these actions of andrographolide in rat VSMCs.

3. Clinical Studies

3.1. Antidiarrheal Effects

In the tropical and subtropical regions of the world, diarrhea is still one of the major causes of death. In developing countries, it is a principal cause of death in children under 5 years of age and the causes include infectious agents, plant toxins, and gastrointestinal disorders [130]. Many Western medicines, such as kaolin-pectin, bismuth, and loperamide, have long been used to alleviate the symptoms but have included undesirable side effects. It was reported that the ethanol extract of *A. paniculata* cured 88.3% of acute bacillary dysentery and 91.3% of acute gastroenteritis cases [91]. Administering andrographolide was reported to cure 91% of acute bacillary dysentery cases. The same cure rate (91.1%) was also achieved by administering a compound tablet containing andrographolide and neoandrographolide (at a ratio of 7 : 3) in cases of bacillary dysentery. This was reported to be higher than cure rates obtained with furazolidrne or chloramphenicol [91]. This compound has also been used traditionally to sluggish live as an antidote for colic dysentery and dyspepsia, and has been employed successfully in cases of general debility in convalescence after fever, livero disorders and advanced stages of dysentery. The juice of fresh leaves of *A. paniculata*, which generally contains andrographolide, is used as a domestic remedy to treat colic pain, loss of appetite, irregular stool, and diarrhea [131].

3.2. Anti-HIV Effects

Studies on the development of new anti-HIV drugs have begun worldwide in the past few years [132]. The growing incidence of drug-resistant HIV strains is one of the main problems in treating HIV infection, although current anti-HIV drugs can inhibit HIV infection. To avoid existing therapeutic difficulties, current searches for new anti-HIV agents are focused on discovering compounds with novel structures and different mechanisms of action [133]. Natural products and their derivatives have long been invaluable as a source of therapeutic agents for the development of medicine. The development of anti-HIV drugs derived from natural products is an area of research in which considerable effort should be dedicated in the future [134]. A clinical trial of andrographolide was conducted to examine 13 HIV-positive patients and five HIV-negative healthy volunteers. A planned protocol began with a dose of 5 mg/kg body weight for the first 3 weeks, increased to 10 mg/kg body weight for 3 weeks, and then increased to 20 mg/kg body weight for the final 3 weeks. Andrographolide administration significantly improved the $CD4^+$ lymphocyte count from a baseline mean of 405 cells/mm^3 to 501 cells/mm^3 in HIV-positive patients. There was no statistically significant change in mean plasma HIV-1 RNA levels [3]. A recent study summarized that andrographolide derivatives may be promising candidates for preventing

2.51; 95% CI 1.82–3.46) as compared with the placebo group in a duration-dependent manner. They have found that *Andrographis paniculata* extract treatment for 4 days significantly decreases in the intensity of all symptoms than in 2-day treatment group.

4. Dosage and Safety of Andrographolide

Numerous studies have been performed in different countries on the toxicity of *A. paniculata,* finding that it is extremely nontoxic, even at high doses (Table 2). Sakila et al. [137] conducted an antifertility study and found no toxicity, even at a high dose of *A. paniculata* that was administered to rats. The LD50 of andrographolide in male mice through the intraperitoneal route was reported to be 11.46 g/kg [138]. In a study of HIV-positive patients, a dose of 1,500–2,000 mg of andrographolide was administered daily for 6 weeks. The study was discontinued early despite some improvements in $CD4^+$ counts [3], and the side effects were common. Intravenous administration of andrographolide (10 mg/kg) to rabbits showed no abnormal cardiovascular responses. Results from liver enzyme tests indicated that the heart, liver, kidney, and spleen of these rabbits were found to be normal [139]. Mice receiving an oral plant extract (10 g/kg) once a day for 7 days proved that no mortality was observed. In another test for toxicity, rats or rabbits receiving 1 g/kg of andrographolide orally showed no changes in body weight, blood count, or the functions of the liver, kidney, or other vital organs [94]. Singha et al. [56] noticed that pretreatment of *A. paniculata* and andrographolide at 500 mg/kg body weight and 125 mg/kg body weight, respectively, could minimize the toxicity when compared with the ethanol-treated group, as evidenced by different enzymatic assays in the liver and kidney tissues; the results were comparable with those of administering silymarin.

Our recent study show that andrographolide concentrations of 22 μg/kg and 55 μg/kg markedly lowered the mortality rate in mice challenged with ADP (700 mg/kg) from 90% to 60%, respectively, indicating that andrographolide effectively prevents thromboembolism (Table 1) [120]. Suo et al. [140] investigated the pharmacokinetics of andrographolide (10 mg/kg, i.v.) in rats and observed that the blood concentration of andrographolide was approximately 11 μg/mL (approximately 30 μM). Moreover, administering andrographolide causes no cytotoxic effects on platelets at concentrations between 35 and 150 mM [15]. Therefore, andrographolide is recommended to be clinically tested as a pharmaceutical agent.

Table 1

Effect of andrographolide on mortality of acute pulmonary thrombosis caused by intravenous injection of ADP in experimental mice.

Table 2

Dosage and toxicity of *Andrographis paniculata* and its major natural product andrographolide.

common cold, and rheumatoid arthritis. Nevertheless, summarizing the effects on cardiovascular disease, NF-κB, and platelet activation of this natural product is worthy of review, and additional studies must be conducted to confirm the toxicological properties of this novel molecule before taking place in clinical studies in patients. This summary offers pharmaceutical chemists and plant scientists additional thoughts for drug discovery. The combined drug discovery of andrographolide analogues will likely transform them into an effective assemblage of inflammation and cancer treatment in the future.

Table 3

Experimental and clinical pharmacology of *Andrographis paniculata* and its major phytoconstituent andrographolide.

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References

1. R. A. Kumar, K. Sridevi, N. Vijaya Kumar, S. Nanduri, and S. Rajagopal, "Anticancer and immunostimulatory compounds from *Andrographis paniculata*," *Journal of Ethnopharmacology*, vol. 92, no. 2-3, pp. 291–295, 2004.
View at: Publisher Site | Google Scholar

2. S. Rajagopal, R. A. Kumar, D. S. Deevi, C. Satyanarayana, and R. Rajagopalan, "Andrographolide, a potential cancer therapeutic agent isolated from *Andrographis paniculata*," *Journal of Experimental Therapeutics and Oncology*, vol. 3, no. 3, pp. 147–158, 2003.
View at: Publisher Site | Google Scholar

3. C. Calabrese, S. H. Berman, J. G. Babish et al., "A phase I trial of andrographolide in HIV positive patients and normal volunteers," *Phytotheraphy Research*, vol. 14, no. 5, pp. 333–338, 2000.
View at: Google Scholar

4. P. K. Singha, S. Roy, and S. Dey, "Antimicrobial activity of *Andrographis paniculata*," *Fitoterapia*, vol. 74, no. 7-8, pp. 692–694, 2003.
View at: Publisher Site | Google Scholar

5. K. Jarukamjorn and N. Nemoto, "Pharmacological aspects of *Andrographis paniculata* on health and its major

View at: Google Scholar

8. M. Rajani, N. Shrivastava, and M. N. Ravishankara, "A rapid method for isolation of andrographolide from *Andrographis paniculata* Nees (Kalmegh)," *Pharmaceutical Biology*, vol. 38, no. 3, pp. 204–209, 2000.
View at: Google Scholar

9. Q. Du, G. Jerz, and P. Winterhalter, "Separation of andrographolide and neoandrographolide from the leaves of *Andrographis paniculata* using high-speed counter-current chromatography," *Journal of Chromatography A*, vol. 984, no. 1, pp. 147–151, 2003.
View at: Publisher Site | Google Scholar

10. L. Cui, F. Qiu, and X. Yao, "Isolation and identification of seven glucuronide conjugates of andrographolide in human urine," *Drug Metabolism and Disposition*, vol. 33, no. 4, pp. 555–562, 2005.
View at: Publisher Site | Google Scholar

11. L. Lomlim, N. Jirayupong, and A. Plubrukarn, "Heat-accelerated degradation of solid-state andrographolide," *Chemical & Pharmaceutical Bulletin*, vol. 51, no. 1, pp. 24–26, 2003.
View at: Publisher Site | Google Scholar

12. Y. C. Shen, C. F. Chen, and W. F. Chiou, "Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect," *British Journal of Pharmacology*, vol. 135, no. 2, pp. 399–406, 2002.
View at: Google Scholar

13. Y. F. Xia, B. Q. Ye, Y. D. Li et al., "Andrographolide attenuates inflammation by inhibition of NF-κB activation through covalent modification of reduced cysteine 62 of p 50," *Journal of Immunology*, vol. 173, no. 6, pp. 4207–4217, 2004.
View at: Google Scholar

14. E. Amroyan, E. Gabrielian, A. Panossian, G. Wikman, and H. Wagner, "Inhibitory effect of andrographolide from *Andrographis paniculata* on PAF-induced platelet aggregation," *Phytomedicine*, vol. 6, no. 1, pp. 27–31, 1999.
View at: Google Scholar

15. W. J. Lu, J. J. Lee, D. S. Chou et al., "A novel role of andrographolide, an NF-kappa B inhibitor, on inhibition of platelet activation: the pivotal mechanisms of endothelial nitric oxide synthase/cyclic GMP," *Journal of Molecular Medicine*, vol. 89, no. 12, pp. 1263–1271, 2011.
View at: Publisher Site | Google Scholar

16. N. P. Trivedi and U. M. Rawal, "Hepatoprotective and antioxidant property of *Andrographis paniculata* (Nees) in

19. M. G. Simic, "Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis," *Mutation Research*, vol. 202, no. 2, pp. 377–386, 1988.
View at: Google Scholar

20. E. R. Sherwin, A. L. Branen, P. M. Davidson, and S. Salminen, *Food Additives*, Marcel Dekker, New York, NY, USA, 1990.

21. G. K. Jayaprakasha, T. Selvi, and K. K. Sakariah, "Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extracts," *Food Research International*, vol. 36, no. 2, pp. 117–122, 2003.
View at: Publisher Site | Google Scholar

22. M. Oktay, I. Gülçin, and O. I. Küfrevioğlu, "Determination of *in vitro* antioxidant activity of fennel (Foeniculum vulgare) seed extracts," *LWT-Food Science and Technology*, vol. 36, no. 2, pp. 263–271, 2003.
View at: Publisher Site | Google Scholar

23. N. Verma and M. Vinayak, "Antioxidant action of *Andrographis paniculata* on lymphoma," *Molecular Biology Reports*, vol. 35, no. 4, pp. 535–540, 2008.
View at: Publisher Site | Google Scholar

24. X. F. Zhang and B. K. H. Tan, "Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin-diabetic rats," *Acta Pharmacologica Sinica*, vol. 21, no. 12, pp. 1157–1164, 2000.
View at: Google Scholar

25. Y. C. Shen, C. F. Chen, and W. F. Chiou, "Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide," *Planta Medica*, vol. 66, no. 4, pp. 314–317, 2000.
View at: Publisher Site | Google Scholar

26. F. L. Lin, S. J. Wu, S. C. Lee, and L. T. Ng, "Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide," *Phytotherapy Research*, vol. 23, no. 7, pp. 958–964, 2009.
View at: Publisher Site | Google Scholar

27. W. L. Deng, "Outline of current clinical and pharmacological research on *Andrographis paniculata* in China," *Newsletters Chinese Herbal Medicine*, vol. 10,, pp. 27–31, 1978.
View at: Google Scholar

28. J. Batkhuu, K. Hattori, F. Takano, S. Fushiya, K. I. Oshiman, and Y. Fujimiya, "Suppression of NO production in activated macrophages *in vitro* and *ex vivo* by neoandrographolide isolated from *Andrographis paniculata*," *Biological & Pharmaceutical Bulletin*, vol. 25, no. 9, pp. 1169–1174, 2002.
View at: Publisher Site | Google Scholar

immature dendritic cells to induce antigen-specific tolerance in experimental autoimmune encephalomyelitis," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 318, no. 1, pp. 59–67, 2006.
View at: Publisher Site | Google Scholar

32. A. B. Da Rocha, R. M. Lopes, and G. Schwartsmann, "Natural products in anticancer therapy," *Current Opinion in Pharmacology*, vol. 1, no. 4, pp. 364–369, 2001.
View at: Publisher Site | Google Scholar

33. G. A. Cordell, C. W. W. Beecher, and J. M. Pezzuto, "Can ethnopharmacology contribute to the development of new anticancer drugs?" *Journal of Ethnopharmacology*, vol. 32, no. 1–3, pp. 117–133, 1991.
View at: Google Scholar

34. P. Siripong, B. Kongkathip, K. Preechanukool, P. Picha, K. Tunsuwan, and W. C. Taylor, "Cytotoxic diterpenoid constituents from *A. paniculata* Nees leaves," *Journal of Scientific Soceity of Thailand*, vol. 18, pp. 187–194, 1992.
View at: Google Scholar

35. T. Matsuda, M. Kuroyanagi, S. Sugiyama, K. Umehara, A. Ueno, and K. Nishi, "Cell differentiation-inducing diterpenes from *Andrographis paniculata* NEES," *Chemical & Pharmaceutical Bulletin*, vol. 42, no. 6, pp. 1216–1225, 1994.
View at: Google Scholar

36. S. Nanduri, V. K. Nyavanandi, S. S. R. Thunuguntla et al., "Synthesis and structure-activity relationships of andrographolide analogues as novel cytotoxic agents," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 18, pp. 4711–4717, 2004.
View at: Publisher Site | Google Scholar

37. M. D. Shi, H. H. Lin, Y. C. Lee, J. K. Chao, R. A. Lin, and J. H. Chen, "Inhibition of cell-cycle progression in human colorectal carcinoma Lovo cells by andrographolide," *Chemico-Biological Interactions*, vol. 174, no. 3, pp. 201–210, 2008.
View at: Publisher Site | Google Scholar

38. J. Li, H. Y. Cheung, Z. Zhang, G. K. L. Chan, and W. F. Fong, "Andrographolide induces cell cycle arrest at G2/M phase and cell death in HepG2 cells via alteration of reactive oxygen species," *European Journal of Pharmacology*, vol. 568, no. 1–3, pp. 31–44, 2007.
View at: Publisher Site | Google Scholar

39. S. D. Manikam and J. Stanslas, "Andrographolide inhibits growth of acute promyelocytic leukaemia cells by

Pharmacology & Physiology, vol. 39, no. 3, pp. 300–310, 2012.
View at: Google Scholar

42. S. K. Hung, L. C. Hung, C. D. Kuo et al., "Andrographolide sensitizes Ras-transformed cells to radiation *in vitro* and *in vivo*," *International Journal of Radiation Oncology Biology Physics*, vol. 77, no. 4, pp. 1232–1239, 2010.
View at: Publisher Site | Google Scholar

43. K. Sheeja and G. Kuttan, "Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide," *Immunopharmacology and Immunotoxicology*, vol. 29, no. 1, pp. 81–93, 2007.
View at: Publisher Site | Google Scholar

44. S. Kumar, H. S. Patil, P. Sharma et al., "Andrographolide inhibits osteopontin expression and breast tumor growth through down regulation of PI3 kinase/Akt signaling pathway," *Current Molecular Medicine*, vol. 12, no. 8, pp. 952–966, 2012.
View at: Google Scholar

45. A. Puri, R. Saxena, R. P. Saxena, K. C. Saxena, V. Srivastava, and J. S. Tandon, "Immunostimulant agents from *Andrographis paniculata*," *Journal of Natural Products*, vol. 56, no. 7, pp. 995–999, 1993.
View at: Google Scholar

46. A. Panossian, A. Kochikian, E. Gabrielian et al., "Effect of *Andrographis paniculata* extract on progesterone in blood plasma of pregnant rats," *Phytomedicine*, vol. 6, no. 3, pp. 157–162, 1999.
View at: Google Scholar

47. M. I. Iruretagoyena, J. A. Tobar, P. A. González et al., "Andrographolide interferes with T cell activation and reduces experimental autoimmune encephalomyelitis in the mouse," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 312, no. 1, pp. 366–372, 2005.
View at: Publisher Site | Google Scholar

48. R. A. Burgos, E. E. Caballero, N. S. Sánchez, R. A. Schroeder, G. K. Wikman, and J. L. Hancke, "Testicular toxicity assesment of *Andrographis paniculata* dried extract in rats," *Journal of Ethnopharmacology*, vol. 58, no. 3, pp. 219–224, 1997.
View at: Publisher Site | Google Scholar

49. L. H. Qin, L. Kong, G. J. Shi, Z. T. Wang, and B. X. Ge, "Andrographolide inhibits the production of TNF-α and interleukin-12 in lipopolysaccharide-stimulated macrophages: role of mitogen-activated protein kinases," *Biological & Pharmaceutical Bulletin*, vol. 29, no. 2, pp. 220–224, 2006.

53. J. S. Rajkumar, M. G. Sekar, and S. K. Mitra, "Safety and efficacy of oral HD-03/ES given for six months in patients with chronic hepatitis B virus infection," *World Journal of Gastroenterology*, vol. 13, no. 30, pp. 4103–4107, 2007.
View at: Google Scholar

54. G. Shi, Z. Zhang, R. Zhang et al., "Protective effect of andrographolide against concanavalin A-induced liver injury," *Naunyn's Schmiedebergs Archives of Pharmacology*, vol. 385, no. 1, pp. 69–79, 2012.
View at: Publisher Site | Google Scholar

55. B. Shukla, P. K. S. Visen, G. K. Patnaik, and B. N. Dhawan, "Choleretic effect of andrographolide in rats and guinea pigs," *Planta Medica*, vol. 58, no. 2, pp. 146–149, 1992.
View at: Google Scholar

56. P. K. Singha, S. Roy, and S. Dey, "Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice," *Journal of Ethnopharmacology*, vol. 111, no. 1, pp. 13–21, 2007.
View at: Publisher Site | Google Scholar

57. A. C. Rana and Y. Avadhoot, "Hepatoprotective effects of Andrograhphis paniculata against carbon tetrachloride-induced liver damage," *Archives of Pharmacal Research*, vol. 14, no. 1, pp. 93–95, 1991.
View at: Google Scholar

58. P. K. S. Visen, B. Shukia, G. K. Patnaik, and B. N. Dhawan, "Andrographolide protects rat hepatocytes against paracetamol-induced damage," *Journal of Ethnopharmacology*, vol. 40, no. 2, pp. 131–136, 1993.
View at: Publisher Site | Google Scholar

59. R. M. Kunwar, K. P. Shrestha, and R. W. Bussmann, "Traditional herbal medicine in far-west Nepal: a pharmacological appraisal," *Journal of Ethnobiology and Ethnomedicine*, vol. 6, article 35, pp. 1–18, 2010.
View at: Publisher Site | Google Scholar

60. M. R. Zaidan, A. Noor Rain, A. R. Badrul, A. Adlin, A. Norazah, and I. Zakiah, "*In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method," *Tropical Biomedicine*, vol. 22, no. 2, pp. 165–170, 2005.
View at: Google Scholar

61. S. P. Voravuthikunchai and S. Limsuwan, "Medicinal plant extracts as anti-Escherichia coli O157:H7 agents and their effects on bacterial cell aggregation," *Journal of Food Protection*, vol. 69, no. 10, pp. 2336–2341, 2006.
View at: Google Scholar

2009.
View at: Publisher Site | Google Scholar

65. N. Poolsup, C. Suthisisang, S. Prathanturarug, A. Asawamekin, and U. Chanchareon, "*Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials," *Journal of Clinical Pharmacy and Therapeutics*, vol. 29, no. 1, pp. 37–45, 2004.
View at: Publisher Site | Google Scholar

66. Y. Xu, R. L. Marshall, and T. K. S. Mukkur, "An investigation on the antimicrobial activity of *Andrographis paniculata* extracts and andrographolide *in vitro*," *Asian Journal of Plant Sciences*, vol. 5, no. 3, pp. 527–530, 2006.
View at: Publisher Site | Google Scholar

67. R. S. Chang, L. Ding, G. Q. Chen, Q. C. Pan, Z. L. Zhao, and K. M. Smith, "Dehydroandrographolide succinic acid monoester as an inhibitor against the human immunodeficiency virus (43225)," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 197, no. 1, pp. 59–66, 1991.
View at: Google Scholar

68. S. Seubsasana, C. Pientong, T. Ekalaksananan, S. Thongchai, and C. Aromdee, "A potential andrographolide analogue against the replication of herpes simplex virus type 1 in vero cells," *Medicinal Chemistry*, vol. 7, no. 3, pp. 237–244, 2011.
View at: Publisher Site | Google Scholar

69. King Spalding LLP, "Andrographolide derivatives to treat viral infections," US20060333785; 2006.
View at: Google Scholar

70. T. P. Lin, S. Y. Chen, P. D. Duh, L. K. Chang, and Y. N. Liu, "Inhibition of the Epstein-Barr virus lytic cycle by andrographolide," *Biological & Pharmaceutical Bulletin*, vol. 31, no. 11, pp. 2018–2023, 2008.
View at: Publisher Site | Google Scholar

71. L. I. C. Tang, A. P. K. Ling, R. Y. Koh, S. M. Chye, and K. G. L. Voon, "Screening of anti-dengue activity in methanolic extracts of medicinal plants," *BMC Complementary and Alternative Medicine*, vol. 12, no. 3, pp. 1–10, 2012.
View at: Google Scholar

72. S. Madav, H. C. Tripathi, Tandan, and S. K. Mishra, "Analgesic, antipyretic and antiulcerogenic effects of andrographolide," *Indian Journal of Pharmaceutical Sciences*, vol. 57, no. 3, pp. 121–125, 1995.
View at: Google Scholar

Journal of Pharmacognosy, vol. 30, no. 4, pp. 263–274, 1992.
View at: Google Scholar

76. B. D. Sheeja, D. Sindhu, J. Ebanasar, and S. Jeeva, "The Larvicidal activity of *Andrographis paniculata* (Burm. f) Nees against Culex quinquefasciatus Say (Insecta: Diptera-Culicidae), a filarial vector," *Asian Pacific Journal of Tropical Disease*, pp. S574–S578, 2012.
View at: Google Scholar

77. R. Babu, K. Murugan, and v, "Interactive effect of neem seed kernel and neem gum extracts on the control of Culex quinquefasciatus say," *Neem Newsletterno*, vol. 15, no. 2, pp. 9–11, 1998.
View at: Google Scholar

78. M. R. Venkatachalam and A. Jebanesan, "Larvicidal activity of Hydrocotyle javanica Thunb. (Apiaceae) extract against Culex quinquefasciatus," *Journal of Experimental Zoology*, vol. 4, no. 1, pp. 99–101, 2001.
View at: Google Scholar

79. T. Pushpanathan, A. Jebanesan, and M. Govindarajan, "Larvicidal, ovicidal and repellent activities of Cymbopogan citratus Stapf (Graminae) essential oil against the filarial mosquito Culex quinquefasciatus (Say) (Diptera : Culicidae)," *Tropical biomedicine*, vol. 23, no. 2, pp. 208–212, 2006.
View at: Google Scholar

80. S. Mandal, "Exploration of larvicidal and adult emergence inhibition activities of Ricinus communis seed extract against three potential mosquito vectors in Kolkata, India," *Asian Pacific Journal of Tropical Medicine*, vol. 3, no. 8, pp. 605–609, 2010.
View at: Publisher Site | Google Scholar

81. C. Kuppusamy and K. Murugan, "Mosquitocidal effect of Andographis paniculata Nees against the malaria vector, *Anopheles stephensi* Liston (Diptera: culicidae)," *International Journal of Integrative Biology*, vol. 5, no. 2, pp. 75–81, 2009.
View at: Google Scholar

82. K. Chenniappan and M. Kadarkarai, "Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* Nees against the malarial vector Anopheles stephensi Liston (Diptera: Culicidae)," *Entomological Research*, vol. 38, no. 2, pp. 119–125, 2008.
View at: Publisher Site | Google Scholar

83. M. Govindarajan, "Evaluation of *Andrographis paniculata* Burm.f. (Family:Acanthaceae) extracts against Culex quinquefasciatus (Say.) and Aedes aegypti (Linn.) (Diptera:Culicidae)," *Asian Pacific Journal of Tropical*

Nees in rats," *Upsala Journal of Medical Sciences*, vol. 114, no. 3, pp. 136–139, 2009.
View at: Publisher Site | Google Scholar

87. R. N. Chopra, S. L. Nayar, and I. C. Chopra, *Glossary of Indian Medicinal Plants*, Publications and Information Directorate, CSIR, New Delhi, India, 1990.

88. M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra, and C. Ray, "Screening of Indian plants for biological activity: I," *Indian Journal of Experimental Biology*, vol. 6, no. 4, pp. 232–247, 1968.
View at: Google Scholar

89. D. S. Bhakuni, M. L. Dhar, M. M. Dhar, B. N. Dhawan, and B. N. Mehrotra, "Screening of Indian plants for biological activity. II," *Indian Journal of Experimental Biology*, vol. 7, no. 4, pp. 250–262, 1969.
View at: Google Scholar

90. M. Shamsuzzoha, M. S. Rahman, M. M. Ahmed, and A. K. Islam, "Antifertility effect in mice of medicinal plant of family acanthaceae," *The Lancet*, vol. 2, no. 8095, p. 900, 1978.
View at: Google Scholar

91. H. M. Chang and P. P. H. But, *Pharmacology and Applications of Chinese Materia Medica*, vol. 2, World Scientific, Singapore, 1987, English translation by Shem Chang-Shing Yeung, Sih Cheng-Yao and Lai-Ling Wang (Chinese Medicinal Material Research Centre, the Chinese University of Hong Kong).

92. M. S. Zoha, A. H. Hussain, and S. A. Choudhury, "Antifertility effect of *Andrographis paniculata* in mice," *Bangladesh Medical Research Council Bulletin*, vol. 15, no. 1, pp. 34–37, 1989.
View at: Google Scholar

93. M. A. Akbarsha, B. Manivannan, K. S. Hamid, and B. Vijayan, "Antifertility effect of *Andrographis paniculata* (Nees) in male albino rat," *Indian Journal of Experimental Biology*, vol. 28, no. 5, pp. 421–426, 1990.
View at: Google Scholar

94. M. A. Akbarsha and P. Murugaian, "Aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats: effect on the testis and the cauda epididymidal spermatozoa," *Phytother Research*, vol. 14, no. 6, pp. 432–435, 2000.
View at: Google Scholar

95. J. Sattayasai, S. Srisuwan, T. Arkaravichien, and C. Aromdee, "Effects of andrographolide on sexual functions, vascular reactivity and serum testosterone level in rodents," *Food and Chemical Toxicology*, vol. 48, no. 7, pp. 1934–1938, 2010.
View at: Publisher Site | Google Scholar

View at: Google Scholar

99. R. Husen, A. H. L. Pihie, and M. Nallappan, "Screening for antihyperglycaemic activity in several local herbs of Malaysia," *Journal of Ethnopharmacology*, vol. 95, no. 2-3, pp. 205–208, 2004.
View at: Publisher Site | Google Scholar

100. A. M. Dandu and N. M. Inamdar, "Evaluation of beneficial effects of antioxidant properties of aqueous leaf extract of *Andrographis paniculata* in STZ-induced diabetes," *Pakistan Journal of Pharmaceutical Sciences*, vol. 22, no. 1, pp. 49–52, 2009.
View at: Google Scholar

101. B. A. S. Reyes, N. D. Bautista, N. C. Tanquilut et al., "Anti-diabetic potentials of Momordica charantia and *Andrographis paniculata* and their effects on estrous cyclicity of alloxan-induced diabetic rats," *Journal of Ethnopharmacology*, vol. 105, no. 1-2, pp. 196–200, 2006.
View at: Publisher Site | Google Scholar

102. B. C. Yu, C. R. Hung, W. C. Chen, and J. T. Cheng, "Antihyperglycemic effect of andrographolide in streptozotocin-induced diabetic rats," *Planta Medica*, vol. 69, no. 12, pp. 1075–1079, 2003.
View at: Publisher Site | Google Scholar

103. A. Wibudi, B. Kiranadi, W. Manalu, A. winarto, and S. Suyono, "The traditional plant, *Andrographis paniculata* (Sambiloto), exhibits insulin-releasing actions *in vitro*," *Acta medica Indonesiana*, vol. 40, no. 2, pp. 63–68, 2008.
View at: Google Scholar

104. R. Subramanian, M. Z. Asmawi, and A. Sadikun, "*In vitro*α-glucosidase and α-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide," *Acta Biochimica Polonica*, vol. 55, no. 2, pp. 391–398, 2008.
View at: Google Scholar

105. J. Stamler, M. L. Daviglus, D. B. Garside, A. R. Dyer, P. Greenland, and J. D. Neaton, "Relationship of baseline serum cholesterol levels in 3 large cohorts of younger men to long-term coronary, cardiovascular, and all-cause mortality and to longevity," *Journal of the American Medical Association*, vol. 284, no. 3, pp. 311–318, 2000.
View at: Google Scholar

106. M. Briel, I. Ferreira-Gonzalez, J. J. You et al., "Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis," *British Medical Journal*, vol. 338, no. 7693, p. b92, 2009.
View at: Publisher Site | Google Scholar

View at: Publisher Site | Google Scholar

110. A. E. Nugroho, M. Andrie, N. K. Warditiani, E. Siswanto, S. Pramono E, and Lukitaningsih, "Antidiabetic and antihiperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats," *Indian Journal of Pharmacology*, vol. 44, no. 3, pp. 377–381, 2012.
View at: Google Scholar

111. H. W. Wang, H. Y. Zhao, and S. Q. Xiang, "Effects of *Andrographis paniculata* component on nitric oxide, endothelin and lipid peroxidation in experimental atherosclerotic rabbits," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 17, no. 9, pp. 547–549, 1997.
View at: Google Scholar

112. L. Y. Huang, "The effects of andrographolide on experimental blood deficiency of cardiac muscle," *Chinese Herbal Medicine*, vol. 18, pp. 26–28, 1987.
View at: Google Scholar

113. A. Y. H. Woo, M. M. Y. Waye, S. K. W. Tsui, S. T. W. Yeung, and C. H. K. Cheng, "Andrographolide up-regulates cellular-reduced glutathione level and protects cardiomyocytes against hypoxia/reoxygenation injury," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 325, no. 1, pp. 226–235, 2008.
View at: Publisher Site | Google Scholar

114. K. Awang, N. H. Abdullah, A. H. Hadi, and Y. S. Fong, "Cardiovascular activity of labdane diterpenes from *Andrographis paniculata* in isolated rat hearts," *Journal of Biomedicine and Biotechnology*, vol. 2012, Article ID 876458, 5 pages, 2012.
View at: Publisher Site | Google Scholar

115. D. Wang and H. Zhao, "Experimental studies on prevention of atherosclerotic arterial stenosis and restenosis after angioplasty with *Andrographis paniculata* Nees and Fish Oil," *Journal of Tongji Medical University*, vol. 13, no. 4, pp. 193–198, 1993.
View at: Google Scholar

116. H. Zhao and W. Fang, "Protective effects of *Andrographis paniculata* Nees on post-infarction myocardium in experimental dogs," *Journal of Tongji Medical University*, vol. 10, no. 4, pp. 212–217, 1990.
View at: Google Scholar

117. G. Hsiao, K. H. Lin, Y. Chang et al., "Protective mechanisms of inosine in platelet activation and cerebral ischemic damage," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 9, pp. 1998–2004, 2005.
View at: Publisher Site | Google Scholar

ERK2 cascade," *Biochemical Pharmacology*, vol. 84, pp. 914–924, 2012.
View at: Publisher Site | Google Scholar

121. Y. Z. Zhang, J. Z. Tang, and Y. J. Zhang, "Study of *Andrographis paniculata* extracts on antiplatelet aggregation and release reaction and its mechanism," *Zhongguo Zhong Xi Yi Jie He Za Zhi*, vol. 14, no. 1, pp. 28–5, 1994.
View at: Google Scholar

122. M. Karin, Y. Yamamoto, and Q. M. Wang, "The IKK NF-κB system: a treasure trove for drug development," *Nature Reviews Drug Discovery*, vol. 3, no. 1, pp. 17–26, 2004.
View at: Google Scholar

123. K. T. Ku, Y. L. Huang, Y. J. Huang, and W. F. Chiou, "Miyabenol A inhibits LPS-induced NO production via IKK/IκB inactivation in RAW 264.7 macrophages: possible involvement of the p38 and PI3K pathways," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 19, pp. 8911–8918, 2008.
View at: Publisher Site | Google Scholar

124. Y. H. Hong, W. W. Chao, M. L. Chen, and B. F. Lin, "Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation *in vitro* and *in vivo*," *Journal of Biomedical Science*, vol. 16, no. 1, article 64, 2009.
View at: Publisher Site | Google Scholar

125. K. J. Yun, D. J. Koh, S. H. Kim et al., "Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygase-2, and proinflammatory cytokines expressions via nuclear factor-κB inactivation," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, pp. 10265–10272, 2008.
View at: Publisher Site | Google Scholar

126. Y. Yamamoto and R. B. Gaynor, "Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer," *The Journal of Clinical Investigation*, vol. 107, no. 2, pp. 135–142, 2001.
View at: Google Scholar

127. M. A. Hidalgo, A. Romero, J. Figueroa et al., "Andrographolide interferes with binding of nuclear factor-κB to DNA in HL-60-derived neutrophilic cells," *British Journal of Pharmacology*, vol. 144, no. 5, pp. 680–686, 2005.
View at: Publisher Site | Google Scholar

128. Z. Bao, S. Guan, C. Cheng et al., "A novel antiinflammatory role for andrographolide in asthma via inhibition of the nuclear factor-κb pathway," *American Journal of Respiratory and Critical Care Medicine*, vol. 179, no. 8, pp. 657–665, 2009.
View at: Publisher Site | Google Scholar

vol. 1, no. 2, pp. 1–5, 2010.
View at: Google Scholar

132. Y. El Safadi, J. C. Paillarte, G. Laumond et al., "5-modified-2′-dU and 2′-dC as mutagenic anti HIV-1 proliferation agents: synthesis and activity," *Journal of Medicinal Chemistry*, vol. 53, no. 4, pp. 1534–1545, 2010.
View at: Publisher Site | Google Scholar

133. X. K. Zhu, J. Guan, Z. Xiao, L. M. Cosentino, and K. H. Lee, "Anti-AIDS agents. Part 61: anti-HIV activity of new podophyllotoxin derivatives," *Bioorganic and Medicinal Chemistry*, vol. 12, no. 15, pp. 4267–4273, 2004.
View at: Publisher Site | Google Scholar

134. Y. Mehellou and E. De Clercq, "Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go?" *Journal of Medicinal Chemistry*, vol. 53, no. 2, pp. 521–538, 2010.
View at: Publisher Site | Google Scholar

135. M. M. Uttekar, T. Das, R. S. Pawar et al., "Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding," *European Journal of Medicinal Chemistry*, vol. 56, pp. 368–374, 2012.
View at: Publisher Site | Google Scholar

136. D. D. Cáceres, J. L. Hancke, R. A. Burgos, F. Sandberg, and G. K. Wikman, "Use of visual analogue scale measurements (VAS) to asses the effectiveness of standardized *Andrographis paniculata* extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study," *Phytomedicine*, vol. 6, no. 4, pp. 217–223, 1999.
View at: Google Scholar

137. S. Sakila, N. Begum, S. Kawsar, Z. A. Begum, and M. S. Zoha, "Relationship of anti-fertility effects of