Pharmacognosy Reviews
Vol 1, Issue 2, Jul-Dec, 2007

PHCOG REV.
An official Publication of Phcog.Net

Phcog Rev.: Plant Review
Andrographis paniculata (Kalmegh): A Review

Siddhartha K. Mishra, Neelam S. Sangwan and Rajender S. Sangwan *

Central Institute of Medicinal and Aromatic Plants (CIMAP), P O CIMAP, Luckow-226015 U P India
Phone: +91-0522-2717434 (Lab), 9451246764 (M)
Author for correspondence: sangwan.lab@gmail.com

ABSTRACT

Andrographis paniculata (Burm.f.) Nees (Acanthaceae) is a medicinal plant traditionally used for the treatment of cold, fever, laryngitis and several infectious diseases ranging from malaria to dysentery and diarrhea in China, India and other south east Asian countries. The plant is claimed to possess immunological, antibacterial, antiinflammatory, antithrombotic and hepatoprotective properties. In Malaysia, the plant is used in folk medicines to treat diabetes and hypertension. The contents of diterpenoids like andrographolide, neoandrographolide and dehydroandrographolide are the chief criteria for monitoring the quality of *A. paniculata*. Andrographolide, though found in all plant parts, is most concentrated in the leaves. It is a diterpene containing a γ-lactone ring connected to a decalin ring system *via* an unsaturated C-2 moiety. It has multiple pharmacological properties such as protozoacidal, antihepatotoxic, anti-HIV, anticancer, antitumor, hypoglycemic and hypotensive activities. Andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential to be developed as an anticancer chemotherapeutic agent as well. In this review, we have explored the various dimensions of the *Andrographis paniculata* plant and compiled its vast pharmacological applications to comprehend and synthesize the subject of its potential image of multipurpose medicinal agent. The plant is widely cultivated to large regions of the world and its importance as a medicinal plant is growing up substantially with increasing and stronger reports in support of its multifarious therapeutic uses.

KEY WORDS - *Andrographis paniculata*, andrographolide, neoandrographolide, hepatoprotective, anticancer, antitumor, anti-HIV, medicinal plant.

INTRODUCTION

Andrographis paniculata (Burm. F.) Wall. Ex Nees (AP) also called Kalmegh or "King of Bitters" belongs to family *Acanthaceae*. It has been used for centuries in Asia to treat gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. *Indian Pharmacopoeia* narrates that it is a predominant constituent of at least 26 Ayurvedic formulations. In Traditional Chinese Medicine (TCM), *Andrographis* is considered as the herb possessing an important "cold property" useful to treat the heat of body in fevers, and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common colds.

TAXONOMICAL CLASSIFICATION

Kingdom	*: Plantae, Plants;*
Subkingdom	*: Tracheobionta, Vascular plants;*
Super division	*: Spermatophyta, Seed plants;*
Division	*: Angiosperma*
Class	*: Dicotyledonae*
Sub class	*: Gamopetalae*
Series	*: Bicarpellatae*
Order	*: Personales*
Tribe	*: Justicieae*
Family	*: Acanthaceae*
Genus	*: Andrographis*
Species	*: paniculata*

Botanical Description: AP is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, easily broken fragile texture stem. Leaves are simple, opposite, lanceolate, glabrous, 2–12cm long, 1–3cm wide with margin acute and entire or slightly undulated and upper leaves often bractiform with short petiole. Inflorescence of the plant is characterized as patent, terminal and axillary in panicle, 10–30 mm long; bract small; pedicel short. The flowers possess botanical features of calyx 5-particle, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with a yellowish top; lower lip broadly cuneate, 3-lobed, white with violet markings; stamens 2, inserted in the throat and far exserted; anther basally bearded. Superior ovary, 2-celled; style far exserted. Capsule of the plant is erect, linear-oblong, 1–2 cm long and 2–5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thinly glandular-hairy. Seeds are very small, subquadrate (1-5).

Habitat: It grows abundantly in southeastern Asia: India (and Sri Lanka), Pakistan and Indonesia but it is cultivated extensively in China and Thailand, the East and West Indies, and Mauritius. AP is normally grown from seeds ubiquitously in its native areas where it grows in pine, evergreen and deciduous forest areas, and along roads and in villages. In India, it is cultivated during rainy phase of summer season (Kharif) crop. Any soil having fair amount of organic matter is suitable for commercial cultivation of this crop. About 400 g seed are sufficient for one hectare. The spacing is maintained 30×15cm. No major insect and disease infestation has been reported. The plants at flowering stage (90–120 days after sowing) are cut at the base leaving 10–15cm stem for plant regeneration. About 50–60 days after first harvest, final harvest is performed. In Indian condition, the yield varies

between 2000–2500 Kg dry herb per hectare.

Plant Parts used: The aerial parts of the plant (leaves and stems) are used to extract the active phytochemicals and thus used for its medicinal importance. Very rarely roots are also used.

Synonyms:

Arab: Quasabhuva; *Bengali:* Kalmegh; *English:* The Creat, King of Bitters; *Gujarathi:* Kariyatu; *Hindi:* Kirayat; *Kannada:* Nelaberu; *Malayalam:* Kiriyattu; *Marathi:* Oli-kiryata; *Oriya:* Bhuinimba; *Persian:* Naine-havandi; *Sanskrit:* Kalmegha, Bhunimba; *Tamil:* Nilavembu; *Telugu:* Nilavembu

PHYTOCHEMISTRY

The characteristic secondary metabolites encountered in the plant have considerably enhanced its importance in the arena of medicinal plants and medicines. It is specifically rated very high in therapeutic action in curing liver disorders and common cough and cold in humans. A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolated from *Andrographis*, mainly the most bitter compounds among them are andrographolide, neoandrographolide, deoxyandrographolide. Other such phytochemicals amassed by the plant are 14-deoxyandro - grapholide, 14-deoxy-11,12-didehydroandrographolide, andrographiside, deoxyandrographiside, homoandrogra-pholide, andrographan, andrographon, andro-graphosterin and stigmasterol (6). The leaves of *Andrographis* contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest (7). Andrographolide has highly bitter taste, is colorless crystalline in appearance, and possess a "lactone function". Both growing region and seasonal changes have a strong impact on formation of the diterpene lactones. The highest concentration of the active components is found just before the plant blooms, making early fall the best time to harvest. In those parts of Asia where *Andrographis* is sold commercially as medicine, a variety of lab level methodologies are used to ensure a standardized level of andrographolides: thin-layer chromatography, ultraviolet spectrophotometry, liquid chromatography, and volumetric and colorimetric techniques and HPLC among them is most robust and reliable for quantitative and qualitative profiles of andrographolides. For extraction of andrographolides, a solvent extraction method is usually employed using ethanol, and liquid extracts or tinctures are the most common form of dispensing the product. Varied methodologies for isolation and extraction of andrographolides from the plant have been adopted by different working groups. In general, extraction of andrographolides is aqueous or aqueous methanolic and the extract is further fractionated with methanol-chloroform, dichloromethane and/or petroleum ether or hexane in accordance with the andrographolide fractions/moieties desired to be enriched for specific application(s). Usually aqueous or little amount of ethanol/methanol is used to isolate andrographolides for pharmacological investigations. To advance, solid-liquid extraction of stem and leaves of *Andrographis paniculata* is in precisely defined relative proportions of water and ethanol in order to obtain andrographolide (8).

The structure of andrographolide comprises (i) an a-alkylidene c-butyrolactone moiety, (ii) two olefin bonds $\Delta^{8(17)}$ and $\Delta^{12(13)}$, and (iii) three hydroxyls at C-3, C-19, and C-14. Of the three hydroxyl groups, the one at C-14 is allylic in nature, and the others at C-3 and C-19 are secondary and primary, respectively. Systematic studies on chemistry of *Andrographis* have been carried out by various researches during last several years. Analysis of the whole plant, in a study (9), has given the following lactones (dry weight basis): andrographolide ($C_{20}H_{30}O_5$; mp 230-239°C), 0.6%; 14-deoxy-11-oxoandrographolide ($C_{20}H_{28}O_5$, mp 98-100°C), 0.12%; 14-deoxy-11, 12-didehydroandrographolide ($C_{20}H_{30}O_4$, mp 203-204°C), 0.06%; 14-deoxyandrographolide ($C_{20}H_{30}O_4$, mp 175°C), 0.02%; and a non-bitter constituent, neoandrographolide ($C_{26}H_{40}O_8$, mp 167-168°C), 0.005%. From the petroleum ether extract of the leaves collected from Bangladesh, the following phytochemicals have been isolated: α-,ß-unsaturated lactone, homoandrographolide ($C_{22}H_{32}O_9$, mp 115°C), andrographosterol ($C_{23}H_{38}O$, mp 135°C), andrographane ($C_{40}H_{82}$, mp 67-68°C), andrographone ($C_{32}H_{64}O$, mp 85°C), a wax and two esters containing hydroxyl groups. The roots of the plant have been found to contain apigenin-7, 4'- di-O-methyl ether, andrographolide and a flavone, 5-hydroxy 7,8,2',3'- tetramethoxy flavone ($C_{19}H_{18}O_7$, mp 150-151°C; yield, 0.006%). They also contain a monohydroxy trimethyl flavone, andrographin ($C_{18}H_{16}O_6$, mp 190-191°C) and a dihydroxy-di-methoxyflavone, panicolin ($C_{17}H_4O_6$, mp 263-264°C). The presence of α-sitosterol is also reported.

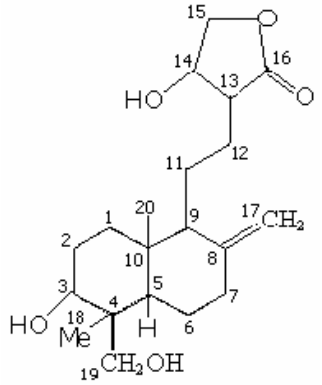
An investigation by Matsuda and associates in 1994 (9) revealed six new diterpenoids of *ent*-labdane type, 14-*epi*-andrographolide, isoandrographolide, 14-deoxy-12-methoxy - andrographolide, 12-*epi*-14-deoxy-12methoxy-andrographolide, 14-deoxy-12 -hydroxy andrographolide and 14-deoxy-11-hydroxyandrographolide as well as two new diterpene glucoside, 14-deoxy-11,12-didehydroandrographi-side and 6'-acetylneoandrographolide, and four new diterpene dimers, bisandrographolide A, B, C and D, were isolated along with six known compounds. Phytochemical investigation of the roots and aerial parts of *Andrographis paniculata* Nees yielded a new flavone, 5-hydroxy-7,20,60-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12didehydroandrographolide together with five known flavonoids and four known diterpenoids (10) and one deoxyandrographolide-19ß-D-glucoside (11) . For the drug analysis and process development, several sensitive and accurate analytical methods are used for the quantitation of important diterpenoids especially andrographolides such as gravimetric (12, 13), colorimetric (14), spectrophotometric (15, 16) and titrimetric (17, 18) but many of these procedures are time consuming. Meanwhile HPLC (19) and HPTLC methods are also applied for the quantitation of andrographolide where a simple reversed phase liquid chromatographic method has been used for the simultaneous determination of the three major andrographolides *viz*. 14-deoxy-11,12-didehydroandrographolide, andrographolide and neoandrographolide with UV detection at 230 nm by

employing an isocratic binary mobile phase (19). Other advanced methods of analysis include: the development of a micellar electro kinetic chromatographic (MEKC) method for simultaneous determination of andrographolide, deoxyandrographolide and neoandrographolide in ethanol extracts of *Andrographis paniculata*. Separations were carried out in a fused-silica capillary tube with UV detection at 214 nm. Good separation was achieved using a 20 m*M* borate buffer, containing 20 m*M* sodium dodecyl sulphate and 10 m*M* sodium cholate, adjusted to pH 8.3 at an operating voltage of 25 kV, temperature of 358°C and a hydrodynamic injection of 5 sec. This method could be used for speedy and accurate qualitative and quantitative analysis of bioactive diterpenoids in *Andrographis* herb and its derived products (21). A novel technique based on dynamic microwave-assisted extraction (DMAE) coupled on-line with high-performance liquid chromatography (HPLC) through a flow injection interface has been developed for determination of andrographolide and dehydroandrographolide in *Andrographis paniculata* Nees. A TM010 microwave resonance cavity built in the laboratory was applied to concentrating the microwave energy. An extraction vessel was placed in microwave irradiation zone. The extraction was performed in a re-circulating system. When a number of extraction cycles were completed, the fractional extract (20μL) was driven to the analytical column by 65% aqueous methanol and was measured by diode array detector (DAD) at 225 nm. The optimized extraction conditions are follows: extraction solvent 60% aqueous methanol; microwave forward power 80W; extraction time 6 min; extraction solvent flow-rate 1.0mL min^{-1}. Mean recoveries for andrographolide and dehydroandrographolide are 97.7% and 98.7%, respectively. Compared with ultrasonic extraction used in the Chinese pharmacopoeia, the proposed method was demonstrated to obtain higher extraction yield in a shorter time. In addition, only small quantities of solvent (5 mL) and sample (10 mg) were required (22).

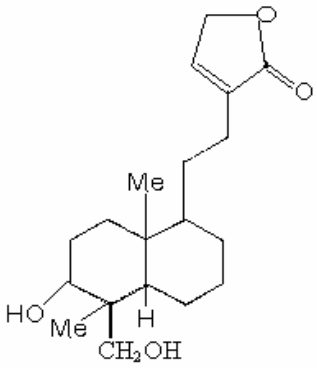
PHARMACOLOGY

Researches conducted in past decades have confirmed that *Andrographis*, if properly administered, has a surprisingly broad range of pharmacological effects, some of them are extremely beneficial:

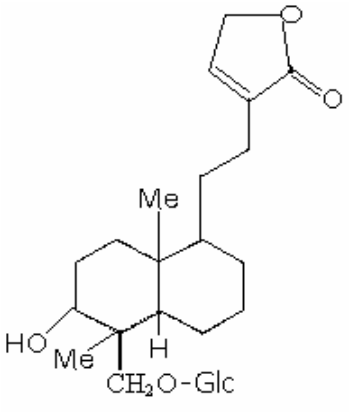
- ***Abortifacient*** (can abort pregnancy; although Ayurvedic tradition allows it to be taken for short duration during pregnancy thus all women are advised to avoid its use during pregnancy as a precaution. In almost every other respect *Andrographis* has an extremely low toxicity.
- **Vermicidal** (kills intestinal worms)
(hot: in this case, slightly rubifacient to the skin)
- ***Analgesic*** (pain killer)
reduces swelling and cuts down exudation from capillaries; antiflammatory action probably mediated, in part, by adrenal function)
- ***Antibacterial*** (fights bacterial activity; although *Andrographis* appears to have weak direct antibacterial action, it has remarkably beneficial effect in reducing diarrhea and symptoms arising from bacterial infections.)
- ***Antiperiodic*** (counteracts periodic/intermittent diseases, such as malaria)
- ***Antipyretic*** (fever reducer - both in humans and animals, caused by multiple infections or by toxins)
- ***Antithrombotic*** (blood clot preventative)
- ***Antiviral*** (inhibits viral activity)
- ***Cancerolytic*** (fights, even kills, cancer cells)
- ***Cardioprotective*** (protects heart muscles)
- ***Choleretic*** (alters the properties and flow of bile)
- ***Depurative*** (cleans and purifies the system, particularly the blood)
- ***Digestive*** (promotes digestion)
- ***Expectorant*** (promotes mucus discharge from the respiratory system)
- ***Hepatoprotective*** (protects the liver and gall bladder)
- ***Hypoglycemic*** (blood sugar reducer)
- ***Immune Enhancement*** (increases white cell phagocytosis, inhibits HIV-1 replication, and improves $CD4^+$ and T lymphocyte counts)
- ***Laxative*** (aids bowel elimination)
- ***Sedative*** (relaxing herb, though not with the same effect as the accepted herbal sedatives, valerian root, hops, skullcap, etc.)
- ***Thrombolytic*** (blood clot buster)



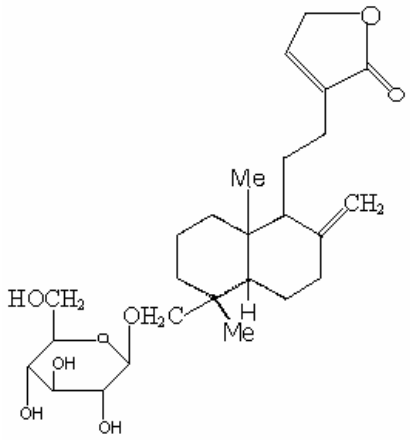
Andrographolide (the main carbon skeleton)



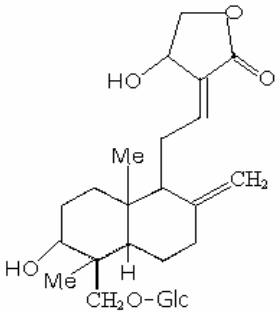
14-deoxyandrographolide



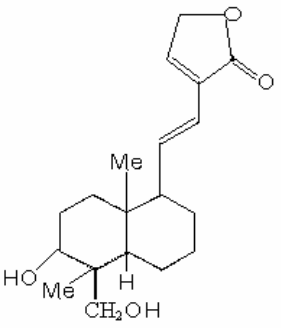
14-deoxyandrographiside



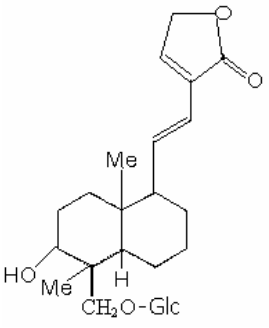
Neoandrographolide



Andrographiside



14-deoxy-11,12-didehydroandrographolide



14-deoxy-11,12-didehydroandrographiside

Chemical structures of the main diterpenoids in *Andrographis paniculata*

MECHANISMS OF ACTION OF ANDROGRAPHOLIDES

Andrographis paniculata has been extensively studied, particularly with much focus "AP's" pharmacological composition, safety, efficacy, and mechanisms of action during the second half of the 20th century (23, 24, 25). All cells in the body contain receptors on the surface of the cell membrane that surrounds the cell. These receptors function to bind hormones, growth factors, neurotransmitters, and other molecules that regulate (or in the case of cancer, disturb) cell function. Once a molecule binds to the receptor, a chemical message is transmitted to targets in the cell or to other molecules in the cell, which carry the message further. The message eventually reaches the nucleus of the cell where the genetic expression gets modulated and the cell function response is a cell-type specific manner. An example would be a message to make a particular protein, such as insulin, by a cell in the pancreas. The receptor, its cellular target, and any intermediary molecules are referred to as a "signal transduction pathway." Signal transduction technology involves the study of these pathways that affect cell function. Any point in this pathway may be affected by cancer-causing toxins or by viruses. In the case of cancer, changes in the components or in the timing of cellular events can cause abnormal cell division. Uncontrolled cell division results in a tumor or in the spread of cancerous cells. Other diseases can also develop when the signals are disturbed.

Many of the steps are involved in signal transduction are well understood, although researches concerning their fine-tuned regulation are still not well understood for understanding pathways holistically. Investigations to understand what and how these pathways can go odd way at a basic level (intracellularly) would allow detection of diseases at a much earlier stage -before there are obvious symptoms and when there is still a good chance to correct the problem. "Scientists at many U.S. companies are using signal transduction technology to determine the effects of natural and synthetic components on the signal transduction pathways in the cell, in particular those involved in cell division. Several applications of signal transduction technology in the development of compounds with therapeutic potential have been reviewed in an excellent editorial published in *Genetic Engineering News* in January 1996 (26). One of the criticisms made by the conventional medical and scientific community regarding dietary supplements is that their development and use have been based on folklore tradition, not on sequence of scientific evidences in modern set-ups of controls and placebos with statistical tests of hypotheses.

Using signal transduction technology, extracts of

Andrographis paniculata have been found to counteract interference with the cell cycle. Such interference is the basis for the development of cancer or infection with viruses such as HIV-1. Andrographolides are thought to enhance immune system functions such as production of white blood cells (scavengers of bacteria and other foreign matter), release of interferon, and activity of the lymph system. Interferon is a protein (called a cytokine) made by cells in response to viruses. It is a potent antiviral agent and is also anti-proliferative (stops the growth of viruses). The lymph system is an important part of the immune system. Briefly, it is another circulatory system (like the vascular system) that carries a fluid, the lymph. The lymph carries away the by-products of cellular metabolism and also acts as a shuttle for invading bacteria and viruses, taking them to the lymph nodes where the white blood cells (lymphocytes) destroy them. *Andrographis*, a superb immune system enhancer, is even more effective when combined with immune stimulators, such as the herb Echinacea, and with zinc and vitamin C.

Several studies have traced the disposition of andrographolide in various organs of the body (27). Bio-distribution experiments have been done in experimental animals. Following injection of radioactively labeled andrographolide, this compound appears to be widely distributed in the body. High concentrations are noted in the central nervous system (brain and spinal cord) and other organs with high blood flow, including the colon, spleen, heart, lungs, and kidneys. Andrographolide appears to have a relatively short half-life of approximately two hours. The term "half-life" refers to the time when the concentration of the compound in the body is half of what it originally was when it entered the body. This is what is left after the compound has been metabolized (broken down), changed into other forms (called metabolites), and excreted by one of several routes (urine, feces, exhaled air, sweat, or other body excretions). Compounds with short half-lives need to be given often since they do not stay in the body for long. Andrographolides are excreted fairly rapidly from the body via the urine and gastrointestinal tract. In some studies, 80 percent of the administered dose of andrographolide is removed from the body within eight hours, with excretion rates of more than 90 percent of the compound within forty-eight hours. The wide tissue and organ distribution and the immune-stimulating and regulatory actions of AP make it an ideal candidate in the prevention and treatment of many diseases and conditions. When consumed, andrographolides appear to accumulate in organs throughout the viscera. In one study, after 48 hours, the concentration of labelled andrographolide was 20.9%, brain; 14.9%, spleen; 11.1%, heart; 10.9%, lung; 8.6%, rectum; 7.9%, kidney; 5.6%, liver; 5.1%, uterus; 5.1%, ovary; and 3.2%, intestine (28). Absorption and excretion is rapid: 80% is removed within 8 hours via the kidney (urine) and G.I. tract. Ninety percent is eliminated within 48 hours.

Immunological applications in Cancer

Studies on mice have shown that *Andrographis paniculata* is a potent stimulator of the immune system in two ways: **(1) *Antigen-specific response:*** antibodies are made to counteract invading microbes, and ***(2) Nonspecific immune response:*** macrophage cells scavenge and destroy invaders. AP activates both responses - making it effective against a variety of infectious and oncogenic (cancer-causing) agents (29). The initial interests with AP was two-fold: its hepatoprotective (liver protecting), as well as its anti-cancer properties. Similarly, AP has a record of effective treatment rooted in its mechanisms of immune boosting. Cancer results when cells do not respond to signals that are intended to limit growth. When cells develop normally, at each stage of development the cells become more specialized in order to be able to perform the duties of that particular cell. For example, cells that will make insulin will develop the cellular machinery to do so. When cancer upsets normal development, cells do not mature; they more closely resemble immature body cells. The more they resemble immature cells, the more unfavorable the outcome: the cancer grows and spreads (metastasizes) more rapidly. If a cancer cell can be made to mature (or differentiate), it will not have the ability to grow out of control. Researchers are therefore searching for substances that can cause cancer cells to mature. In one study of mice, researchers searched for naturally occurring substances that would cause differentiation of leukemia cells. Leukemia is a cancer of the white blood cells. AP was chosen because it contained substances (terpenes) that were similar to substances found in other plants and were known to cause differentiation of cancer cells. The results of the study demonstrated that AP had potent cell differentiation-inducing activity on leukemia cells (9). In addition to causing cancer cell maturity or differentiation, AP extracts from the leaves of the plant are also cytotoxic (cell-killing) against cancer cells. This cancer cell-killing ability was demonstrated against human epidermoid carcinoma (squamous cell carcinoma) of the skin lining of the nasopharynx and against lymphocytic leukemia cells (30). It was the andrographolide component that was found to have the cancer cell-killing ability. This ability for killing cancer cells was superior to the levels of the effectiveness recommended by the National Cancer Institute for a cytotoxic substance.

A group of Japanese researchers have reported that AP stopped stomach cancer cells from multiplying. After three days, there were less than 8 cancer cells growing in the presence of AP while the untreated cancer cells numbered 120. Another group of Japanese researchers tested AP on sarcoma cells. These usually very malignant cancers affect muscle, connective tissue, and bones. When tumor samples were examined under the microscope, AP was found to inhibit the growth of the tumors. Laboratory tests conducted in Buffalo, New York, demonstrated that AP inhibited the growth of human breast cancer cells at levels similar to the drug tamoxifen. Extracts of AP are much less toxic than most chemotherapeutic agents used to fight cancer. Although more studies need to be done to determine just which types of cancer respond to AP, the results so far have been promising.

In 1977, a human study was conducted using AP in sixty skin cancer patients, including forty-one with confirmed metastases (the cancer was spreading). As reported in the *Journal of Chinese Medicine*, twelve patients given AP and its compounds alone, recovered. All other patients were given AP

along with standard drugs; there was no tumor re growth in forty-seven of these patients. Based on this report, American investigators obtained investigational new drug status from the FDA to test AP extract. In 1996, early trials showed that the extract safely and effectively blocked growth of prostate and breast cancer, as well as non-Hodgkin's lymphomas. Based on the results of using AP on breast cancer cells grown in the laboratory, researchers believe that AP probably inhibits synthesis of cancer cell DNA. Additional details of cancer trials are given in the book, *Miracle Herbs* by Stephen Holt, M.D., wherein cancer studies done at Roswell Park Cancer Institute in Buffalo, New York, showed that AP extract has an antiprostate cancer action comparable to that of the widely used and highly toxic agent cisplatin - without the toxicity.

In a study, the methanolic extract of *Andrographis paniculata* was evaluated as the anticancer and immunomodulatory activity in human cancer and immune cells. The methanolic extract of *Andrographis paniculata* was fractionated into dichloromethane, petroleum ether and aqueous extracts and screened for bioactivity. The results indicated that the dichloromethane fraction of the methanolic extract retains the active compounds contributing for both the anticancer and immunostimulatory activity. Dichloromethane fraction significantly inhibited the proliferation of HT-29 (colon cancer) cells and augments the proliferation human peripheral blood lymphocytes (HPBLs) at low concentrations. On further fractionation of the dichloromethane extract, three diterpene compounds were isolated, i.e. andrographolide, 14-deoxyandrographolide and 14-deoxy-11,12didehydroandrographolide. Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers. Whereas all the three molecules showed enhanced proliferation and interleukin2 (IL-2) induction in HPBLs (31).

Human cancer cell lines representing different types of cancers were incubated in complete medium with and without test compounds for 48 h and the percentage growth determined by SRB method. Percentage growth of the treated cells was calculated compared to the control untreated cells and the concentration required to inhibit the 50% growth (GI50 concentration). The numbers represent the GI50 values in micromolar concentration. Among the three molecules isolated andrographolide inhibited the proliferation of cancer cells with GI50 values ranging from 10 to 28µM. 14-Deoxyandrographolide showed moderate inhibition on proliferation of NCI/ADR-RES and A498 cells and did not inhibit the proliferation of other cancer cells. 14-deoxy-11,12-didehydroandrographolide did not inhibit the proliferation of any cancer cell line tested. These data suggests that the anticancer activity shown by the plant extract is mainly due to the presence of andrographolide and the other two compounds are not contributing significantly for the anticancer activity. Similar results were reported by Siripong *et. al.*, 1992 (6) which elaborated the cytotoxic activity of andrographolide against KB (human epidermoid carcinoma) and P388 (lymphocytic leukaemia) cells. The growth inhibitory and differentiating activity of the methanol extract of aerial parts of *Andrographis paniculata* and some of the isolated compounds on M1 (mouse myeloid leukaemia) cells was also reported (9). From above results it is evident that the major constituent andrographolide shows anticancer and immunostimulatory activities. The in vivo results from hollow fiber assay conducted in immunocompetent Swiss albino mice, demonstrated that andrographolide significantly inhibits the cancer cell proliferation without showing any signs of toxicity in mice even at high doses. Although 14-deoxy11,12-didehydroandrographolide and 14-deoxyandrographlide did not show in vitro anticancer activity, studies are in progress to check whether the observed in vitro immunostimulatory activity of these compounds will result in any objective in vivo anticancer activity indirectly through stimulation of host immune system in terms of tumor growth inhibition, preventing the metastasis and increasing survival time. We conclude that, owing to its potent anticancer and immunostimulatory activities, the diterpenoid andrographolide, can serve as a scaffold for design and synthesis of novel, potent, non-toxic anticancer and/or immunomodulatory molecules.

In a recent study by Xu *et. al.*, 2007 (32) explored that Gavage of mice, immunised with an inactivated S. typhimurium vaccine, with *Andrographis paniculata* (AP) extract or andrographolide (AD) resulted in an enhancement of Salmonella-specific antibody response and induction of cell-mediated response against salmonellosis. Mice were vaccinated with either one or two doses of killed S. typhimurium vaccine and fed two different quantities of AP or AD, for 14 days in mice immunized with one dose of the vaccine, and for 28 days in mice immunized with two doses of vaccine, respectively. Both AP and AD were found to enhance IgG antibody levels against *S. typhimurium*, the enhancement being statistically significant in mice receiving two doses of the vaccine. Splenocyte cultures, prepared from mice immunized with the killed Salmonella vaccine and treated with APE or AND, showed a remarkable increase in the production IFN-α following stimulation with the bacterial lysate, indicating an induction of Salmonella-specific cell-mediated response/immune response.

In a study (33) it is reported that andrographolide in comparison to other two diterpenoids, deoxyandrographolide and neoandrographolide, had more potent anti-cancer activity against human leukemia HL-60 cells and other cancer cells. As demonstrated by chromosomal DNA fragmentation, accumulation of HL-60 cells in the sub-G1 cell cycle stage, disappearance of mitochondrial cytochrome c and an increased expression of Bax and downregulation of Bcl-2 in the inhibited cells, it induced apoptosis of HL-60 cells.

The cytotoxicity of andrographolide to HepG2 human hepatoma cells was investigated by Jieliang Li *et. al.*, 2007 (34) exposed that growth of HepG2 cells was affected in the presence of andrographolide with an IC_{50} of 40.2 ìM after 48 h treatment. Flow cytometric analysis and DNA fragmentation assay revealed that andrographolide induced cell cycle arrest at G2/M phase and a late apoptosis of the cells. The occurrence of cell cycle arrest was accompanied by the collapse of mitochondrial membrane potential (MMP) and an intracellular increase of hydrogen peroxide (H2O2) but a

decrease of superoxide radicals ($O_2^{\cdot -}$) and reduced glutathione. In the treated cells, expression of Bax as well as the transcriptional controller of this pro-apoptotic gene, p53, was upregulated but not other apoptotic proteins such as Bad, Bcl-2 and Bcl-XL. Although the activity of caspase-3, which has direct effect on apoptosis, was also enhanced by the presence of andrographolide, cell death of HepG2 could neither be prevented by a specific inhibitor of capsase-3 nor the pan-caspase inhibitor-zVAD (Val-Ala-Asp), indicating that it was a caspase-independent cell death. Since the overall percentage of apoptotic cells was relatively small throughout the experimental studies, we conclude that the cytotoxic effect of andrographolide on HepG2 cells is primary attributed to the induction of cell cycle arrest via the alteration of cellular redox status.

Effects on HIV and other Viruses

Immune deficiency is at the root of susceptibility to a variety of infections, and it is the basis of the Acquired Immune Deficiency Syndrome (AIDS). Impairments of immune function result in variable clinical symptoms. To understand how to treat the disease and why infection with the human immuno-deficiency virus (HIV-1) is resistant to conventional and alternative therapies, we need to understand just what AIDS is. AIDS appears to have first arisen in Africa. It may have started when the HIV virus that previously only affected African primates most likely mutated and was able to infect humans. In this modern age of fast intercontinental travel, the virus spread all over the world. The initial cases in North America were reported in 1981, before the condition had even been named. Studies of hospital records and frozen tissue samples, however, indicate that AIDS was present as early as 1969. Two strains of HIV have been since identified: HIV-1 and HIV-2 (which seems confined to Africa).

HIV, like all viruses, cannot reproduce itself or even live, without using the resources of other cells. When HIV virus finds a suitable cell, it attaches to the cell, using proteins on its cell surface. In the case of human cells, the HIV virus enters the cells by binding two molecules on the cell's surface. The first of these to be identified was CD4; other, more recently identified molecules are CCR5 and CXCR4. The brain and certain skin tissues are areas where the HIV virus tends to concentrate. HIV also attacks and debilitates cells in the immune system. Helper T cells - the "T" represents the thymus gland where the cells are produced - are a main target of the virus. These cells signal the lymph nodes and the spleen to produce more antibodies against the NIV virus. Once the antibodies inactivate the virus, suppressor T cells produce chemicals that stop further production of antibodies. The HIV virus, however, attaches itself to the helper T cell. Through a series of manipulations of the helper cell's genetic machinery, the virus tricks the cell into producing chemicals that the virus needs. HIV takes over the "machinery" of the helper T cell and thus becomes a virus production factory that is no longer part of the immune system. Without the T-cells, the other components of the immune system do not receive any messages to produce antibodies and resistance to HIV is seriously compromised.

Conventional treatment consists of a combination of drugs designed to achieve maximum viral suppression. Often referred to as a "cocktail," this mixture consists of compounds called protease inhibitors and reverse transcriptase inhibitors. Without going into detail, a protease is an enzyme needed by the HIV virus for replication and assembly of new virus parts. Reverse transcriptase is another enzyme that the HIV virus uses to copy its genetic material when inside the T cell. While inhibiting these enzymes has been effective treatment in many cases, reducing the amount of HIV in the blood does not mean that a patient will suffer from fewer AIDS-related diseases. Researchers are not certain how long a new combination of drugs will work before virus strains become resistant to the treatment. It is always the case with drug treatment that a few resistant virus particles will survive and go on to reestablish the infection. Protease inhibitors (Invirase, Norvir, Viracept, and Crixivan) do not work on everyone and are not well absorbed. Large doses (36 pills a day) may be required with costs as high as $16,000 a year. Dangerous side effects, such as diabetes and hypertension, can develop or become worse in patients taking protease inhibitors.

Another therapy: AZT - an antiviral that can slow the HIV infection -has limited use because of the high incidence of side effects, which include kidney stones, bone marrow depression and brain and liver toxicity. Scientists are therefore looking for better therapies. Protease inhibitors are abundant in plants: soybeans, rice, corn, beans, wild tomatoes, and other vegetables. Reverse transcriptase inhibitors are also found in nature. Quercetin, a bioflavonoid found in red apples and red onions, has activity against viruses that cause AIDS, herpes, and polio. The long history of using herbs with immune-enhancing properties in TCM prompted scientists to look further into this area of potential therapies. Exciting recent research has indicated that extracts of *Andrographis paniculata* may have great promise for interfering with the viability of the HIV virus. Scientists now believe that AP can join with modern technology in the fight against AIDS. An important place to look for a way to stop HIV was in the human cell where the virus was using the cell's machinery to reproduce itself. Cells, when they grow and reproduce, go through a series of steps collectively termed the "cell cycle." During this process, chemical messages are carried to various parts of the cell in order to "turn on" functions. This process is called "signal transduction." The HIV virus actually subverts the cell's messengers, tricking them into producing more viral particles. Using signal transduction technology (methods to investigate cell message systems), scientists found that AP contained substances that destroyed the virus's communcations mechanism. One component of the herb - andrographolide, prevented transmission of the virus to other cells and stopped the progress of the disease by modifying cellular signal transduction. Andrographolide probably does this by inhibiting enzymes that facilitate the transfer of phosphates. Phosphates are molecules that are the energy storehouses of the cell. During the cell cycle, phosphates are created or chemically changed and energy is produced. This energy is used in the regulation of the cell cycle and for the many

cellular functions that go on during reproduction of the cell. AP can thus interfere with key enzymes that result in viral reproduction (35). HIV alters regulation of the cell cycle by causing the process to stop at a particular phase. What the virus specifically does is to alter the action of a central information-processing enzyme that coordinates all events relating to cell division. This regulatory enzyme (actually a class of enzymes) is called cyclin-dependent kinase (CDK). A particular CDK, CDK-1 is the prime target of HIV. When the cell moves through its cycle, all information about cellular activities is sent to CDK-1. Several diseases in addition to AIDS, such as cancer, heart disease, and viral infections, are associated with aberrant functioning of CDK-1. The virus causes CDK-1to misfunction by attaching molecules to it, a process called phosphorylation. Agents that can prevent this phosphorylation can less the severity of AIDS. The new class of antiviral compounds with this ability is called tyrosine kinase inhibitors. This class includes the andrographolides. Work done at the National Institutes of Health (NIH, USA) in 1995 has showed that T-cells infected with HIV accumulate high levels of overphosphorylated CDK-1. An extract of AP can, in fact, inhibit CDK-1 that has been altered by HIV. In April 1992, NIH researchers reported that these inhibitors could halt the disease-causing components of HIV. These compounds are amino acids that can inhibit the viral enzymes involved in the production of high-energy phosphates. Cooperative research at the National Cancer Institute has shown that andrographolide can also inhibit HIV's toxic effect on cells. It does this by inhibiting c-mos, a genetic component involved in HIV propagation and T-cell death. C-mos is integrated into the DNA of the cell and usually is inactive. Normally found only in reproductive system cells, c-mos is not expressed in CD4 cells or other body cells. When CD4 cells are infected by the HIV virus, c-mos expression is activated. For this to happen, an enzyme (c-mos kinase) is needed. *Andrographis* extract can inhibit this enzyme and so can support normal immune function. A hypothesis for the mechanism of action of AP in AIDS is that the herbal extract appears to induce apoptosis or programmed cell death. In this process, cells break up into particles which are then scavenged by immune system cells. The HIV virus may generate apoptotic signals to uninfected immune cells. This would explain the extensive T-cell destruction caused by HIV infection, which is far more than the amount of virus present. Testing of AP done at the Frederick Research Center demonstrated that extracts of AP increased AZT's ability to inhibit replication of HIV. The effect of the combination was greater than that of either compound alone. An added benefit is that lower doses of AZT could be used. Some researchers believe that AP extracts may also be useful in combating other viruses, including the Ebola virus and the viruses associated with herpes, hepatitis, and influenza. In a study examining 27 types of "heat clearing" and detoxifying medicinal herbs, researchers at the China Academy of Traditional Chinese Medicine in Beijing reported that AP was one of the herbs that had an inhibitory effect on HIV replication (24). A phase I dose-escalating clinical trial of andrographolide from *Andrographis paniculata* has been conducted by Carlo *et. al.*, 2000 (36) in 13 HIV positive patients and five HIV uninfected, healthy volunteers. Their objectives were primarily to assess safety and tolerability and secondarily to assess affects on plasma virion HIV-1 RNA level and $CD4^+$ lymphocyte levels. No subjects were antiretroviral medications during the trial. Those with liver or renal abnormalities were excluded. The planned regimen was 5 mg/kg bodyweight for 3 weeks, escalating to 10 mg/kg bodyweight for 3 weeks, and to 20 mg/kg bodyweight for a final 3 weeks. The trial was interrupted at 6 weeks due to adverse events including an anaphylactic reaction in one patient. A significant rise in the mean $CD4^+$ lymphocyte level of HIV subjects occurred after administration of 10 mg/kg andrographolide (from a baseline 405 cells/mm^3 to 501 cells/mm^3; p=0.002). There were no statistically significant changes in mean plasma HIV-1 RNA levels throughout the trial. Andrographolide may inhibit HIV-induced cell cycle dysregulation, leading to a rise in $CD4^+$ lymphocyte levels in HIV-1 infected individuals.

In MCF-7 breast cancer cells, andrographolide treatment during log growth phase produced a rapid decrease in expression of the cell cycle regulatory proteins phosphorylated cyclin-dependent kinase 1 (cdk1 or $p34^{cdc2}$) and cyclin B. In HIV infected lymphocytes, viral replication is increased in the G2 phase of the cell cycle (37). Andrographolide has been reported to inhibit cell to cell transmission, viral replication and syncytia formation in HIV infected cells (37, 38). Andrographolide also inhibited expression of mitogen-activated protein kinase (MAPK) and apoptotic regulatory proteins c-myc, p53, Bcl-2, Bcl*xl* and Bax. Inhibition of the expression of these apoptosis regulatory proteins correlated with the inhibition of HIV-1 envelope protein-mediated syncytia formation in HL60 cells (39). Thus, andrographolide may exert multiple antiviral effects through inhibiting the dysregulation of signal transduction pathways necessary for viral replication and HIV-1induced T cell cytopathicity.

Effects in Common Cold, Fever & Inflammation

The prevention of the common cold with an extract of AP was shown in a pilot double-blind study. Students were given Kan Jang; a formulation of AP produced by the Swedish Herbal Institute, and was diagnosed for the presence or absence of colds during a three-month period (40). A dose of 200 mg/day was given to the study group. After one month there was no significant difference in the number of colds. However, after the third month of intake of Kan Jang there was a significant decrease in the incidence of colds as compared to the placebo group. The students that got the Kan Jang had a rate of incidence of colds of 30% compared to 62% for students that received the placebo. The relative risk of catching a code indicated that the preventive effect could be due to the presence of andrographolide, which has known immunostimulant effects. The amount of Kan Jang used in the previous study was much less than used in a previous study that produced quicker results. In this study, patients were divided into two groups, one of which received 1,200 mg/day

of Kan Jang (41). These patients already had colds with symptoms including nasal discharge, nasal stuffiness, sore throat, earache, cough, fever, headache, and malaise. At the beginning of the study, the patients receiving Kan Jang, and those receiving a placebo had similar symptoms. The symptoms, such as tiredness, shivering, sore throat, and muscular aches, diminished significantly on the fourth day of treatment with Kan Jang. The researchers concluded that treatment with Kang Jang (standardized to 4% andrographolides) accelerated the recuperation of patients from the common cold. AP is also used as a folk medicine remedy for fever, pain reduction, and disorders of the intestinal tract. The ability of AP to lower fever has been demonstrated independently in several laboratories. Rat studies done in China have shown that andrographolide, neoandrographolide, and dehydroandrographolide can lower the fever produced by different fever-inducing agents, such as bacterial endotoxins (toxic chemicals released from bacteria), pneumococcus, hemolytic streptococcus, typhoid, paratyphoid, and the chemical 2,4-dinitrophenol (42). Researchers tested AP to try and determine whether it did, in fact, work in these conditions (43). Fever was induced in rats. There was a reduction in rectal body temperature for 30, 100, and 300 mg of andrographolide/kg bodyweight. While the analgesic (painkilling) activity of andrographolide extracted from AP was weak compared to aspirin, the antipyretic (fever-reducing) activity was comparable to that of aspirin. The study found that 300 mg/kg bodyweight of andrographolide was as effective as the same amount of aspirin, in fact, the AP extract was found to possess antiulcerogenic activity. It reduced the development of ulcers by 31%, while the standard ulcer drug, cimetidine had an 85.43% reduction rate. Andrographolide caused a significant decrease in total stomach acidity and acid stomach juice secretion, without the cost and side effects associated with ulcer therapy. In another study, AP extracts were found to produce results comparable to 200 mg of aspirin/kg bodyweight (44). The researchers also established that there was a wide margin of safety in using AP extracts, an indication of the lack of toxicity. The anti-inflammatory effects of various AP compounds have been shown in many studies in which the inflammation was produced by chemicals. Inflammation caused by histamine, dimethyl benzene, croton oil (hemolytic necrosis), and acute pneumocystis produced by adrenaline was significantly reduced or relieved (45). This effect was observed for all major andrographolides: deoxyandrographolide, andrographolide, neoandrographolide, and dehydroandrographolide. Dehydroandrographolide had the most pronounced effect, followed by neoandrographolide and andrographolide. This anti-inflammatory effect seemed to work by a mechanism that involved the adrenal glands. The effect disappeared when adrenal glands were removed from experimental animals (46). Further study confirmed that the anti-inflammatory action of dehydroandrographolide was due to its effect on increasing the synthesis and release of adrenocorticotrophic hormone (ACTH) of the pituitary gland of the brain. ACTH signals the adrenal gland to make cortisol, a natural anti-inflammatory (45). In research done on the anti-inflammatory activity of naturally occurring products, AP was found to inhibit edema (swelling due to fluid trapped in tissues). At a concentration of 200 mg/kg bodyweight, AP significantly inhibited (by 60%) edema at three hours. With 400 mg/kg bodyweight, 62.7 percent was inhibited (50).

Andrographolide has been shown to have several biological activities including analgesic, antipyretic and antiinflammatory effects. Since the upregulation of adhesion molecules expression and endothelial-leucocytes adhesion are key steps in the development of inflammation, a study examined whether andrographolide modulates these biological processes *in vitro*. Incubation of endothelial cells with non-toxic concentrations (0.16–16.7 mg/mL) of andrographolide attenuated the tumor necrosis factor-α (TNF)-induced intercellular adhesion molecule-1 (ICAM-1) expression. Similar concentration ranges of andrographolide also inhibited the TNF-induced endothelial-monocyte adhesion in a concentration dependent manner. These effects of andrographolide may account for its reported *in vivo* anti-inflammatory activity (51).

Yuh-Chiang Shen *et. al.*, 2002 (52) reported that andrographolide (AD) inhibits inflammatory responses by rat neutrophils. To further elucidate the possible mechanism(s) underlying the AD's effect, N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced adhesion and transmigration of isolated peripheral human neutrophils were studied. Pretreatment with AD (0.1-10μM) concentration-dependently prevented fMLP-induced neutrophil adhesion and transmigration. Further examined the up-expression of surface Mac-1 (CD11b/CD18), an essential integrin mediated in neutrophil adhesion and transmigration. AD pretreatment significantly decreased fMLP-induced up-expression of both CD11b and CD18. Accumulation of reactive oxygen species (ROS) as well as quick intracellular calcium ($[Ca^{++}]i$) mobilization induced by fMLP displays two important signaling pathways in regulating the up-expression of Mac-1by neutrophils. That AD pretreatment diminished fMLP-induced production of H2O2 and $O2.^-$, but failed to block that of $[Ca^{++}]i$ mobilization suggested that the ROS but not $[Ca^{++}]i$ signaling could be modulated by AD. To clarify whether ROS production impeded by AD could be an antagonism of fMLP binding, phorbol-12myristate-13-acetate (PMA), a direct protein kinase C (PKC) activator, was introduced to activate ROS production. PMA triggered remarkable ROS production and adhesion, and were partially reversed by AD. This indicated that a PKC-dependent mechanism might be interfered by AD. They conclude that the prevention of ROS production through, at least in part, modulation of PKC-dependent pathway could confer AD the ability to down-regulate Mac1 up-expression that is essential for neutrophil adhesion and transmigration.

The potential use of AP and its components are important especially because bacteria are showing resistance to drugs. Each time bacteria are exposed to an antibiotic, most are killed, but a few survive. These survivors go on to multiply and establish infections that cannot be treated with the

original antibiotic, and in some cases there are no existing drugs to stop the bacteria. Although AP and other herbs are not substitutes for antibiotics, these plants and other herbs could have a complementary effect when used along with antibiotics. In fact, according to Dr. Stephen Holt, we may be seeing natural remedies combined with synthetic medications being used in therapies that are more effective and safer. Malaria is still a prevalent disease in many tropical and subtropical countries. It is difficult to eradicate because the parasites that carry malaria become resistant to the drugs used to treat the disease. Extracts of AP containing the four major active components were evaluated for antimalarial activity against *Plasmodium berghei*, one of the parasites that transmit malaria. The extract was found to produce considerably inhibition of multiplication of the parasites (53). Two of the AP components, neoandrographolide and deoxandrographolide, were found to be the most effective of the four. Pretreating animals with neoandrographolide for fifteen to twenty-one days prior to exposure as well as after infection was found to be more effective than treatment started only after infection. Effects were better than treatment after infection with chloroquine, a commonly used antimalarial drug. In a subsequent study, researchers repeated the effects of AP and indicated that the protective action of AP may be due to reactivation of superoxide dismutase (SOD), a key antioxidant enzyme that protects the liver. AP extracts are also efffective in killing filaria (microscopic worms) that obstructs lymph channels in the body, leading to gross swelling termed elephantiasis. The study was done in dogs. Since no toxic effects were apparent, researchers believed that the AP plant extract would be safe for humans. No plant has previously been shown to have antifilarial action. Screening of aqueous extracts of A*ndrographis*, andrographolide and arabinogalactan proteins showed significant antibacterial and antifungal activities in comparison to some known antibiotics. The investigations revealed the biological value of the cumulative effects of AP and AD resulting in enhanced antimicrobial activity (54). Studies on compounds other than andrographolide revealed four xanthones; 1,8-di-hydroxy-3,7-dimethoxy-xanthone, 4,8-dihydroxy-2,7dimethoxy-xanthone, 1,2-dihydroxy-6,8-dimethoxy-xanthone and 3,7,8-trimethoxy-1-hydroxy xanthone from the roots of *Andrographis paniculata*. Among them the compound 1,2-dihydroxy-6,8-dimethoxy-xanthone possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with its IC50 value of 4μgml^{-1} . Xanthones bearing hydroxyl group at 2 position demonstrated most potent activity while xanthones with hydroxyl group at 1,4 or 8 position possessed very low activity. In vivo anti-malarial sensitivity test of this compound on Swiss Albino mice with *Plasmodium berghei* infection using Peters' 4-day test gave substantial reduction (62%) in parasitaemia after treating the mice with 30mg kg^{-1} dose. In vitro cytotoxicity against mammalian cells revealed that 1,2-dihydroxy-6,8-dimethoxy-xanthone is non-cytotoxic with its IC50 > 32μgml^{-1} (55).

Antidiarrheal & Intestinal Effects

Experiments on animals demonstrate that AP can prevent or stop diarrhea. Diarrhea-type diseases are one of the top ten causes of death worldwide and are a leading cause of death in children in developing countries, especially those that are under five years of age. The use of antibiotics is producing antibiotic-resistant strains of bacteria. While there are many drugs used to relieve the symptoms of diarrhea (kaolin-pectin, bismuth, Lomotil, loperamide hydrochloride, and others), many have undesirable side effects. An inexpensive and easily obtained herbal remedy would benefit many, especially people in developing countries where diarrheal disease is almost catastrophic. Extracts of AP have been shown to have significant effects against the diarrhea associated with *E. coli* bacterial infections (23). The AP components, andrographolide and neoandrographolide, showed similar activity to loperamide (Imodium), the most common antidiarrheal drug. Acute bacterial diarrhea in patients was treated with a total dose of 500 mg andrographolide divided over three dosing periods per day for six days (2.5 to 3.0 mg/kg of body weight). This regimen was combined with rehydration. There were 66 cures of 80 patients treated - an 82.5% cure rate. Seven additional patients responded favorably to the treatment and only seven patients (8.8%) did not respond. The effectiveness of the treatment was confirmed by laboratory tests of stool samples (46). In another study, AP was used to treat 1,611cases of bacterial dysentery and 955 cases of diarrhea with overall effectiveness of 91.3% (45). It had been believed that AP was effective against bacterial dysentry and diarrhea because it was antibacterial, but studies could not confirm this effect. However, the andrographolides were very effective in stopping the diarrhea. How this is accomplished is not completely understood at present. Chronic inflammation of the colon was treated with a combination of AP (60 g) and *Rehmannia glutinosa* (30 g), decocted. Rehmannia is a Chinese herb used to treat anemia, fatigue, and to promote the healing of injured bones. It is also a demulcent. The liquid part of the mixture was used as an enema at doses of 100 to 150 ml each night for fourteen days. Of a total of 85 patients, 61 (72%) were considered clinically cured and 22 (26%) had symptomatic relief (46).

An ethanol, chloroform or 1-butanol extract of the aerial parts (300mg/ml) inhibited the *E. coli* enterotoxin-induced secretory response—which causes a diarrhoeal syndrome -in the rabbit and guinea-pig ileal loop assay (47, 48). However, an aqueous extract of the aerial parts was not active (48). The constituent diterpene lactones, andrographolide and neoandrographolide, exhibited potent antisecretory activity in vivo against *E. coli* enterotoxin-induced diarrhoea (48). Andrographolide (1 mg/loop) was as active as loperamide when tested against heat-labile *E. coli* enterotoxin-induced diarrhoea and more effective than loperamide when tested against heat-stable *E. coli* enterotoxin-induced diarrhoea (48). Neoandrographolide (1 mg/loop) was as effective as loperamide when tested against heat-labile *E. coli* enterotoxin-induced diarrhoea and slightly less active than loperamide when tested against heat-stable *E. coli*

enterotoxin-induced diarrhoea (48). The mechanism of action involves inhibition of the intestinal secretory response induced by heat-labile *E. coli* enterotoxins, which are known to act through the stimulation of adenylate cyclase, and by inhibition of the secretion induced by heat-stable *E. coli* enterotoxins, which act through the activation of guanylate cyclase (47). Incubation of murine macrophages with andrographolide (1–50mM) inhibited bacterial endotoxin-induced nitrite accumulation in a concentration- and timedependent manner. Western blot analysis demonstrated that andrographolide inhibited the expression of an inducible isoform of nitric oxide synthase linked to endotoxin-induced circulatory shock (49).

The aerial parts have been used for the treatment of acute bacillary dysentery and enteritis (62, 63, 64). In clinical studies, the combination of andrographolide and neoandrographolide was reported to be more effective than either furazolidine or chloramphenicol in the treatment of bacillary dysentery (62). A randomized, double-blind clinical study of 200 patients compared the efficacy of the powdered aerial parts with tetracycline in the treatment of acute diarrhoea and bacillary dysentery (62, 63). Patients received capsules of either the aerial parts or tetracycline (both 500 mg, four times daily) for 3 days. Compared with tetracycline, the aerial parts decreased the diarrhoea (both the frequency and amount of discharge) (62). Furthermore, the aerial parts were more effective in treating diarrhoea resulting from shigellosis than from cholera (62).

Cardiovascular Benefits

In 1964, angioplasty was developed. This technique has been used to treat blocked blood vessels (usually arteries). A balloon is inserted into the artery and then inflated to clear away fatty deposits, widen the artery, and improve blood flow. In 1967, surgeons at the Cleveland Clinic developed another treatment for coronary artery obstructions: bypass surgery. In this procedure, a new vein (from another part of the body, from an animal, or a synthetic) replaces the obstructed artery. Today, angioplasty and bypass surgery are routine, with about 800,000 such procedures done in the United States each year. These treatments are not, however, a cure-all. For, with angioplasty, restricted blood flow recurs in 30% of patients within six months; 50% of patients will require a repeat procedure. Many of these patients eventually require bypass surgery, which is successful in only 50 to 65% of cases. Clot-dissolving drugs used in the emergency treatment of heart attacks appear to be as effective as angioplasty and may prevent some of the heart attacks or strokes that occur within one month of angioplasty. The process of blood clotting in the body is not yet fully understood. It is a delicate balance between the clotting necessary to achieve healing and processes that will cause abnormal and unwanted clotting. Research to understand the signals involved in bleeding and blood vessel development is making use of signal transduction technology. It has been demonstrated that extracts of AP can increase the time it takes for blood clots to form, thus decreasing the risk of subsequent closing of blood vessels (restenosis) seen after angioplasty procedures. In studies done on rabbits given angioplasty, AP extracts was shown to significantly prevent constriction of blood vessels. The rabbits received AP for three days before angioplasty and for four weeks after surgery. While the arterial narrowing occurred in 100% of animals not given AP, only 70% of those receiving AP showed narrowing (56). Narrowing caused by injury to the inner lining of the blood vessel and by high cholesterol in the diet was also found to be decreased by AP. It appears that AP may be quite effective in preventing repeated narrowing of vessels after coronary angioplasty. In 80 to 90% of patients with destroyed heart muscle resulting from an acute myocardial infarction (heart attack), clots are found in the heart shortly after the beginning of symptoms. When heart muscle is deprived of its blood supply, and therefore of oxygen, the tissue dies. Physicians and researchers believe that the best treatment is to limit the size of the myocardial infarction (the area of tissue damage) in order to preserve the pump function of the heart. Agents that dissolve the clots and increase blood flow through the blocked artery are constantly being sought. AP may have the potential to be part of the treatment plan in such cases. Researchers at the Tongi Medical University in China have demonstrated that AP given to dogs one hour after developement of myocardial infarction decreased the damage that occurred to the heart muscle (57). Such damage occurs *after* the blood supply is restored to the muscle. This is due to a sudden influx of oxygen (which produces free radicals that damage tissue) and abnormally high amounts of calcium. In subsequent studies at the same university, the researchers demonstrated by electrocardiograph that abnormal changes in heart readings were prevented by pretreatment with AP. Also, clumping of platelets (the blood particles that initiate clotting) was inhibited and no clot (thrombus) that could cause infarction was induced (57). An added effect of AP was that it activated fibrionolysis, the natural process in the body that dissolves clots (58). Another way to prevent cardiovascular disease is to correct high blood pressure. Researchers have reported that an extract of AP produced antihypertensive (blood pressure lowering) effects (58). The extract was given intravenously to hypertensive rats. Noradrenaline, a hormone secreted by the brain, acts to constrict blood vessels and increase heart rate, blood pressure, and blood sugar levels. AP inhibited the increase in blood pressure that is caused by noradrenaline. Researchers believe that AP has this antihypertensive effect because it relaxes the smooth muscle in the walls of blood vessels. This relaxation prevents the blood vessel from constricting and limiting blood flow to the heart, brain, and other organs in the body. AP keeps blood, and therefore oxygen, flowing to the brain. Diminished blood flow to the brain can cause short-term memory loss, ringing in the ears, dizziness, headaches, depression, and impaired mental performance. The effects of AP are produced without toxicity and at a reasonable cost, making this miracle herb a good option for cardiovascular therapy.

The cardiovascular activities of crude water extract (WE) of *Andrographis paniculata*, its three semi-purified ethyl acetate (EA), n-butanol (BU) and aqueous (AQ) fractions and andrographolide as such were elucidated in anaesthetized

Sprague-Dawley (SD) rats. EA and andrographolide, which possesses multiple pharmacological activities, elicited no drop in mean arterial blood pressure (MAP), while WE, BU and AQ produced a significant fall in MAP in a dose-dependent manner without significant decrease in heart rate. The ED50 values for WE, BU and AQ were 11.4, 5.0 and 8.6 mg/kg respectively. These suggested that the hypotensive substance(s) of the crude water extract was concentrated in BU. The hypotensive action of BU was not mediated through effects on the βadrenoceptor, muscarinic cholinergic receptor and angiotensin-converting enzyme, for it was not affected by propranolol, atropine and captopril, respectively. However, it seems to work via α-adrenoceptors, autonomic ganglion and histaminergic receptors, since the hypotensive effect of BU was negated or attenuated in the presence of phentolamine, hexamethonium as well as pyrilamin and cimetidine (59).

Inhibition of angiogenesis is currently perceived as one of the promising strategies in the treatment of cancer. In a recent study, Sheeja *et. al.*, 2007 (60) analyzed the antiangiogenic activity of *Andrographis paniculata* extract (AP) and its major component andrographolide (AD) using both in vitro and in vivo models. Intraperitoneal administration of AP and AD significantly inhibited the B16F-10 melanoma cell line induced capillary formation in C57BL/6 mice. Analysis of serum cytokine profile showed a drastic elevation in the proinflammatory cytokines such as IL-1β, IL-6, TNF-α and GM-CSF and the most potent angiogenic factor VEGF in angiogenesis induced animals. Treatment of AP and AD significantly reduced this elevated level. Moreover, VEGF mRNA levels in B16F-10 cell line showed a reduced level of expression in the presence of AP and AD. Serum NO level, which was increased in B16F10 melanoma injected control animals, was also found to be significantly lowered by the administration of AP and AD. Antiangiogenic factors such as TIMP-1 and IL-2 level was elevated in AP and AD treated angiogenesis induced animals. In the rat aortic ring assay AP and AD inhibited the microvessel outgrowth at non-toxic concentrations. Results demonstrate that AP and AD inhibit the tumor specific angiogenesis by regulating the production of various pro and antiangiogenic factors such as proinflammatory cytokine, nitric oxide, VEGF, IL-2 and TIMP-1.

The three active diterpenoids from *Andrographis* and its aqueous plant extracts, were investigated for the inhibitory effect for *in vitro* platelet aggregation by Thisoda *et. al.*, 2006 (82). The results indicated that andrographolide (AD) and 14-deoxy-11,12-didehydroandrographolide (ADD) significantly inhibited thrombin-induced platelet aggregation in a concentration (1–100μM) and time-dependent manner while neoandrographolide (ADN) had little or no activity. ADD exhibited higher antiplatelet activity than AD with IC50 values ranging from 10 to 50 μM. The inhibitory mechanism of AD and ADD on platelet aggregation was also evaluated and the results indicated that the inhibition of extracellular signal-regulated kinase1/2 (ERK1/2) pathway may contribute to antiplatelet activity of these two compounds. In addition, standardized aqueous extracts of *A. paniculata* containing different amounts of ADD inhibited thrombin-induced aggregation to different degrees. The extracts significantly decreased platelet aggregation in a concentration (10–100 μg/ml) and time-dependentmanner. However, the extract with high level of ADD (Extract B) (IC50 values=50–75μg/ml) showed less inhibitory activity against thrombin than the extract with lower level of ADD (Extract A) (IC50 values=25–50μg/ml). These results indicate that the standardized *A. paniculata* extract may contain other antiplatelet compounds rather than AD and ADD, which contribute to high antiplatelet activity. Therefore, the consumption of *A. paniculata* products may help to prevent or treat some cardiovascular disorders i.e. thrombosis; however, it should be used with caution by patients (82).

Antifertility Effects

AP has clear antifertility as well as pregnancy-terminating effects. In India, where AP is used for common ailments such as diarrhea, fever, and other digestive disorders, it is recommended that the herb be used only for short-term treatment. This is due to the content of compounds that are contraceptive in nature. To determine the actual effects on fertility, studies were done in male rats. In one study, it was found that AP, given as dry leaf powder (105mg powder/kg body weight) each day for 60 days, stopped spermatogenesis (development and maturation of sperm cells) (61). The authors suggested an antispermatogenic (sperm production blocking) or antiandrogenic (blocking effects of androgens) ability of the plant. It should be noted that many herbal extracts have effects on reproductive functions and thus should not be used during pregnancy. Studies by Zoha and colleagues, also in India, reported antifertility effects on female mice (62). When 2 gm/kg body weight of sun-dried AP powder were given to the rats every day for six weeks, none of the animals were pregnant after mating (five times) with proven fertile males who did not receive the AP. The mice who did not receive the AP had normal litters when bred with similar males. The effect of AP may have been to prevent ovulation. The potential for its use as an antifertility agent in Bangladesh, where the plant is easily available, motivated scientists to perform these experiments. Studies done in cultured human placental tissue showed that andrographolide sodium succinate (derived from AP) was effective in inhibiting human progesterone production (46). This hormone is necessary for pregnancy to be successful. The form of AP used was tissue specific, meaning it only affected the tissue it was intended for. There were no detrimental effects on other normal human tissue, even at the highest doses tested.

The researchers concluded that the derivatives appeared to be promising contraceptives. Other studies in female mice using dehydroandrographolide indicated that the dose required to affect pregnancy was 250 mg/kg body weight. This amount of pure compound would not be found in the 105 mg/kg body weight dose of AP given to male animals or the 2g (2,000 mg/kg body weight) given to the female animals in the studies described above. Thus, it appears unlikely that the active compound in AP causing infertility is a member of the andrographolide series of compounds.

Liver & Gall bladder Protection; abdominal benefits

In Ayurvedic medicine, there are 26 different formulations

containing AP that are used to treat liver disorders. AP's four related medicinal compounds were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride (a cleaning solvent), alcohol, or other toxic chemicals (65). These chemicals damage the liver by causing lipid peroxidation. This is a process whereby free radicals (reactive molecules) produced by the chemical attack and destroy cellular membranes that surround liver cells. When the AP compounds were given to animals three days before the toxic chemicals, there was a significant protective effect in the liver. This effect was attributed to the antioxidant ability of the AP compounds, which was effective as silymarin (another plant antioxidant from milk thistle; *Silybum marianum*). In another study, andrographolide from AP was shown to produce a significant increase in bile flow (66). Bile is produced in the liver and stored in the gallbladder and aids in digestion. When a chemical, paracetamol, was given to animals pretreated with andrographolide, the usual decrease in bile production seen with this chemical was prevented. In this case, andrographolide was found again more potent than silymarin. Infective hepatitis is an acute inflammatory condition of the liver. It is often followed by liver cirrhosis and may progress to a coma and death. In India, where ancient physicians used AP to treat similar liver ailments, a study was conducted to evaluate the effect of AP in infective hepatitis. There was marked improvement in the majority of patients tested, when given a decoction or infusion of AP. Appetite improved on the fifth day of treatment, jaundice (yellow color of conjunctive of the eye and skin) gradually diminished and completely disappeared within 24 days, and fever subsided after 7 days on average. Other indications of effectiveness of AP included improvement in liver function tests. The researchers concluded that AP was a useful remedy for treatment of infective hepatitis.

The andrographolides present in AP are potent stimulators of gallbladder function. In animal experiments, those that received andrographolides for seven consecutive days showed an increase in bile flow, bile salts, and bile acids. These increases are beneficial and result in enhanced gallbladder function. Use of AP might, therefore, decrease the probability of gallstone formation and might also aid fat digestion. The andrographolides also prevented decreases in the amount of bile that are caused by acetaminophen toxicity (35).

Modulatory influence of *Andrographis paniculata* crude extract on cytochrome P450 (CYP) enzymes was performed by administration of the crude extract of *Andrographis paniculata* to ICR male mice. Total hepatic P450 content was not significantly modified by either the aqueous or the alcoholic extracts of *Andrographis paniculata*. Assessment of hepatic microsomal P450 activities by alkoxyresorufin *O*-dealkylations noted that both the aqueous and alcoholic extracts of *Andrographis paniculata* significantly increased ethoxyresorufin *O*-dealkylase and pentoxyresorufin *O*-dealkylase activities, while those of methoxyresorufin *O*-dealkylase activities were not elevated. These results suggested that *Andrographis paniculata* might effectuate hepatic cytochrome P450 enzymes of which CYP1A1and CYP2B are the responsive P450 isoforms (67).

The aerial parts and their constituent andrographolides have antihepatotoxic activity *in vitro* and *in vivo*. Intraperitoneal administration of a methanol extract of the aerial parts (861.3 mg/kg body weight) to mice reduced hepatotoxicity induced by carbon tetrachloride (CCl4), and reversed CCl4-induced histopathological changes in the liver (19). Intraperitoneal administration of andrographolide (100 mg/kg body weight) to mice inhibited the CCl4-induced increase in the activity of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, bilirubin and hepatic triglycerides (19). Intraperitoneal administration of a methanol extract of the aerial parts (500 mg/kg body weight) to rats also suppressed the CCl4induced increase in the activity of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase and bilirubin (70). Intragastric administration of an aqueous extract of the aerial parts (500mg/kg body weight) to ethanol-treated rats decreased the activity of serum transaminases and suppressed histopathological changes in the liver (69). Andrographolide, the major antihepatotoxic component of the plant, exerted a pronounced protective effect in rats against hepatotoxicity induced by CCl4 (65), D-galactosamine (71), paracetamol (68) and ethanol (69). Andrographolide was more effective than silymarin, the standard hepatoprotective agent (65, 68).

Effects on Central Nervous System and Brain

Many compounds do not penetrate the blood-brain barrier. However, andrographolide does so and concentrates in the brain and particularly in the spinal cord (24). Several studies have shown that AP products have a sedative effect. In mice given barbital as anesthesia, the animals became sedated more quickly and the anesthesia lasted longer. Also, it was possible to give less of the anesthesia if it was given along with AP (45). The studies indicate that AP products may act at the barbital receptors in the brain.

Effects on Respiratory System

Andrographolide has been used to treat tonsillitis, respiratory infections, and tuberculosis. In one study, AP was used to treat 129 cases of acute tonsillitis. Sixty-five percent of patients responded to the therapy. The same authors used andrographolide to treat 49 pneumonia patients. Thirty five cases were found to show positive changes and nine patients completely recovered. In another study, andrographolide was used to treat 111 patients with pneumonia and twenty with chronic bronchitis and lung infection. The overall effectiveness of AP treatment was 91%. Fever subsided within three days in 72% of the patients and 40% of these patients had smaller areas of infection within one week.

Tuberculosis is usually treated within the antibiotic rifampin. When used alone, rifampin therapy still results in 22.5% of patients dying. In a study using an injectable solution of 2.5% andrographolide given so as to provide 50 to 80 mg/kg body weight per day for two months, results were improved. Of seventy cases of tubercular meningitis, 30% of patients were considered cured with a fatality rate of 8.6%. The combination of andrographolide plus rifampin resulted in a 2.6-fold decrease in fatality rates.

Effects in Other Diseases

Leptospirosis is a disease caused by the bacterium *Leptospira interrogens*. Infection with this organism results in fever, hemorrhagic (blood-containing) lesions, central nervous system dysfunction, and jaundice. Several studies have reported efficacy in approximately 80% of patients treated with deoxyandrographolide, andrographolide, and neoandrographolide tablets. In a study reported from India, twenty cases of infective hepatitis (hepatitis A) in men and women were treated with a decoction of AP (Kalmegh) equivalent to 40 g of the crude compound for over twenty-four days. In all twenty patients, the yellowing of the conjunctiva of the eye and of the urine returned to normal coloration. Ninety percent of the patients regained their appetite and 83% had relief from general depression. Overall, 80% of the patients were considered cures and 20% improved based on biochemical tests and changes in symptoms (72). In a similar study in China, 112 cases of hepatitis were successfully treated in 83% of patients (45). Acute pyelonephritis is an inflammation of the kidney, particularly due to local bacterial infection. In a study evaluating the effectiveness of AP in treating this disease, AP was compared with nitrofurantoin, a standard clinical drug for pyelonephritis therapy. AP was found to be as effective as the standard drug, but with fewer side effects. Chorioepithelioma is a highly malignant tumor derived from the placenta. It is surrounded by "lakes" of blood. Hemorrhagic metastases develop relatively early in the course of the illness, and are frequently found in the lungs, liver, brain, vagina, and various other pelvic organs; one where a hydatidiform mole is present. AP was found to have a unique effect on these conditions. In one study, sixty patients with these conditions were treated with AP and AP-derived compounds. Forty-one of these patients had confirmed metastasis (spread of the cancer) of the lesions. Twelve patients treated with AP alone recovered. Of these patients, four women subsequently became pregnant (this condition usually results in difficulties in trying to get pregnant). Of patients treated with other drugs in addition to AP, forty-seven did not experience a re-growth of the tumor during the time of the study. In a case study of patient with an anal tumor, results were reported as "satisfactory" when the tumor was treated with a decoction of AP. In this therapy, a 500 ml. decoction was prepared from 100 g of AP and 1,000 ml water, filtering out residue, and mixing the liquid with 10 ml of vinegar. When the temperature of the liquid was below 40 degrees C., the anal tumor was treated in a sitz bath for fifteen minutes twice-daily (73). Additional diseases reported to be effectively treated by herbal combinations that include AP are Japanese B encephalitis, cervical erosion, pelvic infection (46), otitis media purulence, cutaneous gangrene in infants (74), leprosy, herpes, chicken pox, and mumps (75, 76), neurodermatitis, eczema, and burns. When cobra venom was given to mice, AP prolonged survival time and postponed the occurrence of respiratory failure caused by the venom (77). *Andrographis* has also found to have antipyretic activity when intragastric administration of an ethanol extract of the aerial parts (500mg/kg body weight) to rats decreased yeast-induced pyrexia (44). The extract was reported to be as effective as 200 mg/kg body weight of aspirin, and no toxicity was observed at doses up to 600 mg/kg body weight (44). Intragastric administration of andrographolide (100 mg/kg body weight) to mice decreased brewer's yeast-induced pyrexia (43). Intragastric administration of deoxyandrographolide, andrographolide, neoandrographolide or 11,12-didehydro-14-deoxyandrographolide (100 mg/kg body weight) to mice, rats or rabbits reduced pyrexia induced by 2,4-dinitrophenol or endotoxins (80).

Since ancient time *Andrographis* is said to have antivenom activity which was observed by intraperitoneal injection of an ethanol extract of the aerial parts (25 g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of respiratory failure and death (81). The same extract induced contractions in guinea-pig ileum at concentrations of 2 mg/ml. The contractions were enhanced by physostigmine and blocked by atropine, but were unchanged by antihistamines. These data suggest that extracts of the aerial parts do not modify the activity of the nicotinic receptors but produce significant muscarinic activity, which accounts for its antivenom effects (81).

Safety & Contraindications

In Traditional Chinese Medicine (TCM) and in systems of healing in Thailand and India, AP has long been perceived as safe. Although trial and error in humans may not be considered scientific, it is a way of determining whether a substance is effective or harmful. When scientists began to investigate the safety of AP, formal toxicological studies in animal models and in animal and human clinical trials confirmed that andrographolide and other members of this AP family of compounds have very low toxicity. In mice that received oral extracts of AP (10 g/kg body weight) once a day for seven days, could survive and none of the mice died (78). This very high amount did produce decreased activity and general lethargy. Heart, kidney, liver, and spleen were found to be normal in these animals. When 500 mg/kg of AP were given daily for ten days to mice, there was no effect on growth, appetite, or stool production. The animals were energetic and results of complete blood counts were normal. In rabbits intravenous andrographolide (10 mg/kg) when given, there were no abnormal cardiovascular responses. Liver enzyme tests and heart, liver, kidney, and spleen were normal in these animals (79). In other tests for toxicity, rats or rabbits received 1 g/kg orally of andrographolide or neoandrographolide for seven days. This amount did not affect body weight, blood counts, liver or kidney function, or other important organs (46, 61). As with all herbs, some people will have an allergic reaction to AP. The other side effect, as discussed above, is antifertility. Overall, evidence to date indicates that andrographolides are naturally occurring compounds with low toxicity when used appropriately. The use of AP has been associated with allergic reactions ranging from minor skin rashes to more serious anaphylaxis, which is a potential problem at high doses. Whether or not these reactions are due to AP *per se* or other matter in herbal preparations is not clearly understood.

CONCLUSIONS

We conclude from the vast literature study and experimental results analysis that *Andrographis paniculata*, is a traditional remedy for fever and various infections, employs various immunological applications in cancer and viral disease like HIV and others. The plant is beneficial in treating cardiovascular disease and in preventing liver toxicity thus improving functions of heart and liver. It also finds immense utility in abdominal problems, body pain, snake biting, respiratory disorders, allergic reactions, and central nervous system and brain functioning. *Andrographis* is reported to decrease fertility in both male and female animals and human being. Taking great concern of the useful benefits of the plant, it can be advocated as a safe, highly important, medicinal plant for general mankind.

REFERENCES

1. Standard of ASEAN herbal medicine. Vol. 1. 1993. Jakarta, ASEAN Countries.
2. Pharmacopoeia of the People's Republic of China. Vol. 1 (English ed.) 1997. Beijing, Chemical Industry Press.
3. Thai herbal pharmacopoeia. Vol. 1. 1995. Bangkok, Prachachon Co.
4. Manual for cultivation, production and utilization of herbal medicines in primary healthcare. Nonthaburi, Department of Medical Sciences, Ministry of Public Health (1990).
5. Medicinal plants in Viet Nam. Manila, World Health Organization (WHO Regional Publications, 1990). Western Pacific Series, No. 3.
6. P. Siripong, B. Kongkathip, K. Preechanukool, P. Picha, K. Tunsuwan, and W.C. Taylor. Cytotoxic diterpenoid constituents from *Andrographis paniculata*, Nees leaves, *J. Sci. Soc. Thailand*. **18(4)**: 187-94 (1992).
7. A. Sharma, L. Krishan, and S.S. Handa. Standardization of the Indian crude drug Kalmegh by high pressure liquid chromatographic determination of andrographolide. *Phytochem. Anal*. **3**: 129-31 (1992).
8. R. Wongkittipong, L. Prat, S. Damronglerd, C. Gourdon. Solid–liquid extraction of andrographolide from plants—experimental study, kinetic reaction and model. *Sep. Pur. Technol.* **40:**147–154 (2004).
9. T., Matsuda, M. Kuroyanagi, S. Sugiyama, K. Umehara, A. Ueno, and K. Nishi. Cell differentiation-inducing diterpenes from *Andrographis paniculata* Nees. *Chem. Pharm. Bull* (Tokyo). **42(6)**: 1216-25 (1994).
10. M.K. Reddy, M.V.B. Reddy, D. Gunasekar, M.M. Murthy, C. Caux and B. Bodo. A flavone and an unusual 23-carbon terpenoid from *Andrographis paniculata*. *Phytochem*. **62:** 1271–1275 (2003).
11. C. Weiming and L. Xiaotion. Deoxyandrographolide 19β-D-glucoside from the leaves of *A. paniculata*. *Planta Medica* **15:** 245-246 (1982).
12. S.K. Srivastava, P.C. Bose, G.K. Ray and B. Mukherjee. *Ind. J. Pharm*. **21:** 229–230 (1959).
13. S.P. Sengupta, S. Banerjee and D. Chakravarty. *Ind. J. Pharm.* **11:** 77–78 (1949).
14. P.C. Maiti, S.K. Kanji and R. Chatterjee. Studies in kalmegh extracts. *Ind. J. Pharm*. **21:** 169 (1959).
15. K.N. Gaind, R.N. Dar and R.N. Kaul. *Ind. J. Pharm*. **25:** 225–226 (1963).
16. Z. Pinye, P. Nuanbing and J. Weijie. *Chin. J. Pharm. Anal*. **4:** 34–36 (1984).
17. V. Subba Rao. *Ind. J. Pharm.* **24:** 134 (1962).
18. P.B. Talukdar, S. Banerjee, P.K. Chatterjee and A.K. Dutta. *Ind. J. Chem*. **6:** 359–363 (1968).
19. S.S. Handa and A. Sharma. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Ind. J. Med. Res.***92:** 276–283 (1990).
20. D.C. Jain, M.M. Gupta, S. Saxena and S. Kumar. LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata. J. Pharma. Biomed. Anal*. **22:** 705–709 (2000).
21. H.Y. Cheung, C.S. Cheung and C.K. Kong. Determination of bioactive diterpenoids from *Andrographis paniculata* by micellar electrokinetic chromatography. *J. Chrom. A*, **930:** 171–176 (2001).
22. L. Chen, H. Jin, L. Ding, H. Zhang, X. Wang, Z. Wang, J. Li, C. Qu, Y. Wang and H. Zhang. On-line coupling of dynamic microwave-assisted extraction with high-performance liquid chromatography for determination of andrographolide and dehydroandrographolide in *Andrographis paniculata* Nees. *J. Chrom. A*. **1140:** 71–77 (2007).
23. S. Gupta, M.A. Choudhry, J.N.S. Yadav, V. Srivastava and J.S. Tandon. Antidiarrheal activity of diterpenes of *Andrographis paniculata* (Kalmegh) against *Escherichia coli* enterotoxin in *in vivo* models. *Int. J. Crude Drug Res*. **28:** 4:273-83 (1990).
24. L. Weibo. Prospect for study on treatment of AIDS with traditional Chinese medicine. *J. Trad. Chinese Med*. **15(1):** 3-9 (1995).
25. Y.H. Wang. The pharmacology and application of traditional Chinese medicine. Beijing: People's Health Press (1983).
26. Signal Transduction Companies (editorial). *Genetic Engineering News.* **16(1):** 1 January 1996.
27. W. Tang, and G. Eisenbrandt. Chinese drugs of plant origin: Chemistry, pharmacology, and use in traditional and modern medicine (1992). New York: Springer-Verlag.
28. Z.Y. Zheng. Pharmacokinetic studies on 3H-andrographolide. *Chinese Herbal Med.* **13(9):** 33-36 (1982).
29. A. Puri, R. Saxena, R.P. Saxena, and K.C. Saxena. Immunostimulant agents from *Andrographis paniculata. J. Nat. Prod.* **56(7):** 995-99 (1993).
30. P.B. Talukdar and S. Banerjee. Studies on the stability of andrographolide. *Ind. J.Chem.* **6:** 252-54 (1968).
31. R.A. Kumar, K. Sridevi, N.V. Kumar, S. Nanduri and S. Rajagopal. Anticancer and immunostimulatory compounds from *Andrographis paniculata. J. Ethnopharm*. **92:** 291–295 (2004).
32. Y. Xu, A. Chen, S. Fry, R.A. Barrow, R.L. Marshall and T.K.S. Mukkur. Modulation of immune response in mice immunised with an inactivated Salmonella vaccine and gavaged with *Andrographis paniculata* extract or andrographolide. *Int. Immunopharmacology* (in Press) (2007).
33. H.Y. Cheung, S.H. Cheung, J. Li, C.S. Cheung, W.P. Lai, W.F. Fong and F.M. Leung. Andrographolide isolated from *Andrographis paniculata* induces cell cycle arrest and mitochondrial-mediated apoptosis in human leukemic HL-60 cells. *Planta Med*. **71:** 1106–1111 (2005).
34. 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. *Eur. J. Pharma*. **568:** 31–44 (2007).
35. Holt, M.D. Stephen, and L. Comac. Miracle Herbs: How Herbs Combine with Modern Medicine to Treat Cancer, Heart Disease, AIDS, and More (1998). Caro Publishing Group.
36. C. Calabrese, S.H. Berman, J.G. Babish, X. Ma, L. Shinto, M. Dorr, K. Wells, C.A. Wenner and L.J. Standish. A Phase I Trial of Andrographolide in HIV Positive Patients and Normal Volunteers. *Phytother. Res.* **14:** 333–338 (2000).
37. W.C. Goh, M.E. Rogel and C.M. Kinsey. HIV-1 Vpr increases viral expression by manipulation of the cell cycle: a mechanism for selection of Vpr in vivo. *Nat. Med*. **4:** 65-71 (1998).
38. R.S. Chang and H.W. Yeung Inhibition of growth of human immunodeficiency virus in vitro by crude extracts of Chinese medicinal herbs. *Antiviral Res*. **9:** 163-175 (1988).
39. X. Ma, J. Rininger, P. Chigurupati, H. Dong and J.G. Babish. Biochemical pathways of inhibiting human immunodeficiency virus-1 replication and cytopathicity by andrographolide (abstract no. T12). In Gen. Meet. Am. Soc. Microbiol., Vol. 97, pp. 538, American Society of Microbiology Miami Beach, FL (1997).
40. D.D. Caceres, J.L. Hancke, R.A. Burgos and G.K. Wikman. Prevention of common colds with *Andrographis paniculata* dried extract: A pilot double-blind trial. *Phytomedicine.* **4(2):** 101-4 (1997).
41. R.A. Burgos and D.D. Caceres. A double-blind study with a new mono drug: Kan-Jang: decrease of symptoms and enhancement of resistance in common colds. Research performed at the University of Chile, Departments of Pharmacology and School of Public Health, Santiago, Chile and funded by the Swedish Herbal Institute (August 1994).
42. W.L. Deng. Preliminary studies on the pharmacology of the Andrographis product dihydroandrographolide sodium succinate. *Newsletters of Chinese Herbal Med.* **8:** 26-28 (1978a).
43. H.C. Madav, T. Tripathi, and S.K. Mishra. Analgesic, antipyretic, and antiulcerogenic effects of andrographolide. *Ind. J. Pharm. Sci*. **57(3):** 121-25 (1995).
44. S. Vedavathy and K.N. Rao. Antipyretic activity of six indigenous medicinal plants of Tirumala Hills, Andhra Pradesh, India. *J. Ethnopharma.* **33:** 193-96 (1991).
45. W.L. Deng. Outline of current clinical and pharmacological research on *Andrographis paniculata* in China. *Newsletters of Chinese Herbal Med.* **10:** 27-31 (1978b).
46. J. Yin and L. Guo. *Contemporary Traditional Chinese Medicine*, pp. 517-524. Xi e Yuan, Beijing (1993).
47. S. Gupta *et al*. Antisecretory (antidiarrhoeal) activity of Indian medicinal plants against *Escherichia coli* enterotoxin-induced secretion in rabbit and guinea-pig ileal loop models. *Int. J. Pharma.***31:**198–204 (1993).
48. S. Gupta *et al*. Antidiarrhoeal activity of diterpenes of *Andrographis paniculata* (kalmegh) against *Escherichia coli* enterotoxin in in vivo models. *Int. J. Crude Drug Research* **28:** 273–283 (1990).
49. W-F Chiou, J-J Lin and C-F Chen. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophages and restores the vasoconstriction in rat aorta treated with lipopolysaccharide. *British J. Pharmacol.* **125:** 327–334 (1998).
50. S. Manez, J.J. Alcaraz, J. Paya, J.L. Rios and J.L. Hancke. Selected extracts from medicinal plants as anti-inflammatory agents (1990).
51. S. Habtemariam. Andrographolide Inhibits the Tumour Necrosis Factor-α-induced Upregulation of ICAM-1 Expression and Endothelial-monocyte Adhesion. *Phyto. Res.* **12:** 37–40 (1998).
52. 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 J. Pharmacol.* **135:** 399-406 (2002).
53. P. Misra, N.L. Pal, P.Y. Guru, J.C. Katiyar, V. Srivastava and J.S. Tandon. Antimalarial activity of *Andrographis paniculata* (Kalmegh) against *Plasmodim berghei* NK 65 in Mastomys natalensis. *Int. J. Pharmacog*. **30(4):** 263-74 (1992).

54. P.K. Singha, S. Roy and S. Dey. Antimicrobial activity of *Andrographis paniculata. Fitoterapia* **74:** 692–694 (2003).
55. V.K. Dua, V.P. Ojha, R. Roy, B.C. Joshi, N. Valecha, C. Usha Devi, M.C. Bhatnagar, V.P. Sharma and S.K. Subbarao. Anti-malarial activity of some xanthones isolated from the roots of *Andrographis paniculata. J. Ethnopharmacol*. **95:** 247–251 (2004).
56. D. Wang, and H. Zhao. Experimental studies on prevention of atherosclerotic arterial stenosis and restenosis after angioplasty with *Andrographis paniculata* Nees and fish oil. *J. Tongji Medical University* **13(4):** 193-98 (1993).
57. H. Zhao, and W. Fang. Protective effects of *Andrographis paniculata* Nees on post-infarction myocardium in experimental dogs. *J. Tongji Medical University* **10(4):** 212-17 (1990).
58. L.Y. Huang. The effects of andrographolides on experimental blood deficiency of cardiac muscle. *Chinese Herbal Med*. **18(7):** 26-28 (1987).
59. C.Y. Zhang and B.K.H. Tan. Mechanisms of cardiovascular activity of *Andrographis paniculata* in the anaesthetized rat. *J. Ethnopharmacol.* **56:** 97-101 (1997).
60. K. Sheeja, C. Guruvayoorappan and G. Kuttan. Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. *Int. Immunopharmacol*. **7:** 211–221 (2007).
61. M.A. Akbarsha, B. Manivanan, K.S. Hamid and B. Vijayan. Antifertility effect of *Andrographis paniculata* (Nees) in male albino rat. *Ind. J. Exp. Biol*. **28:** 421-26 (1990).
62. M.S. Zoha, A.H. Hussain, and S.A. Choudhury. Antifertility effects of *Andrographis paniculata* in mice. *Bangladesh Med. Res. Council Bull.* **15:** 34-37 (1989).
63. H.M. Chang. P.P.H. But, eds. Pharmacology and applications of Chinese materia medica. Vol. 1. Singapore, World Scientific 918–928 (1986).
64. C. Chaichantipyuth, B. Thanagkul. *Andrographis paniculata* Nees as antidiarrhoeal and antidysentery drug in Thailand. *Asian J. Pharmacy*. **6(Suppl.):** 59–60 (1986).
65. B. Thanagkul, C. Chaichantipayut. Double-blind study of *Andrographis paniculata* Nees and tetracycline in acute diarrhoea and bacillary dysentery. *Ramathibodi Medical J.* **8:** 57–61 (1985).
66. A. Kapil, I.B. Koul, S.K. Banerjee and B.D. Gupta. Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata. Biochem. Pharmacol*. **46(1):**182-85 (1993).
67. B. Shukla, P.K.S. Visen, G.K. Patnaik and B.N. Dhawan. Choleretic effect of andrographolide in rats and guinea pigs. *Planta Med*. **58:** 146-48 (1992).
68. K. Jarukamjorn, K. Don-in, C. Makejaruskul, T. Laha, S. Daodee, P. Pearaksa, B. Sripanidkulchai. Impact of *Andrographis paniculata* crude extract on mouse hepatic cytochrome P450 enzymes. *J. Ethnopharmacol*. **105:** 464–467 (2006).
69. P.K.S. Visen *et al*. Andrographolide protects rat hepatocytes against paracetamolinduced damage. *J. Ethnopharmacol.* **40:** 131–136 (1993).
70. P. Pramyothin *et al*. Hepatoprotective effect of *Andrographis paniculata* and its constituent, andrographolide, on ethanol hepatotoxicity in rats. *Asia Pacific J. Pharmacol.* **9:** 73–78 (1993).
71. A. Sharma *et al*. Antihepatotoxic activity of some plants used in herbal formulations. *Fitoterapia* **22:** 131–138 (1991).
72. B. Saraswat *et al*. Effect of andrographolide against galactosamine-induced hepatotoxicity. *Fitoterapia*. **66:** 415 (1995).
73. G.N. Chaturvedi, G.S. Tomar, S.K. Tiwari and K.P. Singh. Clinical studies on Kalmegh *(Andrographis paniculata* Nees) in infective hepatitis. *J. Int. Instt. Ayurveda* **2:** 208-11 (1983).
74. S.J. Hueng. Treating anal tumor by washing using *Andrographis paniculata* extractions plus vinegar. *Chinese J. Anal. Intest. Dis*. **11(2):** 40 (1991).
75. W.C. Qi. Investigations of forty-five cases of infant cutaneous gangrene treated by Yi-Jian-Xi cream. *Trad. Chinese Med. in Fujian* **4:** 32 (1965).
76. Q.Z. Huang. Treating herpes using *Andrographis paniculata* products. *Guangxi Health* **5:** 43 (1974).
77. Q.Z. Huang. Treating herpes, chicken pox, mumps, and neurodermatitis using *Andrographis paniculata* products. *J. Barefoot Doctor Guangxi* **9:** 21 (1978).
78. T.K. Huang. Handbook of compositions and pharmacology of traditional Chinese medicine (1994) Beijing: China Medical and Technology Press.
79. Y. Chung. *Andrographis paniculata.* Handbook of traditional Chinese medicine (1979). Guangzhou.
80. S.Y. Guo, D.Z. Li, W.S. Li, A.H. Fu, and L.F. Zhang. Study of the toxicity of andrographolide in rabbits. *J. Beijing Med. Univ*. **5:** 422-28 (1988).
81. W. Deng. Comparison of pharmacological effect of four andrographolides. *Chinese Pharm. Bull*. **17:** 195–198 (1982).
82. S.K. Nazimudeen *et al*. Effect of *Andrographis paniculata* on snake venom-induced death and its mechanism. *Ind. J. Pharma. Sci.* **40:** 132–134 (1978).
83. P. Thisoda, N. Rangkadilok, N. Pholphana, L. Worasuttayangkurn, S. Ruchirawat and J. Satayavivad. Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. *Eur. J. Pharmacol*. **553:** 39–45 (2006).