EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Andrographis paniculata* Nees, folium

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Draft

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Andrographis paniculata* Nees, folium
Herbal preparation(s)	
Pharmaceutical forms	
Rapporteur	
Assessor(s)	

Note: This Assessment Report is published to support the release for public consultation of the draft public statement on Andrographis paniculata, folium. It should be noted that this document is a working document, not yet edited, and which shall be further developed after the release for consultation of the public statement. Interested parties are welcome to submit comments to the HMPC secretariat, which the Rapporteur and the MLWP will take into consideration but no 'overview of comments received during the public consultation' will be prepared in relation to the comments that will be received on this assessment report. The publication of this draft assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft public statement.

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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

  Herba Andrographidis consists of the dried aerial parts of *Andrographis paniculata* Nees (Acanthaceae) (WHO monograph 2004,Pharmacopoeia of the People's Republic of China 2005). For Andrographis paniculata, folium no monograph is available.

- Herbal preparation(s)

Special *Andrographis* extracts applied in preclinical and clinical studies:
KalmCold™

The description of the extract preparation for the preparation KalmCold® was given (Saxena et al 2010): 300 kg of the ground ***leaves*** of *Andrographis paniculata* were extracted in a reflux process with 1200 L of methanol over 3 hours. Later on, 1000 L of methanol were added to the extracted drug and the extraction procedure was repeated twice. Then, the extract was concentrated up to a solid content of 40-50% (w/w) and further dried at ≤ 65°C. After milling and sieving, a final weight of 18 kg of powdered extract was achieved. The extracted herb was still further extracted by water which was then spray dried. Both fractions were determined and finally blended and filled into capsules to achieve the medicine.

Paractin®

Each tablet contains 30 mg andrographolide as *A. paniculata* extract. The extract was obtained from ***leaves and aerial parts*** of *A. paniculata.* The solvent used in the extract was alcohol (75% ethanol), and the ratio of herbal drug to extract was 10:1 (Burgos et al. 2009).

HMPL-004

HMPL-004 is obtained from the ethanol / water (90 /10 v/v) extracts of the *A. paniculata* ***leaves***. One capsule contains 200 mg *Andrographis paniculata* extract with 8-10% andrographolide content (Tang et al. 2011).

Combination products containing *Andrographis* extract:

Kan Jang
One capsule comprises 85 mg of the standardized extract of *Andrographis paniculata* **SHA-10** equivalent to 5 mg of andrographolides, as well as 10 mg extract of *Eleutherococcus senticosus* per tablet (Gabrielian et al. 2002, Spasov et al. 2004, Melchior et al. 2000).

ImmunoGuard®

ImmunoGuard®, produced by the Swedish Herbal Institute (SHI), is a combination of *Andrographis paniculata*, *Eleutherococcus senticosus, Schizandra chinensis* and *Glycyrrhiza glabra*. The test medication ImmunoGuard® Clinical A comprised sugar-coated tablets, containing 370 mg of a fixed combination of 50 mg special extract of *Andrographis paniculata* Nees standardized to the content of andrographolide of 4 mg besides the other components.

), A herbal extract prepared by boiling the complete herbal material in a proportion of 1:2, concentration of the liquid, and addition of different further constituents, like 2 % of fennel oil, 2 % of ajowa oil, and 55- 60 % ethanol. The extract is standardized to a content of 0.5 % of andrographolides. Hagers Handbuch der Pharmazeutischen Praxis (1972),

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not relevant.

1.2. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	the plant material is included in food supplements
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered product on the market
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product on the market (only one combination product containing the *herba*)
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal product on the market
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No single-component or multicomponent product on the market with German Standard Marketing Authorisations
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No single-component product on the market (only 2 multicomponent registered products)
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No registered product on the market
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal product on the market
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No single-compound product on the market (only in multicomponent food supplements)
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No THMP or WEU product on the market
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No authorized or registered medicinal product on the market
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No product on the market
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No HMP/THMP product on the market
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

PubMed and Web of Knowledge were assessed udsing the term "Andrographis" in April 2012. Literature provided by AESGP was also used

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

The leaves and aerial parts of *Andrographis paniculata* have been used in traditional systems of Asian medicine for the treatment of different disorders (see section 1.5.). *A. paniculata* was described in "Indigenous drugs of India", a book published in 1896 (Dey & Mair 1896). Fresh and dried leaves, and the juice extracted from this herb are described as official drugs in the Indian Pharmacopoeia (Muniramappa et al. 1997).

However, there is no monocomponent herbal preparation for which 15 years in Europe could be confirmed from the literature or based on the regulatory status overview.

2.2. Information on traditional/current indications and specified substances/preparations

The monograph of the WHO ***Herba*** *Andrographidis* (2004) lists the following medicinal uses:

- Uses supported by clinical data

Prophylaxis and symptomatic treatment of upper respiratory infections, such as the common cold, and uncomplicated sinusitis, bronchitis, and pharyngotonsillitis, lower urinary tract infections, and acute diarrhea.

- Uses described in pharmacopoeias

  Treatment of bacillary dysentery, bronchitis, carbuncles, colitis, coughs, dyspepsia, fevers, hepatitis, malaria, mouth ulcers, sores, tuberculosis and venomous snake bites.

- Uses described in folk medicine, not supported by experimental data

  Treatment of colic, otitis media, vaginitis, pelvic inflammatory disease, chickenpox, eczema and burns.

Andrographis paniculata is well known in Thailand as Fa-Tha-Lai-Jone (Thamlikitkul et al. 1991), as Kalmegh in ayurvedic medicine, especially as a predominant constituent of at least 26 Ayurvedic formulations used to treat liver disorders (Naik et al. 2009, Ganguly et al. 2000, Varma et al. 2011), and has been used as a remedy in Thai traditional medicines for decades (Thamlikitkul et al. 1991) and in general in Asia for centuries (Naik et al. 2009, Poolsup et al. 2004). Is also well known as Chiretta or King of Bitters and has been widely used in India, China and Thailand (Poolsup et al. 2004, Saxena et al. 2010). A long history for the medical use of *Andrographis paniculata* against sore throat has been well known in Thailand (Poolsup et al. 2004). In ancient ayurvedic literature, *Andrographis paniculata* has also been mentioned as a herb for the treatment of neoplasm (Varma et al. 2011).

The traditional indications comprise of the support of the healthy function of the upper respiratory tract, similar to prophylactic and symptomatic treatment of upper respiratory infections such as common cold, sinusitis, bronchitis, pharyngotonsillitis, pneumonia, whooping cough, otitis media, nephritis, lower urinary tract infections and acute diarrhoea or enteritis, tuberculosis, dermatitis, but also the removal of toxins (Ganguly et al. 2000, Panossian et al. 2002, Pharmacopoeia of the People's Republic of China 2005). A decoction of the plant is used as a blood purifier, and as a "cold property" in the traditional Chinese medicine in order to treat body heat, get rid of fever, and dispel toxins from the body (Avani et al. 2008, Pharmacopoeia of the People's Republic of China, 2005). Its ethnomedical use included antipyretic, antidiarrhoeal, tonic, and anti-inflammatory treatments (Thamlikitkul et al. 1991).

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

Assessor's conclusion: based on the market and literature overview, the requirement laid down in Article 16a(1)(d) of Directive 2001/83/EC that "the period of traditional use as laid down on Article 16c(1)(c) has elapsed", is not fulfilled.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

In vitro and *ex vivo* studies

Antiviral effect

Andrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide, ent-labdene diterpenes isolated from Andrographis paniculata showed viricidal activity against herpes simplex virus

I (HSV-1). None of these compounds exhibited significant cytotoxicity at viricidal concentrations (Wiart et al. 2005).

25 µ g/ml of a ethanolic extract from *A. paniculata* and 5 µ g/ml of andrographolide inhibited the expression of Epstein-Barr virus (EBV) lytic proteins, Rta, Zta and EA-D, during the viral lytic cycle in P3HR1 cells. Transient transfection analysis revealed that the lack of expression of Rta, Zta and EA-D is caused by the inhibition of the transcription of BRLF1 and BZLF1, two EBV immediatearly genes that encode Rta and Zta, respectively. The production of mature viral particles was inhibited. Andrographolide was not toxic to P3HR1 cells at the concentrations is 5 µ g/ml (Lin et al. 2008).

14-deoxy-11,12-didehydroandrographolide and 14-deoxyandrographolide, two natural occurring anfrographolide derivatives and eight semisynthetic analogues, modified at the three free OHs of andrographolide, were explored for their anti-HSV-1 activities. The concentrations that produced 80% viable cells were used to test for both pre- and postinfections by using cytopathic effect reduction assays on Vero cell cultures. Three analogues, 14-acetyl-3,19-isopropylideneandrographolide, 14-acetylandrographolide, and 3,14,19-triacetylandrographolide, significantly exhibited preinfection step activity against the virus. For postinfection activity, only 3,19-isopropylideneandrographolide showed absolute inhibition of HSV-1 replication. Meanwhile, andrographolide exhibited slight inhibitory activities of 34.48 +/- 6.93% and 56.90 +/- 2.65% against HSV-1 for pre- and postinfection, respectively. The results confirmed(Aromdee et al. 2011) that the three hydroxyl moieties play a role in the anti-HSV-1 activity of andrographolide. From the study, it can be concluded that 14-acetyl analogues block the viral entry, and 3,19-isopropylideneandrographolide, a cyclic dioxane analogue, exhibits postinfection anti-HSV-1 activity .

Antimicrobial effect

Non-polar (dichloromethane) and polar (MeOH and aqueous) extracts of *Andrographis paniculata* (whole plant) were evaluated for *in vitro* antibacterial activity against 10 skin disease causing bacterial strains (6 gram positive strains; *Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus*) and 4 gram negative strains (*Proteus mirabilis, Proteus vulgaris, Neisseria meningitis, Pseudomonas aeruginosa*) using disc diffusion method at three different concentrations; 1000, 500 and 250 µ g/disc respectively. The extracts showed significant antibacterial activities against both Gram-positive and Gram-negative bacterial strains tested. Highest significant antibacterial activity was exerted by the aqueous extract against *M. luteus* at 1000 µ g/disc and the lowest activity was exhibited by the DCM extract against *N. meningitis* at 250 µ g/disc. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) observed were between 150 to 300 µg/ml and 250 to 400 µ g/ml respectively, depending on microorganism and the type of extract. Time-kill experiments indicated that *A. paniculata* extracts have bactericidal characteristic against most of the Gram positive bacteria and bacteriostatic activity against both Gram negative and Gram positive bacteria (Sule et al. 2011).

The antimicrobial activity of aqueous extract, andrographolides and arabinogalactan proteins from *Andrographis paniculata* were evaluated. The aqueous extract showed significant antimicrobial activity against B*acillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans*, which may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides (Singha et al. 2003).

Four xanthones isolated from the roots of *Andrographis paniculata* were tested in vitro for antiprotozoal activity against *Trypanosoma brucei brucei, Trypanosonia cruzi and Leishmania infantum*. Compound TDR13011 (1,2-dihydroxy-6,8-dimethoxy-xanthone) showed good activity against T. b. brucei and L. infantum with a 50% inhibitory concentration of 4.6 µ g/ml and 8 µ g/ml respectively(Dua et al. 2009).

An ethanol extract of the aerial part of *Andrographis paniculata* was evaluated for antimicrobial activity against eleven bacterial strains by determining minimum inhibitory concentration and zone of inhibition. Minimum inhibitory concentration values were compared with control and zone of inhibition values were compared with standard ciprofloxacin in concentration 100 and 200 mu g/ml. The results revealed that, the ethanol extract (100-1000 µl/disc) is potent in inhibiting bacterial growth of both Gram-negative and Gram positive bacteria (similar inhibition zones in case of higher concentrations) (Mishra et al. 2009).

The study of Murugan et al. reported the antibiofilm activity of different extracts of *Andrographis paniculata* against biofilm forming cystic fibrosis (CF) causative *Pseudomonas aeruginosa* isolated from CF sputum. *P. aeruginosa* was also assessed for their growth and development of the biofilm, phylogenetic relationship and antibiotic susceptibility. Antibiogram of the strains indicated that they were resistant to more than one antibiotic. Six extracts of *A. paniculata* showed significant antibiofilm activity. *P. aeruginosa* strains, KMS P03 and KMS P05, were found to be maximally inhibited by the methanol extract to an extent of 88.6 and 87.5% respectively (Murugan et al. 2011).

Antimalarial effect

Andrographis paniculata was selected for the study based on its ethnomedicinal use. It was screened for anti-malarial activity towards *Plasmodium falciparum* in vitro using the lactate dehydrogenase (LDH) assay. The crude extract of Andrographis exhibited moderate schizonticidal activity (Najila et al. 2002).

The ethanol and methanol extracts of *Andrographis paniculata* were evaluated for their effects on growth, development and reproduction of malarial vector *Anopheles stephensi* Liston. After 8 days of treatment, 88.60 and 85.25% of the larvae treated at 35p.p.m. failed to emerge in ethanol and methanol extracts respectively. In addition, the duration of larval instars and the total development time were prolonged, while female longevity and fecundity were markedly decreased. The suppression of pupation and adult emergence was probably due to juvenile hormone analog similarities in combination with growth regulators and toxicity, which reduced the overall performance of the malaria vector *An. stephensi* (Kuppusamy and Murugan 2010).

Deltamethrin (a synthetic pyrethroid) and different solvent extracts of *Andrographis paniculata* were evaluated under laboratory conditions for larvicidal activity against the malarial vector *Anopheles stephensi* Liston. Ethanolic extract with lethal concentration LC_{50} and LC_{90} of 35.47and 47.43 after 24h and LC_{50} 25.22 and LC_{90} 46.32 ppm after 48h, respectively was found to be the most effective, followed by Acetone, Methanol, Chloroform, Hexane, Petroleum ether and Benzene extracts. LC_{50} and LC_{90} for Deltamethrin were 0.0036 and 0.0097 ppm after 24h and 0.0055 and 0.0085 ppm respectively after 48h of exposure respectively. Combined formulations were evaluated for synergistic activity and a 1:4 ratio of Deltamethrin and Ethanolic extract was observed to be more effective than 1:2 and 1:1 ratios. Combinations *of Andrographis paniculata* extracts with Deltamethrin demonstrated higher larvicidal activity, indicating synergistic activity (Chenniappan and Kadarkarai 2008).

Bacillus thuringiensis var *israelensis* (Bti) and ethanolic extracts (Ee) of *Andrographis paniculata* exhibited both larvicidal as well as pupicidal activity against *Anopheles stephensi* Liston. Ee extract with lethal concentrations (LC_{50} and LC_{90}) values of 36.56 and 53.04 p. p. m. after 24 h and LC_{50} 22.28 and LC_{90} 51.26 p. p. m. after 48h for fourth instar larvae, respectively. LC_{50} and LC_{90} for Bti were 0.0967 and 0.1003 p.p.m after 24h and 0.2944 and 0.4158 p. p. m. after 48 h of exposure for fourth instar larvae, respectively. Combined formulations were evaluated for synergistic activity and a 1:4 ratio of Bti and Ee was observed to be more effective than 1:2 and 1:1 ratios. However the larvicicdal activity is minimal when the mixed formulations contained an equal amount of both constituents (i. e a 1: 1 ratio). A ratio of 1: 4 of Bti and Ee of *A. paniculata* was 51.6 fold more toxic at the LC_{90} for

larvicidal activity, than Bti alone and this high level of activity resulted from synergism between the Bti and Ee of *A. paniculata* Nees (Chenniappan et al. 2011).

Antioxidant effect

A methanolic extract of *Andrographis paniculata* was found to inhibit formation of oxygen derived free radicals such as superoxide (32%) hydroxyl radicals (80%) lipid peroxidation (80%) and nitric oxide (42.8%) in *in vitro* system. In vivo studies using BALB/c mice models also showed significant inhibition in PMA induced superoxide (32.4%) and nitric oxide (65.3%) formation (Sheeja et al. 2006).

Using neonatal rat cardiomyocytes, the cardioprotective actions of several diterpene lactones derived from *A. paniculata* including andrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, and sodium 14-deoxyandrographolide-12-sulfonate were investigated. Pretreatment with andrographolide but not with the other compounds protected the cardiomyocytes against hypoxia/reoxygenation injury and up-regulated the cellular-reduced glutathione (GSH) level and antioxidant enzyme activities. The cardioprotective action of andrographolide was found to coincide in a time-dependent manner with the up-regulation of GSH, indicating the important role of GSH. The cardioprotective action of andrographolide was also completely abolished by buthionine sulfoximine, which acts as a specific gamma-glutamate cysteine ligase (GCL) inhibitor to deplete cellular GSH level. Subsequently, it was found that the mRNA and protein levels of the GCL catalytic subunit (GCLC) and modifier subunit (GCLM) were up-regulated by andrographolide. Luciferase reporter assay also demonstrated that andrographolide activated both the GCLC and the GCLM promoters in the transfected rat H9C2 cardiomyocyte cell line. The 12-O-tetradecanoylphorbo-13-acetate response element or the antioxidant response element may be involved in the transactivating actions of andrographolide on the GCLC and GCLM promoters. The present study pinpoints andrographolide as a cardioprotective principle in *A. paniculata* and reveals its cytoprotective mechanism (Woo et al. 2008).

Aqueous extract of *Andrographis paniculata* (Ap) was examined for antioxidant activity using rat liver subcellular organelles as model systems. The study deals with two important biological oxidative agents, ascorbate-Fe+2 and AAPH generating hydroxyl and peroxyl radical, respectively. Oxidative damage was examined against the inhibition of membrane peroxidation, protein oxidation and restoration in decreased SOD and catalase activity. The antimutagenic activity of Ap was examined following inhibition in AAPH induced strand breaks in plasmid pBR322 DNA. Extract (5-200 µg/ml) was a potent scavenger of DPPH, ABTS radicals, exemplified by ESR signals, O-2(-), OH and H2O2, displayed excellent reducing power, FRAP potentials to reduce Fe (III) -> Fe (II) and had considerable amount of phenolics/ flavonoids contents, an effective antioxidant index. The observed antioxidant effect might be primarily due to its high scavenging ability for ROS. Effect was confirmed ex vivo following inhibition in peroxidation, restoration in SOD enzyme, SOD band intensity and protein degradation in Ap fed liver homogenate. Based on these results, it was concluded that the aqueous extract of *Andrographis paniculata* might is as a potent antiradical agent against various pathophysiological oxidants (Tripathi and Kamat 2007).

In vitro antioxidant studies using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed that compared to aqueous extracts, ethanol extracts of *A. paniculata* leaves have superior free radical scavenging activity with IC(50) value = 10.9 coma and with IC(50) value = 24.65, respectively (Wasman et al. 2011).

Immunomodulatory effect

EtOH extract and purified diterpene andrographolides of *Andrographis paniculata* induced significant stimulation of antibody and delayed type hypersensitivity response to sheep red blood cells (SRBC) in mice. The plant preparations also stimulated nonspecific immune response of the animals measured in

terms of macrophage migration index (MMI) phagocytosis of C-14-leucine labelled Escherichia coli and proliferation of splenic lymphocytes. The stimulation of both antigen specific and nonspecific immune response was lower with andrographolide than with the EtOH extract, suggesting thereby that substance(s) other than andrographolide present in the extract may also be contributing towards immunostimulation (Puri et al. 1993).

The immunomodulatory properties of a diterpene lactone andrographolide and Kan Jang - a standardized fixed combination of *Andrographis paniculata* extract SHA-10 and Eleutherococcus senticosus extract SHE-3 were investigated. Their role on spontaneous and phytohemagglutinin (PHA)-induced proliferation of human peripheral blood lymphocytes (PBL) and on production of interferon-gamma (INF-gamma) and tumor necrosis factor-alpha (TNF-alpha) were determined *in vitro*. Proliferation of PBL induced by PHA was enhanced by co stimulation with andrographolide and Kan Jang. Andrographolide and Kan Jang also inhibited spontaneous proliferation of PBL in vitro. These preparations also have effect on the formation of INF-gamma, TNF-alpha and some immune activation markers such as neopterin (Neo), beta-2-microglobulin (beta2MG), and soluble receptor for interleukin-2 (sIL-2R or sCD25) in blood cells culture. Andrographolide and Kan Jang stimulate the INF-gamma, Neopterin and beta2MG formation, but do not have an effect on the production of INF-gamma and Neopterin in PHA stimulated blood cells. An opposite effect on these immune makers was observed in the PHA-stimulated blood cells: both andrographotide and Kan Jang increase the formation of TNF-alpha and beta2MG in cultivated whole blood cells (Panossian et al. 2002).

Anti-inflammatory effect

The anti-inflammatory activity of chloroform extract of *Andrographis paniculata* stem was determined using a carrageenan induced rat hind paw oedema model for acute inflammation. Ibuprofen was used as a standard drug in this study. The chloroform extract of *Andrographis paniculata* stem showed statistically significant effect in 6(th) hour at a dose of 200 mg/kg and the results were comparable with the standard anti-inflammatory drug Ibuprofen (10 mg/kg) ($t=64.06$, $p<0.001$) (Radhika et al. 2009).

The anti-inflammatory effect of diterpenoids isolated from *Andrographis paniculata*, including dehydroandrographolide (AP1), andrographolide (AP2) and neoandrographolide (AP3), on the production of inflammatory cytokines and COX activities were studied in in an *in vitro* experiment. In addtition, the alteration of gene expression involved in this activity was investigated for the most potent compound in order to elucidate the other possible molecular mechanisms. AP1 (30.1 µ M; 10 µg/ml) and AP2 (28.5 µM; 10 µg/ml) inhibited COX-1 in ionophore A23187-induced human platelets. AP2 (28.5 µM) and AP3 (20.8 mµM; 10 µg/ml) suppressed the LPS-stimulated COX-2 activity in human blood. In addition, AP2 modulated the level of LPS-induced TNF-alpha, IL-6, IL-beta and IL-10 secretion in human blood in a concentration-dependent manner. The results revealed that AP2 exhibited the highest efficacy. Therefore, changes in the levels of mRNA transcripts by AP2 were further investigated using human cDNA microarrays. The molecular response to AP2 was complex and mediated by various processes. Among the altered gene expressions, the genes involved in immune and inflammation processes were selectively down-regulated, such as cytokines and cytokine receptors (TNFSF14, TNF, TNFRSF6, and IL1A), chemokines (CCL8 and CXCL11), JAK/STAT signaling (JAK3 and STAT5A), TLRs family (TLR4 and TLR8) and NF-kappa B (NFKB1). Expression of some genes was validated using RT-PCR. The results demonstrated that AP1, AP2 and AP3 ha a anti-inflammatory effect by interfering COX and inflammatory cytokines and the underlying mechanisms of AP2 may be related to down-expression of genes involved in inflammatory cascade (Parichatikanond et al. 2010).

The suppressive effects of *Andrographis paniculata* on nitric oxide (NO) production in mouse peritoneal macrophages elicited by bacillus Calmette-Guein (BCG) and, stimulated by lipopolysaccharide (LPS) was investigated *in vitro* and *ex vivo*. Incubation of BCG-induced macrophages with the methanol

extract of *A. paniculata* reduced LPS stimulated NO production. The diterpene lactones andrographolide and neoandrographolide were isolated as active components from the extract. These compounds suppressed NO production in a concentration-dependent manner in the concentration range from 0.1 to 100 µM and their IC_{50} values were 7.9 and 35.5 µM. Neoandrographollide also suppressed NO production by 35 and 40% when the macrophages were collected after oral administration of neoandrographolide at doses of 5 and 25 mg/kg/d and LPS stimulated NO production was examined. However, andrographolide did not reduce NO production on oral administration at the same doses. These results indicate that neoandrographolide, which inhibited NO production both in vitro and ex vivo may play an important role in the use of *A. paniculata* as an anti-inflammatory crude drug (Batkhuu et al. 2002).

In the study of Chiou et al., the effect of andrographolide on the expression of inducible NO synthase (iNOS) mRNA, protein, and enzyme activity in RAW 264.7 macrophages stimulated with lipopolysaccharide (LPS) plus interferon-gamma (IFN-gamma) was investigated *in vitro*. RAW 264.7 cells stimulated with LPS/IFN-gamma activated NO production; in this condition andrographolide (1-100 µM) inhibited NO production in a dose-dependent manner with an IC_{50} value of 17.4 +/- 1.1 µ M Andrographolide also reduces the expression of iNOS protein level but without a significant effect on iNOS mRNA. The reduction of iNOS activity is thought to be caused by decreased expression of iNOS protein. In a protein stability assay, andrographolide moderately but significantly reduced the amount of iNOS protein as suggested by accelerating degradation. Furthermore, andrographolide also inhibited total protein de novo synthesis as demonstrated by [S-35]-methionine incorporation. As a whole, these data suggest that andrographolide inhibits NO synthesis in RAW 264.7 cells by reducing the expression of iNOS protein and the reduction could occur through two additional mechanisms: prevention of the de novo protein synthesis and decreasing the protein stability via a post-transcriptional mechanism. It is also possible that inhibition of iNOS protein expression and NO production under immune stimulation and/or bacteria infection may explain, in part, the anti-inflammatory activity of andrographolide (Chiou et al. 2000).

To elucidate the possible mechanism(s) underlying the andrographolides inflammatory effect responses by rat neutrophils, N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced adhesion and transmigration of isolated peripheral human neutrophils were studied. Pretreatment with andrographolide (0.1 - 10 µM) concentration-dependently prevented fMLP-induced neutrophil adhesion and transmigration. Andrographolidepretreatment also significantly decreased fMLP-induced up-expression of both CD11b and CD18. Accumulation of reactive oxygen species (ROS) as well as quick intracellular calcium ($[Ca^{2+}](i)$) mobilization induced by fMLP displays two important signalling pathways in regulating the up-expression of Mac-1 by neutrophils. That andrographolidepretreatment diminished fMLP-induced production of H_2O_2 and O-2(-), but failed to block that of $[Ca^{2+}](i)$ mobilization suggested that the ROS but not [Ca++](i) signalling could be modulated by andrographolide. To clarify whether ROS production impeded by andrographolidecould be an antagonism of fMLP binding, phorbol-12-myristate-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 andrographolide. This indicated that a PKC-dependent mechanism might be interfered by andrographolide. It was concluded that the prevention of ROS production through, at least in part, modulation of PKC-dependent pathway could confer andrographolidethe ability to down-regulate Mac-1 up-expression that is essential for neutrophil adhesion and transmigration (Shen et al. 2002).

Incubation of endothelial cells with non-toxic concentrations (0.16-16.7 mu g/mL) of andrographolide attenuated the tumour necrosis factor-alpha(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 antiinflammatory activity (Habtemariam 1998).

In an *in vitro* study, andrographolide decreased tumor necrosis factor-alpha (TNF-alpha)-induced intercellular adhesion molecule-1 (ICAM-1) expression and adhesion of HL-60 cells onto human umbilical vein endothelial cells (HUVEC), which are associated with inflammatory diseases. Moreover, 1 abolished TNF-alpha-induced Akt phosphorylation. Transfection of an activated Akt1 cDNA vector increased Akt phosphorylation and ICAM-1 expression like TNF-alpha. In addition, 1 and LY294002 blocked TNF-alpha-induced IKB-alpha degradation and nuclear p65 protein accumulation, as well as the DNA-binding activity of NF-kappa 13. Andrographolide exhibits anti-inflammatory properties through the inhibition of TNF-alpha-induced ICAM-1 expression. The anti-inflammatory activity of andrographolide may be associated with the inhibition of the PI3K/Akt pathway and downstream target NF-kappa B activation in HUVEC cells (Chen et al. 2011).

Liu et al. investigated the effect of andrograpanin on the production of nitric oxide (NO) and pro-inflammatory cytokines (TNF-alpha, IL-6 and IL-12p70) and the key signaling pathways involved in lipopolysaccharide (LPS)-activated macrophage cells. They observed that NO and all three pro-inflammatory cytokines were inhibited by andrograpanin (15-90 mu M) in a dose-dependent manner. Andrograpanin inhibited productions of NO and pro-inflammatory cytokines through down-regulating iNOS and pro-inflammatory cytokines gene expression levels. Further studies suggested that down-regulation of p38 mitogen-activated protein kinase (MAPKs) signaling pathways were involved in the anti-inflammatory activities of andrograpanin (Liu et al. 2008).

Andrographolide dose-dependently inhibited OVA-induced increases in total cell count, eosinophil count, and IL-4, IL-5, and IL-13 levels recovered in bronchoalveolar lavage fluid, and reduced serum level of OVA-specific IgE in an animal experimant on BALB/c mice sensitized and challenged with ovalbumin (OVA) after developing airway inflammation. It attenuated OVA-induced lung tissue eosinophilia and airway mucus production, mRNA expression of E-selectin, chitinases, Muc5ac, and inducible nitric oxide synthase in lung tissues, and airway hyperresponsiveness to methacholine. In normal human bronchial epithelial cells, andrographolicle blocked tumor necrosis factor-alpha-induced phosphorylation of inhibitory kappa B kinase-beta, and downstream inhibitory kappa B alpha degradation, p65 subunit of NF-kappa B phosphorylation, and p65 nuclear translocation and DNA-binding activity. Similarly, andrographolicle blocked p65 nuclear translocation and DNA-binding activity in the nuclear extracts from lung tissues of OVA-challenged mice. These findings implicate a potential therapeutic value of andrographolide in the treatment of asthma and it may act by inhibiting the NF-kappa B pathway at the level of inhibitory kappa B kinase-beta activation (Bao et al. 2009).

14-Deoxy-11,12-didehydroandrographolide is an analogue of andrographolide that can be isolated from *A. paniculata*. In contrast to andrographolide, 14-deoxy-11,12-didehydroandrographolide did not elicit any cytotoxic activity in A549 and BEAS-2B human lung epithelial cells and rat basophilic leukemia (RBL)-2H3 cells using a MTS assay. 14-Deoxy-11,12-didehydroandrographolide dose-dependently inhibited ovalbumin (OVA)-induced increases in total and eosinophil counts, IL-4, IL-5, and LL-13 levels in lavage fluid, and serum OVA-specific IgE level in a mouse asthma model, attenuated OVA-induced airway eosinophilia, mucus production, mast cell degranulation, pro-inflammatory biomarker expression in lung tissues, and airway hyperresponsiveness. This substance also blocked p65 nuclear translocation and DNA-binding activity in the OVA-challenged lung and in TNF-alpha-stimulated human lung epithelial cells (Guan et al. 2011).

The study of Chao et al. investigated the effect of andrographolide on the IKK/NF-kappa B signaling pathway, which mediates TNF-alpha-induced ICAM-1 expression in EA.hy926 cells. Andrographolide inhibited TNT-alpha-induced ICAM-1 mRNA and protein levels, its expression on the cell surface, and subsequent adhesion of HL-60 cells to EA.hy926 cells. Andrographolide inhibited TNF-alpha-induced

kappa B inhibitor (I kappa B) kinase (IKK) and I kappa B alpha activation, p65 nuclear translocation, NF-kappa B and DNA binding activity, and promoter activity of ICAM-1. Although andrographolide increased the intracellular cAMP concentration and induced the phosphorylation of cAMP response element-binding protein (CREB), knocking down CREB protein expression by transfecting the cells with CREB-specific small interfering RNA did not relieve the inhibition of ICAM-1 expression by andrographolide. These results suggest that AP down-regulates TNF-alpha-induced ICAM-1 expression at least in part via attenuation of activation of NF-kappa B in EA.hy926 cells rather than through activation of CREB (Chao et al. 2011a).

The effects of ethyl acetate extract from *Andrographis paniculata* on the level of inflammatory mediators were examined first using nuclear factor kappa B (NF-kappa B) driven luciferase assay. The results showed that *Andrographis paniculata* significantly inhibited NF-kappa B luciferase activity and tumor necrosis factor alpha (TNF-alpha), interleukin 6 (IL-6), macrophage inflammatory protein-2 (MIP-2) and nitric oxide (NO) secretions from lipopolysaccharide (LPS)/interferon-gamma stimulated Raw264.7 cells. To further evaluate the anti-inflammatory effects of *Andrographis paniculata in vivo*, BALB/c mice were tube-fed with 0.78 (AP1), 1.56 (AP2), 3.12 (?) and 6.25 (AP4) mg/kg body weight (BW)/day in soybean oil, while the control and PDTC (pyrrolidine dithiocarbamate, an anti-inflammatory agent) groups were tube-fed with soybean oil only. After 1 week of tube-feeding, the PDTC group was injected with 50 mg/kgBW PDTC and 1 h later, all of the mice were injected with 15 mg kg(-1) BW LPS. The results showed that the AP1, AP2, AP3 and PDTC groups, but not AP4, had significantly higher survival rate than the control group. Futher investigation revealed that the *Andrographis paniculata* and PDTC groups had significantly lower TNF-alpha, IL-12p40, MIP-2 or NO in serumor peritoneal macrophages and infiltration of inflammatory cells into the lung of mice. The AP1 group also had significantly lower MIP-2 mRNA expression in brain. This study suggests that *Andrographis paniculata* can inhibit the production of inflammatory mediators and alleviate acute hazards at its optimal dosages (Chao et al. 2011).

The aim of the study of *Chandrasekaran et al.* was to probe the anti-inflammatory/anti-allergic potential of seven phytoconstituents (andrographolide, neoandrographolide, isoandrographolide, andrograpanin, 14-deoxy-11,12-didehydroandrographolide, 7-O-methylwogonin and skullcapflavone-I) isolated from *Andrographis paniculata* on the production of key inflammatory/allergic mediators (NO, PGE(2), IL-1 beta, IL-6, LTB(4), TXB(2) and histamine). The results demonstrated that andrographolide, isoandrographolide, 7-O-methylwogonin and skullcapflavone-I significantly inhibited LPS stimulated NO and PGE(2) release in J774A.1 macrophages. Andrographolide, isoandrographolide and 7-O-methylwogonin showed considerable inhibition of IL-1 beta production in LPS elicited macrophages. LPS induced IL-6 production was significantly inhibited by andrographolide, isoandrographolide and skullcapflavone-I in a concentration dependent manner. Significant dose-dependent reduction in the levels of IL-6 was shown by andrographolide (14.2–57 μM), isoandrographolide (14.2–57 μM) and skullcapflavone-I (63 μM) at the indicated concentrations. The IC_{50} values indicated that, andrographolide (~33.3 μM) was most potent, followed by isoandrographolide (~43 μM). The maximum inhibition of 75.3% for andrographolide, 62% for isoandrographolide and 47.19% for skullcapflavone-I was obtained at the highest concentrations. The results revealed that andrographolide, isoandrographolide and skullcapflavone-I significantly decreased TXB(2) release in A23187 activated HL-60 promyelocytic cells. Furthermore, the anti-allergic properties of the phytoconstituents was investigated on A23187 induced LTB(4) production (HL-60 cells) and histamine release (RBL-2H3 basophilic cells). Only skullcapflavone-I and 7-O-methylwogonin showed marked inhibitory effect on LTB(4) production. 7-O-methylwogonin was the only compound that exerted dose-dependent inhibition towards histamine release. Therefore, this study indicated that some of these phytoconstituents exhibit potent anti-inflammatory/anti-allergic effects by modulating different inflammatory/allergic mediators (Chandrasekaran et al. 2011).

The effects of a methanolic extract of *Andrographis paniculata* leaves on inhibition of lipopolysaccharide (LPS) induced [nitric oxide (NO), prostaglandin E(2) (PGE(2)), interleukin-lbeta (IL-1 beta), and interleukin-6 (IL-6)] and calcimycin (A23187) induced lleukotriene B(4) (LTB(4)), thromboxane B(2) (TXB(2)) and histamine] mediators were studied in diverse cell based models. Andrographis extract illustrated significant alleviation of pro-inflammatory, inflammatory, and allergic mediators. However, no inhibition was observed against histamine release. The result showed that *Andrographis* extract was fairly potent in attenuating the inflammation by inhibiting pro-inflammatory (NO, IL-1 beta and IL-6), inflammatory (PGE(2) and TXB(2)) and allergic (LTB(4)) mediators. *Andrographis* extract showed a significant dose-dependent decline in the levels of NO at concentrations ranging from 10_µg/ml to 30_g/ml. The IC_{50} value obtained was 20_µg/ml with a maximum inhibition of ~69% observed at 30_µg/ml. *Andrographis* extract significantly inhibited PGE_2 levels at the highest concentration of 50_µg/ml, where a maximum inhibition of 53% was attained. *Andrographis* extract displayed inhibition of LPS (5_µg/mL) induced IL-1 beta levels in J774A.1 cells in a dose-dependent manner at concentrations ranging from 20 µg/mL to 40 µg/ml. Maximum inhibition of 27% was observed at 30 µg/mL and 40 µg/mL. A significant dose-dependent reduction in the levels of IL-6 was monitored at concentrations ranging from 20_µg/mL to 40_µg/ml. The IC_{50} value obtained was ~27.5_µg/mL with a maximum inhibition of ~73% observed at 40_µg/ml. Inhibition of A23187 (5_M) induced LTB4 production in HL-60 cells was studied using various concentrations. *Andrographis* extract displayed significant inhibition at concentrations of 20_µg/ml and 40_µg/ml. The IC_{50} value obtained was ~30_µg/ml with a maximum inhibition of 69% at the highest concentration of 40_µg/mL. HL-60 cells were stimulated by calcimycin (A23187) (5_M) to augment the production of TXB2. Pre-treatment of *Andrographis* extract significantly inhibited the levels of TXB2 at concentrations ranging from 10_µg/mL to 40_µg/ml. The treatment produced a maximum inhibition of 99% at the highest concentration of 40_µg/mL with an IC_{50} value of ~12_µg/mL.(Chandrasekaran et al. 2010).

Vasorelaxing effect

14-deoxyandrographolide (DA) and 14-deoxy-11,12-didehydroandrographolide (DDA) stimulated nitric oxide (NO) release from human endothelial cells, DDA compared,vith DA caused a greater production of NO; this is in line with the finding of the earlier study that the vasorelaxant effect of DDA was more dependent on endothelium than DA (Zhang and Tan 1999).

The pharmacological effects of 14-deoxyandrographolide on rat isolated thoracic aorta were examined by Zhang et al. 14-Deoxyandrographolide (2.5-120 mu mol/L) inhibited contractions induced by phenylephrine (PE; 0.1 mu mol/L) and high K+ (80mmol/L) in a concentration-dependent manner in endothelium-intact aorta. The effect was attenuated in endothelium-denuded aorta without modifying the maximal response. Like verapamil, 14-deoxyandrographolide produced a much greater vasorelaxant effect in aorta precontracted by KCI than by PE, 14-Deoxyandrographolide (20-60 mu mol/L) also inhibited responses of the rat aorta to PE, In Ca^{2+}-free medium (KCl 55 mmol/L), 14-deoxyandrographolide (20-80 mu mol/L) antagonized Ca^{2+}-induced vasocontraction in a concentration-dependent manner and transient contractions induced by both caffeine (10 mmol/L) and noradrenaline (1 mu mol/L) were suppressed or almost abolished by 14-deoxyandrographolide. The vasorelaxant effect of 14-deoxyandrographolide was partially antagonized by N-G-nitro-L-arginine methyl ester (25 mu mol/L), a specific and competitive nitric oxide synthase (NOS) inhibitor, and methylene blue (10 mu mol/L), a soluble guanylate cyclase inhibitor, but was not affected by indomethacin (20 mu mol/L), a cyclo-oxygenase inhibitor, or glibenclamide (10 mu mol/L), an ATP-sensitive K+-channel blocker. These results suggest that the vasorelaxant activity of 14-deoxyandrographolide may be mediated via the activation of NOS and guanylate cyclase, as well as the blockade of Ca^{2+} influx through both voltage- and receptor-operated Ca^{2+} channels (Zhang and Tan 1998).

Cytotoxic and antitumour effects

The methanol extract of the aerial part of *Andrographis paniculata* showed potent cell differentiation-inducing activity on mouse myeloid leukemia (M1) cells. From the ethyl acetate-soluble fraction of the methanol extract, six new diterpenoids of ent-labdane type, 14-epi-andrographolide (3), isoandrographolide (4), 14-deoxy-12-methoxyandrographolide (7), 12-epi-14-deoxy-12-methoxyandrographolide (8), 14-deoxy-12-hydroxyandrographolide (9) and 14-deoxy-11-hydroxyandrographolide (10) as well as two new diterpene glucosides, 14-deoxy-11, 12-didehydroandrographi-side (12) and 6'-acetylneoandrographolide (14), and four new diterpene dimers, bis-andrograpolides A (15), B (16), C (17) and D (18), were isolated along with six known compounds. The structures of the diterpenoids were determined by means of spectral methods. Some of these compounds showed potent cell differentiation-inducing activity towards M1 cells (Matsuda et al. 1994).

The cytotoxic activities of several diterpenoid constituents of Andrographis paniculata were evaluated *in vitro*. The seven diterpenoid compounds used were andrographolide, 14-deoxyandrographolide, andrographiside, deoxyandrographiside, 14-deoxy-12-methoxyandrographolide, neoandrographolide, and 14-deoxy- 11,12-didehydroandrographolide. The activities of these compounds were evaluated with various human tumor cell lines such as Caov-3 (human ovarian carcinoma cell line), T-47D (human breast carcinoma cell line), Hs-578T (human breast carcinoma cell line), Hep G2 (human hepatocarcinoma cell line), and NCI-H23 (human lung adenocarcinoma cell line). Cell survival was measured using the MTS assay after 72 h of incubation. Andrographolide, neoandrographolide, andrographiside, deoxyandrographiside, and 14-deoxy-12-methoxyandrographolide appeared to be noncytotoxic against all the cell lines. Only 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide exhibited cytotoxic activities (based on EC50 values), but this was limited to the T-47D cell line (EC50 values of 2.8 mu g/ml and 1.5 mu g/ml, respectively). As one of the principle diterpenoids of Andrographis paniculata, 14-deoxy-11,12-didehydroandrographolide appeared to be the most potent when compared with the rest of the compounds examined. The effects of 14-deoxy-11,12-didehydroandrographolide on T-47D cells were further confirmed to be nonapoptotic, non-necrotic, but programmed in nature, as demonstrated using a DNA fragmentation detection assay, Trypan blue exclusion assay, and annexin V-propidium iodide staining (Tan et al. 2005).

The combination of andrographolide and neoandrographolide with suboptimal concentrations of etoposide revealed compound 2 as chemosensitizer in S-Jurkat and X chromosome-linked inhibitor of apoptosis protein (XIAP)-overexpressing Jurkat cells, a model for chemoresistance (Pfisterer et al. 2010).

A dichloromethane fraction of a methanol extract of *A. paniculata* significantly inhibited the proliferation of HT-29 (colon cancer) cells and augmented the proliferation human peripheral blood lymphocytes (HPBLs) at low concentrations (0.1-100 µg/ml and 0.25 µg/ml, respectively). On further fractionation of the dichloromethane extract three diterpene compounds were isolated, i.e. andrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide. Andrographolide showed anticancer activity on diverse cancer cells representing different types of human cancers. Whereas all the three molecules showed enhanced proliferation and interleukin-2 (IL-2) induction in HPBLs (Kumar et al. 2004).

The cytotoxic activities of flavonoids and labdane diterpenes isolated from *A. paniculata* were evaluated against Jurkat, PC-3, HepG2 and Colon 205 tumor cells, and normal cells PBMCs. The bioactivity assays showed these metabolites exhibited moderate cytotoxic activity against Jurkat, PC-3 and Colon 205 cell lines. Certain compounds selectively blocked the cell cycle progression at G0/G1, while others blocked the same at G2/M phase of the Jurkat cell line (Geethangilil et al. 2008).

The mechanism of the anti-tumor properties of andrographolide, was examined using cell-cycle progression in human colorectal carcinoma Lovo cells. The data from cell growth experiment showed that andrographolide exhibited the anti-proliferation effect on Lovo cells in a time- and dose-dependent

manner. An arrest of the cells at the G1-S phase was observed with andrographolide the tested at concentrations of 0-30 mu M. Cellular uptake of andrographolide was confirmed by capillary electrophoresis analysis and the intracellular accumulation of andrographolide (0.61 +/- 0.07 µM/mg protein) was observed when treatment of Lovo cells with andrographolide for 12 h. In addition, an accumulation of the cells in G1 phase (15% increase for 10 mu M of andrographolide) was observed as well as by the association with a marked decrease in the protein expression of Cyclin A, Cyclin D1, Cdk2 and Cdk4. Andrographolide also inducted the content of Cdk inhibitor p21 and p16, and the phosphorylation of p53. Further immunoprecipitation studies found that, in response to the treatment, the formation of Cyclin D1/Cdk4 and Cyclin A/Cdk2 complexes had declined, preventing the phosphorylation of Rb and the subsequent dissociation of Rb/E2F complex. These results suggest andrographolide inhibits Lovo cell growth by G1-S phase arrest, and was exerted by inducing the expression of p53, p21 and p16 that, in turn, repressed the activity of Cyclin D1/Cdk4 and/or Cyclin A/Cdk2, as well as Rb phosphorylation (Shi et al. 2008).

Pretreatment with andrographolide significantly enhances TRAIL-induced (tumor necrosis factor-related apoptosis-inducing ligand) apoptosis in various human cancer cell lines, including those TRAIL-resistant cells. Such sensitization is achieved through transcriptional up-regulation of death receptor 4 (DR4), a death receptor of TRAIL. In search of the molecular mechanisms responsible for DR4 up-regulation, it was found that the tumor suppressor p53 plays an essential role in DR4 transcriptional activation. andrographolide is capable of activating p53 via increased p53 phosphorylation and protein stabilization, a process mediated by enhanced reactive oxygen species production and subsequent c-Jun NH(2)-terminal kinase activation. Pretreatment with an antioxidant (N-acetylcysteine) or a c-Jun NH2-terminal kinase inhibitor (SP600125) prevented andrographolide-induced p53 activation and DR4 upregulation and eventually blocked the andrographolide-induced sensitization on TRAIL-induced apoptosis. Taken together, these results present a novel anticancer effect of andrographolide and support its potential application in cancer therapy to overcome TRAIL resistance (Zhou et al. 2008).

In an in vitro study is was found that andrographolide significantly decreased the number of surviving hepatoma-derived Hep3B cells in the MTT assay and induced cell apoptosis. Further study showed that andrographolide induced activation of mitogen-activated protein kinases (MAPKs) including p38 kinase, c-Jun N-terminal kinase (JNK) and extracellular signal-related kinases (ERK1/2), but had no significant effect on caspase-3, Bcl-xL and Bcl-2, which are apoptosis-related proteins. Moreover, inhibition of JNK activation partially rescued the toxic effect of andrographolide on Hep3B cells. These results indicate that the JNK signaling pathway plays an important role in the toxic effect of Andro on Hep3B cells (Ji et al. 2007).

Andrographolide induced apoptosis of prostate cancer (PC-3) cells (the most sensitive cell line among the cell lines screened) via the activation of caspase 3, up-regulation of bax, and down-regulation of bcl-2. Furthermore, its inhibitory activity on the level of vascular endothelial growth factor was also verified by ELISA (Zhao et al. 2008).

Andrographolide was found to exhibit growth inhibition and cytotoxicity against the hormone-independent (PC-3 and DU-145) and hormone-dependent (LNCaP) prostate cancer cell lines via the microculture tetrazolium (MTT) assay. Due to its greater cytotoxic potency and selectivity towards PC-3 cells, flow cytometry was used to analyze the cell cycle distribution of control and treated PC-3 cells whereas Annexin V-FITC/PI flow cytometry analysis was carried out to confirm apoptosis induced by andrographolide in PC-3 cells. Cell cycle and apoptotic regulatory proteins were determined by western blot analysis. Andrographolide was found to induce G2/M cell cycle arrest which leads to predominantly apoptotic mode of cell death. Mechanistically, AGP was found to downregulate CDK1 without affecting the levels of CDK4 and cyclin D1. Induction of andrographolide was associated with an increase in activation and expression of caspase 8 which then is believed to have induced cleavage of Bid into

tBid. In addition, activation and enhancement of executioner caspase 9 and Bax proteins without affecting Bcl-2 protein levels were observed (Wong et al. 2011).

Apholidegrapholide has been shown to suppress the growth of HCC cells and trigger apoptosis *in vitro*. To assess the suitability of andrographolide as a chemotherapeutic agent in HCC, its cytotoxic effects have been evaluated both as a single agent and in combination with 5-FU. Andrographolide potentiates the cytotoxic effect of 5-FU in HCC cell line SMMC-7721 through apoptosis. Andrographolide alone induces SMMC-7721 apoptosis with p53 expression, Bax conformation and caspase-3,8,9 activation. Surprisingly, the addition of andrographolide to 5-FU induces synergistic apoptosis, which could be corroborated to the increased caspase-8, p53 activity and the significant changes of Bax conformation in these cells, resulting in increased losses of mitochondrial membrane potential, increased release of cytochrome c, and activation of caspase-9 and caspase-3. Suppression of caspase-8 with the specific inhibitor z-IETD-fmk abrogates largely andrographolide/5-FU biological activity by preventing mitochondrial membrane potential disappearance, caspase-3,9 activation and subsequent apoptosis. The results suggest that andrographolide may be effective in combination with 5-FU for the treatment of HCC cells SMMC-7721 (Yang et al. 2009).

Andrographolide isolated from *Andrographis paniculata* at 0.35 mM, 0.70 mM and 1.40 mM induced DNA fragmentation and increased the percentage of apoptotic cells when TD-47 human breast cancer cell line was treated for 24, 48 and 72 h. The results demonstrated that andrographolide can induce apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increase expression of p53, bax, caspase-3 and decrease expression of bcl-2 determined by immunohistochemical analysis (Harjotaruno et al. 2007).

In an in vitro study it was demonstrated that andrographolide at nontoxic to subtoxic concentrations (0.3-3 mu M) suppressed the invasion ability of CT26 cells in Matrigel-based invasion assays. In addition, the expression of cell adhesion regulators (beta-catenin and ILK) was not altered by andrographolide treatment. However, andrographolide indeed inhibited matrix metalloproteinase 2 (MMP2) activity without affecting its expression. Furthermore, the activation of ERK, but not Akt, was attenuated by andrographolide treatment. Notably, a similar inhibitory effect of andrographolide on the invasion and MMP2 activity of the human colon cancer cell line HT29 was also observed. These results indicate that andrographolide exhibits anti-invasive activity against colon cancer cells via inhibition of MMP2 activity (Chao et al. 2010).

The pro-apoptotic effect of andrographolide was studied on Burkitt p53-mutated Ramos cell line, mantle cell lymphoma (MCL) line Granta, follicular lymphoma (FL) cell line HF-1, and the diffuse large B-cell lymphoma (DLBCL) cell line SUDHL4, as well as primary cells from patients with FL, DLBCL, and MCL. It was found that andrographolide resulted in dose-and time-dependent cell death as measured by MTT. Andrographolide significantly increased reactive oxygen species (ROS) production in all cell lines. To determine mechanism of cell death, apoptosis was measured by Annexin V/propidium iodide in the presence and absence of the antioxidant N-acetyl-L-cysteine (NAC), the glutathione (GSH)-depleting agent buthionine sulfoxamine (BSO), or caspase inhibitors. It was found that apoptosis was enhanced by BSO, blocked by NAC, and accompanied by poly(ADP-ribose) polymerase cleavage and activation of caspase-3, caspase-8, and caspase-9. BAX conformational change and mitochondrial membrane potential were measured, and using mouse embryonic fibroblast (MEF) Bax/Bak double knockouts (MEF(Bax-/-/Bak-/-)), it was found that apoptosis was mediated through mitochondrial pathways, but dependent on caspases in both cell lines and patient samples. Andrographolide caused ROS-dependent apoptosis in lymphoma cell lines and in primary tumor samples, which was enhanced by depletion of GSH and inhibited by NAC or the pan-caspase inhibitor Z-VAD-FMK (Yang et al. 2010).

To investigate the anti-cancer properties of andrographolide, its effect on migration and invasion in human NSCLC A549 cells was examined. The results of a wound-healing assay and *in vitro* transwell

assay revealed that andrographolide inhibited dose-dependently (1-5 µM) the migration and invasion of A549 cells under non-cytotoxic concentrations. Molecular data showed that the effect of andrographolide in A549 cells might be mediated via sustained inactivation of phosphatidylinositol 3-kinase (PI3K)/Akt signal involved in the up-regulation of matrix metalloproteinases (MMPs). The results showed that andrographolide exerted an inhibitory effect on the activity and the mRNA and protein levels of MMP-7, but not MMP-2 or MMP-9. The andrographolide-inhibited MMP-7 expression or activity appeared to occur via activator protein-1 (AP-1) because of its DNA binding activity was suppressed by andrographolide. Additionally, the transfection of Akt over-expression vector (Akt1 cDNA) to A549 cells could result in an increase expression of MMP-7 concomitantly with a marked induction on cell invasion. These findings suggest that the inhibition on MMP-7 expression by andrographolide may be through suppression on PI3K/Akt/AP-1 signaling pathway, which in turn led to the reduced invasiveness of the cancer cells (Lee et al. 2010).

In an *in vitro* study it was observed that andrographolide inhibited the expression of hypoxia-inducible factor-1 alpha (HIF-1 alpha) in A549 cells. HIF-1 alpha plays an important role in tumor growth, angiogenesis and lymph node metastasis of NSCLC. The andrographolide-induced decrease of cellular protein level of HIF-1 alpha was correlated with a rapid ubiquitin-dependent degradation of HIF-1 alpha, and was accompanied by increased expressions of hydroxyl-HIF-1 alpha and prolyl hydroxylase (PHD2), and a later decrease of vascular endothelial growth factor (VEGF) upon the treatment of Andro. The andrographolide-inhibited VEGF expression appeared to be a consequence of HIF-1 alpha inactivation, because its DNA binding activity was suppressed by andrographolide. Molecular data showed that all these effects of andrographolide might be mediated via TGF beta 1/PHD2/HIF-1 alpha pathway, as demonstrated by the transfection of TGF beta 1 overexpression vector and PHD2 siRNA, and the usage of a pharmacological MG132 inhibitor. (Lin et al. 2011).

The well-characterized epidermal growth factor receptor (EGFR) and transferrin receptor (TfR) expressed in epidermoid carcinoma (A-431) cells were utilized as a model to study the effect of andrographolide on receptor trafficking. Receptor distribution, the total number of receptors and surface receptors were analysed by immunofluorescence, Western blot as well as flow-cytometry respectively. Andrographolide treatment inhibited cell growth, down-regulated EGFRs on the cell surface and affected the degradation of EGFRs and TfRs. The EGFR was internalized into the cell at an increased rate, and accumulated in a compartment that co-localizes with the lysosomal-associated membrane protein in the late endosomes. Andrographolide may affect receptor trafficking by inhibiting receptor movement from the late endosomes to lysosomes. The down-regulation of EGFR from the cell surface also indicates a new mechanism by which andrographolide may induce cancer cell death (Tan et al. 2010).

The effect of andrographolide on migration and invasion of Lovo cells was studied in vitro. The results of wound-healing assay and *in vitro* transwell assay revealed that andrographolide inhibited dose-dependently the migration and invasion of Lovo cells under non-cytotoxic concentrations. Using zymographic assay and RT-PCR, the results revealed that andrographolide diminished the activity and the mRNA and protein levels of MMP-7, but not MM P-2 or MMP-9. The down-regulation of MMP-7 appeared to be via the inactivation of activator protein-1 (AP-1) since the treatment with andrographolide suppressed the nuclear protein level of AP-1, which was accompanied by a decrease in DNA-binding level of the factor. Taken together, these results indicated that andrographolide reduces the MMP-7-mediated cellular events in Lovo cells, and provided a new mechanism for its anti-cancer activity (Shi et al. 2009).

Anti-apoptotic effect

In the study of *Chen et al.*, the molecular mechanisms and signaling pathways were investigated by which andrographolide protects human umbilical vein endothelial cells (HUVECs) from growth factor

(GF) deprivation-induced apoptosis. Results demonstrated that HUVECs undergo apoptosis after 18 hr of GF deprivation but that this cell death was suppressed by the addition of andrographolide in a concentration-dependent manner (1-100 muM). Andrographolide suppresses the mitochondrial pathway of apoptosis by inhibiting release of cytochrome c into the cytoplasm and dissipation of mitochondrial potential (Deltapsi(m)), as a consequence, prevented caspase-3 and -9 activation. Treatment of endothelial cells with andrographolide-induced activation of the protein kinase Akt, an anti-apoptotic signal, and phosphorylation of BAD, a down-stream target of Akt. Suppression of Akt activity by wortmannin, by LY-294002 and by using a dominant negative Akt mutant abolished the anti-apoptofic effect of andrographolide. In contrast, the ERK1/2 activities were not affected by andrographolide. The ERK1/2 inhibitor, PD98059 failed to antagonize the protective effect of andrographolide. Andrographolide exerts its anti-apoptotic potential via activation of the Akt-BAD pathway in HUVECs and thus may represent a candidate of therapeutic agent for atherosclerosis (Chen et al. 2004).

Chemopreventive effect

The effects of two doses (50 and 100 mg/kg body wt/day for 14 days) of an 80% hydroalcohol extract of *Andrographis paniculata* and butylated hydroxyanisole (BHA) were examined on drug metabolizing enzymes, antioxidant enzymes, glutatbione content, lactate dehydrogenase (LDH) and lipid peroxidation in the liver of Swiss albino mice (6-8 weeks old). The effect of the extract and BHA were also examined on lung, kidney and forestomach for the activities of glutathione S-transferase (GST), DT-diaphorase (DTD), superoxide dismutase (SOD) and catalase. A significant increase in the levels of acid soluble sulphydryl (-SH) content, cytochrome P450, cytochrome P450 reductase, cytochrome b5 reductase, GST, DTD and SOD were observed at both dose levels of extract treatment while catalase, glutathione peroxidase and glutathione reductase (GR) showed significant increases only at the higher dose in the liver. Both *Andrographis* treated groups showed a significant decrease in activity of LDH and malondialdehyde (MDA) formation. BHA treated mice showed a significant increase in the levels of cytochrome b5, GST, DTD, -SH content, GR and catalase in liver; while LDH and MDA levels were reduced significantly compared with their control values. In the lung, SOD, catalase and DTD, in the kidney catalase, DTD and GST, and in the forestomach SOD and DTD showed a significant increase at both dose levels of treatment. In BHA treated mice GST, DTD and catalase were significantly induced in the lung and along with these enzymes SOD was also induced in the kidney. In the case of the forestomach of BHA treated mice GST, DTD and SOD were enhanced significantly. These findings indicate the chemopreventive potential of *Andrographis paniculata* against chemotoxicity including carcinogenicity (Singh et al. 2001).

The protective effect of *Andrograhis paniculata* and andrographolide (ANDLE) against cyclophosphamide (CTX) induced urothelial toxicity was investigated in this study. Pretreatment of Swiss albino mice with *A paniculata* extract (10 mg/dose/animal intraperitoneally [ip]) and ANDLE (500 mu g/dose/animal ip) could significantly reduce CTX (1.5 nmol/kg body weight)-induced urothelial toxicity. Morphological and histopathological analysis of urinary bladder of CTX-treated mice showed severe inflammation and dark coloration, whereas *A paniculata* and ANDLE-treated mice showed almost normal bladder morphology. Elevation of urinary protein level (7.33 +/- 0.3 g/L) by CTX administration was reduced by *A. paniculata* (3.78 +/- 0.4 g/L) and ANDLE treatment (4.19 +/- 0.1 g/L). Urinary urea N-2 level, which was elevated after 48 hours of CTX administration (24.25 +/- 0.2 g/L) was found to be reduced by the treatment with A paniculata (14.19 +/- 0.5 g/L) and ANDLE (15.79 +/- 0.4 g/L). A decreased level of reduced glutahione (GSH) content in liver (2.81 +/- 0.1 nmol/mg protein) and bladder (1.20 +/- 0.2 nmol/mg protein) after CTX administration was also increased by the treatment with A paniculata (liver: 5.78 +/- 0.3 nmol/mg protein; bladder: 2.96 +/- 0.2 nmol/mg protein) and ANDLE (liver: 5.14 +/- 0.3 nmol/mg protein; bladder: 2.84 +/- 0.2 mnol/mg protein). Production of the proinflammatory cytokine, tumor necrosis factor-alpha, which was

elevated during CTX administration, was found to be inhibited by *A. paniculata* and ANDLE treatment. The lowered level of interleukin-2 and interferon-gamma during CTX treatment was elevated by the administration of *A paniculata* and ANDLE (Sheeja and Kuttan 2006).

In the study of Singh et al. male Wistar albino rats were divided into three groups: normal control, gentamicin control, and aqueous extract of *A. paniculata* (200 mg/kg, per oral (p.o.))-treated. The nephrotoxic model was induced by gentamicin (80 mg/kg, intraperitoeal (i.p.)). Blood samples were examined for serum creatinine, serum urea, and blood urea nitrogen after the 10 days of treatment. Aqueous extract of *A. paniculata* attenuated the gentamicin-induced increase in serum creatinine, serum urea, and blood urea nitrogen levels by 176.92%, 106.27%, and 202.90%, respectively (Singh et al. 2009).

The ameliorative properties of bioactive compound andrographolide, aqueous extract of *Andrographis paniculata* (AE-AP) and vitamin E (vit.E) were tested against nicotine induced liver, kidney, heart, lung and spleen toxicity. A group of mate Wistar rats were intraperitoneally administered vehicle, nicotine (1 mg/kg body weight/day), nicotine + andrographolide (250 mg/kg body weight/clay), nicotine + AE-AP (250 mg/kg body weight/day) and nicotine+ vit.E (50 mg/kg body weight/day) for the period of 7 days. The significantly increased levels of lipid peroxidation, protein oxidation and the decreased antioxidant enzyme status were noted in nicotine treated group as compared to vehicle treated group. Andrographolide, AE-AP and vit.E significantly reduced the lipid peroxidation, protein oxidation and increased the antioxidant enzyme status. According to the authors, *A. paniculata* and vit.E may act as putative protective agent against nicotine induced tissue injury and may pave a new path to develop suitable drug therapy (Neogy et al. 2008).

Hepatoprotective effect

Andrographolide showed a significant dose dependent (0.75-12 mg/kg p.o. x 7) protective activity against paracetamol-induced toxicity on *ex vivo* preparation of isolated rat hepatocytes. It significantly increased the percent viability of the hepatocytes as tested by trypan blue exclusion and oxygen uptake tests. It blocked the toxic effects of paracetamol on certain enzymes (GOT, GPT and alkaline phosphatase) in serum as well as in isolated hepatic cells (Visen et al. 1993).

The pharmacological effects of 14-deoxyandrographolide on rat isolated thoracic aorta were examined. 14-Deoxyandrographolide (2.5-120 mu mol/L) inhibited contractions induced by phenylephrine (PE; 0.1 mu mol/L) and high K^+ (80mmol/L) in a concentration-dependent manner in endothelium-intact aorta. The relaxing action of 14-deoxyandrographolide was attenuated in endothelium-denuded aorta without modifying the maximal response. Like verapamil, 14-deoxyandrographolide produced a much greater vasorelaxant effect in aorta precontracted by KCl than by PE, 14-Deoxyandrographolide (20-60 mu mol/L) also inhibited responses of the rat aorta to PE. In Ca^{2+}-free medium (KCl 55 mmol/L), 14-deoxyandrographolide (20-80 mu mol/L) antagonized Ca^{2+}-induced vasocontraction in a concentration-dependent manner and transient contractions induced by both caffeine (10 mmol/L) and noradrenaline (1 mu mol/L) were suppressed or almost abolished by 14-deoxyandrographolide. The vasorelaxant effect of 14-deoxyandrographolide was partially antagonized by N-G-nitro-L-arginine methyl ester (25 mu mol/L), a specific and competitive nitric oxide synthase (NOS) inhibitor, and methylene blue (10 mu mol/L), a soluble guanylate cyclase inhibitor, but was not affected by indomethacin (20 mu mol/L), a cyclo-oxygenase inhibitor, or glibenclamide (10 mu mol/L), an ATP-sensitive K+-channel blocker. These results suggest that the vasorelaxant activity of 14-deoxyandrographolide may be mediated via the activation of NOS and guanylate cyclase, as well as the blockade of Ca^{2+} influx through both voltage- and receptor-operated Ca^{2+} channels (Zhang and Tan 1998).

Histomorphological, ultrastructural, and biochemical Studies were performed for the effect of the andrographolide on control mice, mice treated with hexachlorocyclohexane (BHC) only and BHC +

andrographolide.. The histological and ultrastructural changes observed upon andrographolide supplementation suggested the recovery of the damaged liver. This recovery was also reflected in the neoplastic nodule formation. The activity of phosphorylase and glucose-6-phosphatase in the liver of the andrographolide-supplemented group suggests improved glycogenolysis in liver. Serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, alkaline phosphatase, acid phosphatase, and gamma-glutamyl transpeptidase showed a significant decrease in andrographolide-supplemented animals as compared with BHC-treated animals, suggesting regenerative effects elicited by andrographolide. This study indicates that the regenerative capability elicited by andrographolide is possibly due to its ability to reactivate liver function enzymes that catalyze the reaction of several biochemical and synthetic processes and that it may be useful for severe liver damage conditions (Trivedi et al. 2009).

14-deoxyandrographolide (DA) and 14-deoxy-11,12-didehydroandrographolide (DDA) are two diterpenoids isolated from *A. paniculata.* As reported in in vivo studies, DDA exhibited a greater hypotensive effect in anaesthetized rats and a vasorelaxant activity in isolated rat aorta, compared with DA. Their vasorelaxant activities were mediated through the activation of the enzymes, nitric oxide synthase (NOS) and guanylyl cyclase. This *in vitro* study demonstrated that both DA and DDA stimulated nitric oxide (NO) release from human endothelial cells, DDA compared,vith DA caused a greater production of NO; this is in line with the finding of the earlier study that the vasorelaxant effect of DDA was more dependent on endothelium than DA (Zhang and Tan 1999).

Spasmolytic effect

The possible blockade of voltage-operated calcium channels (VOCs) by *Andrographis paniculata* dried 70% ethanol extract (DER 5:1) in vas deferens smooth muscle was investigated in rats. The tissues were incubated in Ca^{2+}-free Kreb's solution and stimulated with KCl (40 mM) to produce depolarisation of the membrane. The isometric contractile response to cumulative concentrations of $CaCl_2$ was effectively blockaded by 0.2 and 0.4 mg/ml A. paniculata. In other experiments, the maximum contractile response induced by norepinephrine was not antagonised by 0.2, 0.4 or 0.8 mg/ml A. paniculata. The possible blockade of Ca^{2+} entry by A. paniculata was evaluated with Ca-45(2+) uptake in vas deferens treated with reserpine (5 and 2.5 mg/kg) 48 and 24 h before the experiments. Epididymal segments were incubated with Ca^{2+}-free Kreb's solution with KCl, 25 and 50 mM. The influx was completely blocked with 0.4 mg/m *A. paniculata*. These results suggest that *A. paniculata* selectively blocks VOCs, hence inhibiting the Ca-45(2+) influx (Burgos et al. 2000).

Uterorelaxant effect

The possible relaxation of uterine smooth muscle by *Andrographis paniculata* dried 70% ethanol extract (DER 5:1) via a blockade of voltage operated calcium channels was investigated in rats. Uterine horns pretreated with oestradiol were incubated in Ca^{+2}-free Jalon's solution and stimulated with KCI (20-60 mM) in order to produce depolarization of the membrane. The isometric contractile response to 1 mM or cumulative concentrations of $CaCl_2$ were blockaded by 0.2, 0.4 and 0.8 mg/mL of *A. paniculata*. The maximum contractile response induced by acetylcholine was moderately antagonized by *A. paniculata*, The possible blockade of Ca^{+2} entry by *A, paniculata* was evaluated with Ca-45(+2) uptake in uterine rings incubated with free-Ca^{+2}-Ringer's solution high in K+ (KCl 40 mM), The influx was completely blockaded with 0.4 mg/mL of A, paniculata. These results strongly suggest that *A. paniculata* blockades voltage operated calcium channels inhibiting the entry of Ca^{+2} from the external medium (Burgos et al. 2001).

In a study of *Burgos et al*., the effect of 14-deoxyandrographolide (14-DAP) on calcium channel-dependent rat uterine smooth muscle contraction was evaluated. Using a tissue bath preparation, 14-DAP was able to reduce the contractile response to 0.3 and 3.0 mM of $CaCl_2$, with an IC_{50} of 1.24 +/-

0.23 x 10(-5) M and 5.94 +/- 0.29 x 10(-5) M, respectively. 14-DAP shifted the CaCl2 cumulative dose response curve to the right, increasing the EC50 from 2.08 +/- 0.20 x 10(-4) M to 4.22 +/- 0.22 x 10(-4) M (5 muM 14-DAP) and 2.5 +/- 1.0 x 10(-3) M (50 muM 14-DAP). In order to determine if 14-DAP had any effect on intracellular calcium, the relaxant response to 14-DAP following contraction by oxytocin, PGF(2alpha) and vanadate in Ca+2-free solution was compared with that of isoproterenol and phenylbutazone. While isoproterenol and phenylbutazone relaxed the smooth muscle in a dose-dependent manner, 14-DAP did not have any effect on either the oxytocin, PGF(2alpha) or vanadate-induced smooth muscle contraction. Based on these data, it appears that 14-DAP is an uterine smooth muscle relaxant which produces a selective blockade of voltage operated calcium channels (Burgos et al. 2003).

Antithrombotic effect

Andrographis paniculata leaf extract (leaf standardized for andrographolide content of 3.6%, 100 mg, leaf extracted with 10 ml 40% ethanol) did not infuence the biosynthesis of eicosanoids in isolated human polymorphonuclear leukocytes (PMNL), however, it was found that andrographolide inhibits PAF-induced human blood platelet aggregation in a dose dependent manner (IC_{50} similar to 5 µM) (Amroyan et al. 1999).

The three active diterpenoids from *Andrographis*, and a aqueous plant extracts, were investigated for the inhibitory effect on platelet aggregation in vitro. The results show that andrographolide (AP(1)) and 14-deoxy-11, 12-didehydroandrographolide (AP(3)) significantly inhibited thrombin-induced platelet aggregation in a concentration-(1-100 µM) and time-dependent manner while neoandrographolide (AP(4)) had little or no activity. AP(3) exhibited higher antiplatelet activity than AP, with IC50 values ranging from 10 to 50 µM. The inhibitory mechanism of AP(1) and AP(3) 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 AP(3) inhibited thrombin-induced aggregation to different degrees. The extracts significantly decreased platelet aggregation in a concentration-(10-100 mu g/ml) and time-dependent manner. However, the extract with high level of AP(3) (Extract 13) (IC50 values = 50-75 µg/ml) showed less inhibitory activity against thrombin than the extract with lower level of AP(3) (Extract A) (IC50 values = 25-50 µg/ml). These results indicate that the standardized *A. paniculata* extract may contain other antiplatelet compounds rather than AP(1) and AP(3), which contribute to high antiplatelet activity (Thisoda et al. 2006).

The mechanisms of andrographolide in preventing platelet activation was investigated *in vitro*. Andrographolide (25-75 mu M) exhibited a more potent activity of inhibiting platelet aggregation stimulated by collagen. Andrographolide inhibited collagen-stimulated platelet activation accompanied by relative Ca^{2+} mobilization; thromboxane A_2 formation; and phospholipase C (PLC) gamma 2, protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and Akt phosphorylation. Andrographolide markedly increased cyclic GMP, but not cyclic AMP levels. Andrographolide also stimulated endothelial nitric oxide synthase (eNOS) expression, NO release, and vasodilator-stimulated phosphoprotein (VASP) phosphorylation. ODQ, an inhibitor of guanylate cyclase, markedly reversed the andrographolide-mediated inhibitory effects on platelet aggregation, p38 MAPK and Akt phosphorylation, and the andrographolide-mediated stimulatory effect on VASP phosphorylation. Furthermore, a PI3 kinase inhibitor (LY294002) but not a PKC inhibitor (Ro318220) significantly diminished p38 MAPK phosphorylation; nevertheless, a p38 MAPK inhibitor (SB203580) and LY294002 diminished PKC activity stimulated by collagen. Andrographolide also reduced collagen-triggered hydroxyl radical (OH center dot) formation (Lu et al. 2011)

In vivo studies

Antidiabetic effect

Oral administration of a crude ethanolic extract of *Andrographis paniculata* at different doses (0.1, 0.2, and 0.4 g/body weight) significantly reduced the fasting serum glucose level in STZ-diabetic rats compared to the vehicle (distilled water), but not in normal rats. This effect was dose-dependent. A similar result was seen with metformin (0.5 g/body weight). In the glucose tolerance test, an oral administration of the extract at the same doses suppressed the elevated glucose level in normal and diabetic rats, as did metformin. The effects were also dose-respondent. In the long-term experiment, the extract (0.4 g/body weight), metformin (0.5 g/body weight), and vehicle were given twice daily to diabetic rats for 14 d. On d 15, fasting serum glucose levels were found to be significantly lower in the extract- and metformin-treated groups ($P < 0.001$) than in the vehicle-treated group. The mean food and water intakes over 14 days were significantly lower in the extract-treated group ($P < 0.05$, $P < 0.01$, respectively) and also in the metformin-treated group (both $P < 0.001$) when compared to the vehicle-treated group. No significant change in insulin level was observed among the 3 groups of diabetic rats. The extract, like metformin, maintained the leptin levels after 14-d treatment, whereas this level was significantly decreased ($P < 0.05$) in the vehicle-treated group. The activity of hepatic glucose-6-phosphatase (G-6-Pase) was significantly reduced by the extract as well as by metformin (both $P < 0.05$). No significant difference in hepatic glycogen stores was noted among the 3 groups. The extract caused 49.8 % reduction of fasting serum triglyceride levels, compared to 21.7 % with metformin. However, neither the extract nor metformin significantly affected serum cholesterol level (Zhang and Tan 2000a).

The effect of ethanol extract of Andrographis paniculata (AP) on alpha-glucosidase (EC 3.2.1.20) inhibition was investigated in both normal and streptozotocin-induced diabetic rats. Oral carbohydrate tolerance tests were performed in 18-h fasted rats with starch (3 g/kg), sucrose (4 g/kg), and glucose (2 g/kg) separately, in both normal and diabetic rats, 10 min after administration of 250 (D1), 500 (D2), 1000 (D3) mg/kg ethanol extract of AP, vehicle (control), and acarbose (Acar) 10 mg/kg, respectively. Blood samples were analyzed for blood glucose at 0, 30, 60, and 120 min after respective treatments and the peak blood glucose (PBG) and area under the curve (AUC) determined. The results demonstrate that 500 mg, 1000 mg/kg ethanol extract of AP reduces and prolongs the PBG concentration, simultaneously decreasing AUC after starch and sucrose loading in normal and diabetic rats. Similarly, acarbose also reduced sucrose and starch induced blood glucose excursions, whereas it had no peak blood glucose suppressive effect after exogenous glucose load in both normal and streptozotocin-induced diabetic rats. The results suggest the possibility that the ethanol extract of AP may have PBG suppressive effect, post-carbohydrate challenge as evidenced by reduced PBG and AUC, which can be used effectively as a safer alternative to control postprandial hyperglycemia (PPH), especially in diabetic patients and borderline patients not properly controlled on diet alone (Subramanian and Asmawi 2006).

An ethanolic extract of *A. paniculata* showed appreciable alpha-glucosidase inhibitory effect in a concentration-dependent manner (IC_{50} 17.2 +/- 0.15 mg/ml) and a weak alpha-amylase inhibitory activity (IC_{50} 50.9 +/- 0.17 mg/ml). Andrographolide demonstrated a similar (IC_{50} 11.0 +/- 0.28 mg/ml) alpha-glucosidase and alpha-amylase inhibitory activity (IC_{50} = 11.3 +/- 0.29 mg/ml). The positive in vitro enzyme inhibition tests paved way for confirmatory *in vivo* studies. The *in vivo* studies demonstrated that *A. paniculata* extract significantly ($P < 0.05$) reduced peak blood glucose and area under curve in diabetic rats when challenged with oral administration of starch and sucrose. Further, andrographolide also caused a significant ($P < 0.05$) reduction in peak blood glucose and area under the curve in diabetic rats. Hence alpha-glucosidase inhibition may possibly be one of the mechanisms for the *A. paniculata* extract to exert antidiabetic activity and indicates that AP extract can be

considered as a potential candidate for the management of type 2 diabetes mellitus (Subramanian et al. 2008).

In the study of *Zhang* and *Tan*, the ethanolic extract of the aerial parts of *Andrographis paniculata* was investigated for antihyperglycaemic and antioxidant effects in normal and streptozotocin-induced type I diabetic rats. Normal and diabetic rats were randomly divided into groups and treated orally by gavage with vehicle (distilled water), metformin (500 mg/kg bodyweight) or the extract (400 mg/kg bodyweight), twice a day for 14 days. At the end of the 14 day period, the extract, like metformin, significantly increased bodyweight ($P < 0.01$) and reduced fasting serum glucose in diabetic rats ($P < 0.001$) when compared with vehicle, but had no effect on bodyweight and serum glucose in normal rats. Levels of liver and kidney thiobarbituric acid-reactive substances (TBARS) were significantly increased ($P < 0.0001$, $P < 0.01$, respectively), while liver glutathione (GSH) concentrations were significantly decreased ($P < 0.005$) in vehicle-treated diabetic rats. Liver and kidney TEARS levels were significantly lower ($P < 0.0001$, $P < 0.005$, respectively), whereas liver GSH concentrations were significantly higher ($P < 0.05$) in extract- and metformin-treated diabetic rats compared with vehicle-treated diabetic rats. *Andrographis paniculata* significantly decreased kidney TEARS level ($P < 0.005$) in normal rats. Hepatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were significantly lower in vehicle-treated diabetic rats compared with vehicle-treated normal rats. The extract, as well as metformin, significantly increased the activity of SOD and CAT, but had no significant effect on GSH-Px activity in diabetic rats. The extract and metformin did not produce significant changes in the activity of these anti-oxidant enzymes in normal rats. These results show that oxidative stress is evident in streptozotocin-diabetic rats and indicate that the ethanolic extract of *A. paniculata* not only possesses an antihyperglycaemic property, but may also reduce oxidative stress in diabetic rats (Zhang and Tan 2000b).

The antihyperglycemic action of andrographolide, an active principle in the leaves of *Andrographis paniculata*, was investigated in streptozotocin-induced diabetic rats (STZ-diabetic rats). Oral treatment of andrographolide decreased the plasma glucose concentrations of STZ-diabetic rats in a dose-dependent manner. Similar treatment with andrographolide also decreased the plasma glucose in normal rats and the maximal effect was more marked than that in STZ-diabetic rats. Andrographolide at the effective dose (1.5 mg/kg) significantly attenuated the increase of plasma glucose induced by an intravenous glucose challenge test in normal rats. In the isolated soleus muscle of STZ-diabetic rats, andrographolide enhanced the uptake of radioactive glucose in a concentration-dependent manner. Moreover, the mRNA and protein levels of the subtype 4 form of the glucose transporter (GLUT4) in soleus muscle were increased after repeated intravenous administration of andrographolide in STZ-diabetic rats for 3 days. These results suggest that andrographolide lowers plasma glucose in diabetic rats lacking insulin by increasing the glucose utilization(Yu et al. 2003).

Momordica charantia fruit juice or *Andrographis paniculata* decoction was orally administered to alloxan-induced diabetic rats. Rats that were treated with *Momordica charantia* and *Andrographis paniculata* had higher body weight (BW) compared with diabetic positive control ($P < 0.01$) from day 22 to day 27 (D27) but exhibited lower BW than the non-diabetic control ($P < 0.05$). These rats had lower feed ($P < 0.05$) and liquid intakes ($P < 0.01$) compared with diabetic positive control from day 17 to D27, but similar with the non-diabetic control. The blood glucose levels in these groups were significantly reduced from day 12 to D27 compared with diabetic positive control ($P < 0.01$), however, comparable with non-diabetic control. The diabetic positive control had extended mean estrous cycles (8 days) compared to Momordica charantia and *Andrographis paniculata*-treated diabetic rats (5 days; $P < 0.05$). Our results suggest that the anti-diabetic potentials of Momordica charantia and *Andrographis paniculata* could restore impaired estrous cycle in alloxan-induced diabetic rats (Reyes et al. 2006).

Antioxidant effect

The effect of the aqueous extract of *Andrographis paniculata* (AP) on antioxidant defense system in liver was investigated in lymphoma bearing AKR mice. Oral administration of the aqueous extract of *A. paniculata* in different doses causes a significant elevation of catalase, superoxide dismutase and glutathione S transferase activities. This suggests that the antioxidant action of the aqueous extract of AP, which may play a role in the anticarcinogenic activity by reducing the oxidative stress. LDH activity is known to increase in various cancers due to hypoxic condition. Lactate dehydrogenase is used as tumor marker. A significant decrease in LDH activity was observed after treatment with AP, which indicates a decrease in carcinogenic activity. A comparison with Doxorubicin (DOX), an anticancerous drug, indicates that the aqueous extract of AP is more effective than DOX with respect to its effect on catalase, superoxide dismutase, glutathione S transferase as well as on lactate dehydrogenase activities in liver of lymphoma bearing mice (Verma and Vinayak 2008).

In the study of Lin et al., extracts prepared from cultivated *Andrographis paniculata* (AP) and their active constituent andrographolide were evaluated for antioxidant, antioedema and analgesic activities on rats using different experimental settings. The results showed that the aqueous AP extract (AP-H_2O) exhibited a greater antioxidant activity than the ethanol AP extract (AP-EtOH) in all model systems tested. At a concentration of 50 µg/mL, the free radical scavenging, xanthine oxidase inhibition and antilipid peroxidation activities for AP-H_2O were 66.8%, 57.3% and 65.3%, respectively, and for AP-EtOH were 57.8%, 52.6% and 34.2%, respectively. At a dosage of 100 mg/kg, AP-H_2O and andrographolide, but not AP-EtOH, showed antioedema and analgesic activities. In phytochemical analysis, AP-H_2O showed a higher concentration of total flavanoid but a lower phenol content than AP-EtOH. In conclusion, AP-H_2O was more potent than AP-EtOH in antioxidant activities. Furthermore, compared with andrographolide, AP-H_2O as an extract also appears to possess potent antioedema and analgesic activities (Lin et al. 2009).

Fourteen days oral treatment of rats with the methanol extract of *A. paniculata* leaves (1 g/kg body weight) followed by CCl_4 administration preserved catalase (CAT), and superoxide dismutase (SOD) activities in erythrocytes, whereas plasma lipid peroxidation, alanine transaminase (ALT) and aspartate transaminase (AST) activities were restored to values comparable with control values. Treatment of rats with CCl_4 did not showed significant alteration ($p > 0.05$) in plasma total antioxidant status (TAS) as compare to values of control group (Akowuah et al. 2009).

Anti-inflammatory and antinociceptive effect

Neoandrographolide was tested *in vivo* and *in vitro* for its anti-inflammatory activities and mechanism. Oral administration of neoandrographolide (150 mg/kg) significantly suppressed ear edema induced by dimethyl benzene in mice. Oral administration of neoandrographolide (100-150 mg/kg) also reduced the increase in vascular permeability induced by acetic acid in mice. In vitro studies were performed using the macrophage cell line RAW264.7 to study the effect of neoandrographolide on suppressing phorbol-12-myristate-13-acetate (PMA)-stimulated respiratory bursts and lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha).Respiratory bursts were quantified by chemiluminescence (CL) measurements. Results showed that neoandrographolide suppressed PMA-stimulated respiratory bursts dose-dependently from 30 mu M to 150 mu M. Neoandrographolide also inhibited NO and TNF-alpha production in LPS-induced macrophages, contributing to the anti-inflammatory activity of *A. paniculata* (Liu et al. 2007).

Intra-peritoneal treatment of ovalbumin-immunized and nasally-challenged mice with andrographolide significantly inhibited the elevation of bronchoalveolar fluid (BAF) levels of TNF-alpha and GM-CSF in a dose-dependent manner, with 30 mg/kg producing an inhibition of 92% and 65% of the cytokines,

respectively) and almost completely blocked the accumulation of lymphocytes and eosinophils (Abu-Ghefreh et al. 2009).

An *in vivo* study was performed to evaluate the antinociceptive and antiedematogenic properties of andrographolide isolated from the leaves of *Andrographis paniculata* using two animal models. Antinociceptive activity was evaluated using the acetic acid-induced writhing and the hot-plate tests, while antiedematogenic activity was measured using the carrageenan-induced paw edema test. Subcutaneous (s.c.) administration of andrographolide (10, 25, and 50 mg/kg) did not affect the motor coordination of the experimental animals but produced significant ($p < 0.05$) antinociceptive activity when assessed using both tests. A dose of 2 mg/kg naloxone failed to affect the 25 mg/kg andrographolide activity in both tests, indicating that the activity was modulated via nonopioid mechanisms. Furthermore, andrographolide showed significant ($p < 0.05$) antiedematogenic activity (Sulaiman et al. 2010).

Antiallergic effect

Andrographis paniculata is widely used in traditional remedies in India in a number of clinical conditions including allergic manifestations. Two diterpenes, andrographolide and neoandrographolide, isolated from the plant, were evaluated for their antiallergic activity. These were tested for anti-PCA (Passive cutaneous anaphylaxis) and mast cell stabilizing activities in rats. Significant anti-PCA activity was observed. The compounds also possessed mast cell stabilizing activity against compound 48/80 and in sensitized mast cells, against egg albumin induced degranulation. The activities were found to be comparable to disodium cromoglycate (Gupta et al. 1998).

Immunomodulatory effect

Effects of *Andrographis paniculata* extract and its major component, andrographolide, on cell-mediated immune responses in metastatic tumor bearing animals were studied. NK cell mediated target cell lysis was enhanced by the administration of *Andrographis paniculata* extract (45.0% cell lysis) and andrographolide (40.2% cell lysis) on the 5th day after tumor induction when compared to untreated metastatic tumor bearing animals in which maximum target cell lysis was observed on 11th day (11.4%). Antibody dependent cell-mediated cytotoxicity (ADCC) was also enhanced by treatment with the extract (42.0% cell lysis) and andrographolide (40.2%) in comparison with the untreated case (11.0%). Similarly, the extract (25%) and andrographolide (22%) showed higher ACC activity than the control (14%) and treatment of extract and andrographolide resulted in significant increase in serum IL-2 and TIMP-1 levels. Furthermore, the levels of proinflammatory cytokines such as IL-1 beta, IL-6, GM-CSF and TNF-alpha were effectively reduced by the administration of extract and andrographolide in metastatic tumor bearing animals (Sheeja and Kuttan 2010).

Gastroprotective effect

Gastroprotective activities of aqueous and ethanolic extract of *Andrographis paniculata* leaves in rats have been reported. Sprague Dawley rats, 6 per group were used and rats in groups 1 to 6 were pretreated with (0.25% w/v) carboxymethyl cellulose (negative control, 5 ml/kg), 20 mg/kg omeprazole (positive control), (250 mg/kg and 500 mg/kg) of aqueous leaf extracts (APLAE) and (250 and 500 mg/kg) of ethanol leaf extracts (APLEE) respectively. Animals were orally administered with 95% ethanol (5 ml/kg) 60 min after their pretreatments. Rats were sacrificed 1 h after treatment and gastric contents were collected to measure pH and mucous weight. Stomach was analyzed for gross and histological changes. Ulcer control group showed extensive lesions of gastric mucosal layer, whereas rats pretreated with omeprazole, 250 and 500 mg/kg of APLAE showed significant and dose dependent reduction in gastric lesions with increased pH and mucus content of stomach. Rats pretreated with 250 or 500 mg/kg of APLEE showed significantly better inhibition of gastric mucosal lesions (Wasman et al. 2011).

The aim of a further study was to evaluate the gastroprotective effect of hydroalcoholic extract of *Andrographis paniculata* (HAEAP) in male albino wistar rats. Rats were pretreated with HAEAP (100, 200, 500mg/kg b. wt for 30 days) and then gastric ulcers were induced by ethanol, aspirin, pylorus ligation and cold restraint stress models. Ulcer score was determined in all the ulcer models. pH, gastric volume, titrable acidity, pepsin, mucin, myeloperoxidase, H(+)K(+) ATPase, thiobarbituric acid reacting substances (TBARS) and antioxidant enzyme activities were assayed in ethanol-administered rats. The ulcer score was found to be low in HAEAP-pretreated rats. Among the doses studied, 200 mg/kg b. wt was found to be optimum for significant ulcer reduction. The test drug significantly reduced the acidity, pepsin concentration, myeloperoxidase and H(+)K(+) ATPase activities in ethanol-administered rats. The elevated TBARS and decreased glutathione (GSH) and mucin levels observed during ulcerogenesis were found to be altered in HAEAP-received animals. The ulcer preventing effect of HAEAP may partly be due to its regulating effect on H(+)K(+) ATPase activity and/or mucin preserving effects. The flavonoids present in the HAEAP might be responsible for the gastroprotective action probably by maintaining the antioxidants and thiol status in the gastrointestinal tract (Panneerselvam and Arumugam 2011).

Antiulcer activity of *Andrographis paniculata* was evaluated by cysteamine induced duodenal ulcer model in rats. Male albino Wistar rats were pre-administered with 200 mg/kg body wt. of hydroalcoholic extact of Andrographis paniculata (HAEAP) orally, for 30 days prior to i.p. administration of 420 mg/kg body wt. of cysteamine as a single dose. Rats pre-administered with 30 mg/kg body wt. of ranitidine served as standard drug. Ulcer index, thiobarbituric acid reactive substances, mucin, glutathione peroxidase and myeloperoxidase activities, reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, glycoproteins and membrane bound enzyme activities were measured in duodenum of experimental animals. The ulcer score and myeloperoxidase activity were significantly minimized in rats treated with HAEAP. Mucin content was found to be preserved in rats treated with the extract. GSH/GSSG ratio and glutathione peroxidase activities were found to be maintained by the HAEAP. Level of lipid peroxidation products was found to be significantly low in HAEAP treated rats compared to ulcer control rats. The basolateral and brush border membrane bound enzyme activities which were depleted significantly in ulcer control rats were found to be maintained in rats pre-treated with the extract. The ulcer preventing effect was comparable to that of ranitidine treated rats. Level of glycoproteins was also found to be preserved in rats treated with the extract. The normal rats treated with the HAEAP did not show any abnormal alterations in the parameters studied. Histopathological observations also showed the ulcer preventing effect of the HAEAP (Saranya and Geetha 2011).

The gastroprotective efficacy of andrographolide was evaluated in rats induced with duodenal ulcers. Duodenal ulcers were induced by cysteamine administration in rats pretreated with 3mg kg(-1) BW day(-1) of andrographolide for 30 days. Ulcer score, myeloperoxidase activity, TBARS level, GSH/GSSG ratio and enzyme antioxidants were measured in the duodenal tissue. Brush border and basolateral membranes were isolated to assay sucrase, maltase, alkaline phosphatase and total ATPases. Ulcer score was significantly minimised in rats pretreated with andrographolide. Elevation in myeloperoxidase and TBARS levels were found to be minimised significantly due to andrographolide treatment. Membrane-bound enzyme activities and the thiol redox status of glutathione were significantly maintained in duodenal mucosa of rats that received andrographolide (Panneerselvam et al. 2011).

Effect on the central nervous system

Psychopharmacological studies were conducted on a methanol extract of *Andrographis paniculata* herbs. The extract exhibited a significant alteration in behaviour pattern and a reduction in spontaneous motility. The extract also produced a prolongation of the pentobarbitone-induced sleeping time and lowered the body temperature in different experimental animal models. The extract (100-300

mg/kg) showed a potent central nervous system (CNS) depressant action as indicated by its hypnotic potentiation effect, it produced hypothermia and exhibited an analgesic action against acetic acid-induced writhing in a dose dependent fashion. Reserpine and chlorpromazine have been shown to potentiate barbiturate hypnosis by virtue of their hypothermic action. Thus it was concluded that the extract may have the same mechanism of action. The effect of the extract was further investigated on other psychopharmacological properties, e.g. the exploratory behaviour pattern and muscle relaxant activity. The extract produced a significant inhibitory effect on the head dip and Y-maze tests in a dose dependent manner. A reduction in exploratory behaviour with the extract is in conformity with similar actions produced by other CNS depressant drugs.

The muscle relaxant activity study included chimney, traction and inclined screen tests. The extract exhibited significant motor incoordination and muscle relaxant activity. The residual curiosity as studied in the evasion test, was inhibited by the extract significantly. y (Mandal et al. 2001).

Hepatoprotective effect

The diterpenes andrographolide (I), andrographiside (II) and neoandrographolide (III) isolated from *Andrographis paniculata* were investigated for their protective effects on hepatotoxicity induced in mice by carbon tetrachloride or tert-butylhydroperoxide (tBHP) intoxication. Pretreatment of mice with the diterpenes (I, II & III; 100 mg/kg, i.p.) for 3 consecutive days produced significant reduction in malondialdehyde formation, reduced glutathione (GSH) depletion and enzymatic leakage of glutamic-pyruvate transaminase (GPT) and alkaline phosphatase (AP) in either group of the toxin-treated animals. A comparison with the known hepatoprotective agent silymarin revealed that I exhibited a lower protective potential than II and III, which were as effective as silymarin with respect to their effects on the formation of the degradation products of lipid peroxidation and release of GPT and AP in the serum. GSH status was returned to normal only by III. (KAPIL et al. 1993).

Administration of an alcohol extract of *Andrographis paniculata* (25 mg/kg) and two of its constituent diterpenes, andrographolide and neoandrographolide (6 mg/kg/day for two weeks) showed significant antihepatotoxic action in *P. berghei* K173- induced hepatic damage in M. natalensis. The increased levels of serum lipoprotein-X alkaline phosphatase, GOT, GPT and bilirubin were markedly reduced by *A. paniculata* and its diterpenes. In the liver, these preparations decreased the levels of lipid peroxidation products and facilitated the recovery of superoxide dismutase and glycogen. The protective effects of andrographolide were comparable to those of neoandrographolide (Chander et al. 1995).

Hepatoprotective effect of *Andrographis paniculata* and its constituent, andrographolide, was studied using ethanol as the hepatotoxin, Acute and subacute hepatotoxicities were induced in rats by varying doses of ethanol (2-6 g kg(-1)) and time of treatment (0-21 days). Serum transaminases (AST and ALT) and histopathology changes in the livers were estimated to monitor the hepatoprotective activity. Single dose pretreatment of andrographolide (20, 50, 100, 200 mg kg(-1), i.p.) and aqueous extract of *Andrographis paniculata* leaves (300, 500, 800, 1000 mg kg(-1), p.o.) 48 h and 4 h respectively, before ethanol, significantly reduced the toxicity induced by ethanol (4 g kg(-1), p.o.). Same results were obtained with 7 days pretreatment of andrographolide (100 mg kg(-1) day(-1), i.p.) and aqueous extract of *Andrographis paniculata* leaves (500 mg kg(-1) day(-1), p.o.). Since hepatic alcohol dehydrogenase activity was unaffected by these pretreatments, it is suggested that hepatoprotective mechanisms of andrographolide and *Andrographis paniculata* may not involve the metabolism of ethanol (Pramyothin et al. 1994).

The study of *Trivedi et al* aimed to analyze antioxidant properties of andrographolide. This study investigated the effect of andrographolide on the hepatocellular antioxidant defense system and lipid peroxidation of control mice, mice treated with hexachlorocyclohexane (BHC) only, and

andrographolide + BHC. Glutathione (GSH), glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GSH-Px), gamma-glutamyl transpeptidase (gamma-GTP), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation (LPO) are studied by spectrophotometric methods. The BHC experimental model forms an irreversible liver tumor in male mice. The activities of GSH, GR, GSH-Px, SOD, and CAT show significant (P <= .05) increases, while gamma-GTP and GST show significant decreases (P <= .05) in andrographolide supplemented mice as compared with BHC-treated mice. Antioxidant effect of andrographolide could be due to its ability to activate antioxidant enzymes that catalyze the reaction of oxidants and are effective in severe liver damage (Trivedi et al. 2007).

The ability of the extracts of *A. paniculata* plants to offer protection against acute hepatotoxicity induced by paracetamol (150 mg/kg) was studied in Swiss albino mice. Oral administration of *A. paniculata* extract (70% EtOH dry extract, yield: 5.8% w/w, 100-200 mg/kg) offered a significant dose dependent protection against paracetamol induced hepatotoxicity as assessed in terms of biochemical and histopathological parameters. The paracetamol induced elevated levels of serum marker enzymes such as serum glutamate pyruvate transaminase (GPT), serum glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP), and bilirubin in peripheral blood serum and distorted hepatic tissue architecture along with increased levels of lipid peroxides (LPO) and reduction of superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and glutathione peroxidase (GPx) in liver tissue. Administration of the plant extract after paracetamol iontoxication restored the levels of serum marker enzymes to control (untreated) levels (Nagalekshmi et al. 2011).

In an *in vivo* study, carbon tetrachloride challenge of rats at a dose of 1.2 ml/kg body weight-induced oxidative stress in the liver. This was evidenced by augmentation in lipid peroxidation, which was accompanied by a decrease in the activities of antioxidant enzymes and depletion in the level of reduced glutathione ($P<0.05$). Parrallel to these changes, CCl_4 challenge too, enhanced hepatic damage as evidenced by sharp increase in serum transaminases (e. g. alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase) ($P<0.05$). Additionally, the impairment of liver function corresponded to histolopathological changes. However, most of these changes were reversed in a dose-dependent fashion by pre-treatment of animals with *A. paniculata* ($P<0.05$). The ability of *A. paniculata* to scavenge the 2,2-Diphenyl-2-picrylhydrazyl radical was determined through its EC_{50} value. The EC_{50} value of *A. paniculata* was 583.60 +/- 4.25 mu g/ml. In addition, *A. paniculata* was found to contain 65.37 +/- 1.20 mg/g total phenolics expressed as gallic acid equivalent. From these studies, it is concluded that *A. paniculata* could be used as a hepatoprotective agent and possesses the potential to treat or prevent degenerative diseases where oxidative stress is implicated (Koh et al. 2011).

Neuroprotective effect

An *in vivo* study examined potential therapeutic effects of andrographolide on cerebral ischaemia using a rat model with permanent middle cerebral artery occlusion (pMCAO). The middle cerebral artery in rats was permanently occluded (by cautery), and 24 h later neurological effects were assessed with behavioural scores. Infarct volume and microglial activation were determined histologically. The p65 form of the transcription factor, nuclear factor-kappa B (NF-kappa B), was measured by Western blot, and cytokines by immunoassay of brain extracts. Andrographolide, given i.p. 1 h after pMCAO, reduced infarct volume with a maximum reduction of approximately 50% obtained at 0.1 mg center dot kg(-1). Neurological deficits were also reduced by andrographolide, reflecting a correlation between infarct volume and neurological deficits. pMCAO was found to induce activation of microglia and elevate tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 beta and prostaglandin (PG)E(2) in the ischaemic brain areas. Andrographolide (0.1 mg center dot kg(-1)) significantly attenuated or abolished these effects. In addition, andrographolide suppressed the translocation of p65 from cytosol

to nucleus, indicating reduced NF-kappa B activation. Andrographolide exhibited neuroprotective effects, with accompanying suppression of NF-kappa B and microglial activation, and reduction in the production of cytokines including TNF-alpha and IL-1 beta, and pro-inflammatory factors such as PGE(2). These findings suggest that andrographolide may have therapeutic value in the treatment of stroke (Chan et al. 2010).

Antithrombotic effects

In 16 dogs the endothelium of the left anterior descending coronary artery was injured mechanically. Then a copper wire was inserted into the lumen as a choke of flow and the vessel was slightly compressed from outside by a constrictor. Eight dogs had been pretreated with a preparation of flavone extracted from the root of *Andrographis paniculata* (TFAP). In the control group, saline solution was given, the epicardially recorded ST segment started to elevate within 15 minutes, the platelet aggregation rate and the plasma levels of TXB_2 increased rapidly, whereas the level of 6-k-PGF1-alpha remained stable. Platelet cGMP rose continuously; however, platelet cAMP rose only at 60 minutes. Histological findings confirmed the occurrence of arterial thrombus and myocardial necrosis. Contrariwise, in the pretreated group there was no elevation of the ST segment, plasma 6-k-PGF1-alpha and platelet cAMP were increased, the production of TXB_2 and aggregation of platelets were inhibited, and no thrombus or myocardial infarction was induced. All data suggest that TFAP might promote the synthesis of PGI_2, inhibit the production of TXA_2, stimulate the synthesis of cAMP in platelets, impede aggregation of platelets, and thereby prevent the formation of thrombi as well as the development of myocardial infarction (ZHAO and FANG 1991).

Cardiovascular effects

The cardiovascular activities of crude water extract (WE) of *Andrographis paniculata*, its three semi-purified ethyl acetate (FA), n-butanol (FB) and aqueous (FC) fractions, as well as andrographolide, a major plant constituent, were elucidated in anaesthetized Sprague-Dawley (SD) rats. FA and andrographolide, elicited no drop in mean arterial blood pressure (MAP), while WE, FB and FC produced a significant fall in MAP in a dose-dependent manner without significant decrease in heart rate. The ED_{50} values for WE, FB and FC 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 FB. Pharmacological antagonist studies were consequently only tested in FB (5 mg/kg). The hypotensive action of FB was not mediated through effects on the beta-adrenoceptor, muscarinic cholinergic receptor and angiotensin-converting enzyme, for it was not affected by propranolol, atropine and captopril, respectively. However, it seems to work via alpha-adrenoceptors, autonomic ganglion and histaminergic receptors, since the hypotensive effect of FB was negated or attenuated in the presence of phentolamine, hexamethonium as well as pyrilamine and cimetidine(Zhang and Tan 1997).

The hypotensive activity of an aqueous extract of *Andrographis paniculata* was studied using chronic intraperitoneal (i.p.) infusions by osmotic pumps. The extract exhibited a dose-dependent hypotensive effect on the systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR). The optimum hypotensive dose determined was repeated in a study in SHR and their normotensive controls, Wistar-Kyoto (WKY) rats, to demonstrate its comparative effects on the SBP, plasma and lung angiotensin-converting enzyme (ACE) activities, as well as on lipid peroxidation in the kidneys, as measured by the thiobarbituric acid (TBA) assay. The extract significantly lowered the SBP of both SHR and WKY rats. Plasma, but not lung, ACE activity and kidney TBA level were significantly lower in extract-treated SHR when compared with vehicle-treated SHR controls. Plasma and lung ACE activities as well as kidney TBA levels were not significantly different between extract- and vehicle-treated WKY rats. This study indicated that the aqueous extract of *A. paniculata* lowers SBP in the SHR possibly by reducing circulating ACE in the plasma as well as by reducing free radical levels in the kidneys, The mechanism(s) of hypotensive action seems to be different in WKY rats (Zhang and Tan 1996)

The cardiovascular activity of 14-deoxy-11,12-didehydroandrographolide (DDA) from *Andrographis paniculata* was elucidated in anaesthetised Sprague-Dawley (SD) rats and isolated rat right atria. In anaesthetised rats, DDA pro duced significant falls in mean arterial blood pressure (MAP) and heart rate in a dose-dependent manner with the maximum decrease of 37.6 +/- 2.6% and 18.1 +/- 4.8%; respectively. The ED_{50} value for MAP was 3.43 mmol/kg. Pharmacological antagonist studies were done using this dose. The hypotensive action of DDA was not mediated through effects on the alpha-adrenoceptor, muscarinic cholinergic and histaminergic receptors, for it was not affected by phentolamine, atropine as well as pyrilamine and cimetidine. However, it seemed to work via adrenoceptors, autonomic ganglia receptor and angiotensin-converting enzyme, since the hypotensive effect of DDA was negated or attenuated in the presence of propranolol, hexamethonium and captopril. In the isolated right atria, DDA caused negative chronotropic action and antagonised isoproterenal-induced positive chronotropic actions in a non-competitive and dose-dependent manner. These results support the bradycardia-inducing and beta-adrenoceptor antagonistic properties of DDA *in vivo*(Zhang et al. 1998).

The hypotensive activity of an aqueous extract of *Andrographis paniculata* was studied using chronic intraperitoneal (i.p.) infusions by osmotic pumps. The extract exhibited a dose-dependent hypotensive effect on the systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR). The optimum hypotensive dose determined was repeated in a study in SHR and their normotensive controls, Wistar-Kyoto (WKY) rats, to demonstrate its comparative effects on the SBP, plasma and lung angiotensin-converting enzyme (ACE) activities, as well as on lipid peroxidation in the kidneys, as measured by the thiobarbituric acid (TBA) assay. The extract significantly lowered the SBP of both SHR and WKY rats. Plasma, but not lung, ACE activity and kidney TBA level were significantly lower in extract-treated SHR when compared with vehicle-treated SHR controls. Plasma and lung ACE activities as well as kidney TBA levels were not significantly different between extract- and vehicle-treated WKY rats. This study indicates that the aqueous extract of A. paniculata lowers SBP in the SHR possibly by reducing circulating ACE in the plasma as well as by reducing free radical levels in the kidneys, The mechanism(s) of hypotensive action seems to be different in WKY rats(Zhang and Tan 1996).

The effects of *Andrographis paniculata* (Ap) and fish oil (FO, omega3 polyunsaturated fatty acids over 70%) on atherosclerotic stenosis and restenosis after experimental angioplasty and the relevant mechanisms of Ap and FO were studied *in vivo*. Preliminary results showed that Ap can significantly alleviate atherosclerotic iliac artery stenosis induced by both deendothelialization and high cholesterol diet (HCD) and restenosis following angioplasty in rabbits. FO showed the same but milder effects than Ap did. Both Ap and FO significantly inhibited blood monocytes to secrete growth factors in vivo. Ca^{2+} -ATPase activity of cell membrane of atherosclerotic rabbits was significantly decreased, while APN or FO, especially the former alleviated this reduction. Refined extract of Ap significantly decreased in vitro resting platelet $[Ca^{2+}]i$ and in vivo the resting and thrombin-stimulated platelet $[Ca^{2+}]i$ after oral administration of APN for 2 weeks. Ap significantly inhibited cell growth or DNA synthesis in dose-dependent manner. In conclusion because of the mechanisms described above, APN can alleviate atherosclerotic artery stenosis induced by both deendothelialization and HCD as well as lower restenosis rate after experimental angioplasty. The effects of APN are superior to those of FO (Wang and Zhao 1994).

Chiou et al investigated whether andrographolide, a diterpenoid lactone found at *Andrographis paniculata*, influences the induction of the inducible nitric oxide synthase (iNOS) in RAW264.7 cells activated by bacterial endotoxin (LPS), as well as in the rats with endotoxic shock and in aortic rings treated with LPS. Incubation of RAW264.7 cells with andrographolide (1 to 50 mu M) inhibited the LPS (1 mu g ml(-1))-induced nitrite accumulation in concentration- and time-dependent manners. Maximum inhibition was observed when andrographolide was added together with LPS and decreased progressively as the interval between andrographolide and LPS was increased to 20 h. Western blot

analysis demonstrated that iNOS expression was markedly attenuated in the presence of andrographolide for 6-24 h, suggesting that andrographolide inhibited iNOS protein induction. Thoracic aorta incubation with LPS (300 ng ml(-1)) for 5 h in vitro exhibited a significant decrease in the maximal contractile response to phenylephrine (10(-9)-10(-5) M). Andrographolide (30 mu M) restored the contractile response to control level. In anaesthetized rats, LPS (10 mg kg(-1), i.v.) caused a fall in mean arterial blood pressure (MAP) from 116 +/- 4 to 77 +/- 5 mmHg. The presser effect of phenylephrine (10 mu g ml(-1), i.v.) was also significantly reduced at 30, 60, 120 and 180 min after LPS injection. In contrast, animals pretreated with andrographolide (1 mg kg(-1), i.v., 20 min prior to LPS) maintained a significantly higher MAP when compared to LPS-rats given with vehicle. Administration of andrographolide 60 min after LPS caused a increase in MAP and significantly reversed the reduction of the presser response to phenylephrine. These results indicated that andrographolide inhibits nitrite synthesis by suppressing expression of iNOS protein in vitro. And, this inhibition of iNOS synthesis may contribute to the beneficial haemodynamic effects of andrographolide in endotoxic shock (Chiou et al. 1998).

The cardiovascular activities of crude water extract (WE) of *Andrographis paniculata*, its three semi-purified ethyl acetate (FA), n-butanol (FB) and aqueous (FC) fractions, as well as andrographolide were elucidated in anaesthetized Sprague-Dawley (SD) rats for the very first time. FA and andrographolide, which possesses multiple pharmacological activities, elicited no drop in mean arterial blood pressure (MAP), while WE, FB and FC produced a significant fall in MAP in a dose-dependent manner without significant decrease in heart rate. The ED50 values for WE, FB and FC 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 FB. Pharmacological antagonist studies were consequently only tested in FB (5 mg/kg). The hypotensive action of FB was not mediated through effects on the beta-adrenoceptor, muscarinic cholinergic receptor and angiotensin-converting enzyme, for it was not affected by propranolol, atropine and captopril, respectively. However, it seems to work via alpha-adrenoceptors, autonomic ganglion and histaminergic receptors, since the hypotensive effect of FB was negated or attenuated in the presence of phentolamine, hexamethonium as well as pyrilamine and cimetidine (Zhang and Tan 1997).

Choleretic effects

Andrographolide, a diterpene, isolated from *Andrographis paniculata* exhibited a strong choleratic action when administered intraperitoneally (i.p.) to albino rats. It induced an increase in bile flow together with a change in the physical properties of the bile secretion (Triphathi and Tripahti 1991).

Wound healing effect

An *in vivo* study was carried out to study the effect of topical application of *Andrographis paniculata* on the rate of wound enclosure and its histological features. A wound was created in four groups of rat in posterior neck region. Blank placebo was applied topically to the wounds of Group 1. Groups 2 and 3 were dressed with placebo containing 5% and 10% extracts of *A. paniculata*, respectively. Intrasite gel was applied topically to the wounds of Group 4. Macroscopical examination revealed that the rate of wound healing was significantly accelerated in the wound dressed with *A. paniculata* extract compared to the blank placebo. The wounds dressed with 10% extract or Intrasite gel healed earlier compared to the wounds dressed with placebo containing 5% A. paniculata extract. Histologically, wounds dressed with *A. paniculata* extracts showed markedly less scar width and contained large amounts of fibroblast proliferation. More collagen and less angiogenesis with absence of inflammatory cells were seen for wounds dressed with 10% *A. paniculata* compared to the blank placebo (Al-Bayaty et al. 2012).

Effect on progesterone level

The effect of the powdered extract of *Andrographis paniculata* leaves on blood progesterone content in rats was studied. Peroral administration of APE during the first 19 days of pregnancy in doses of 200, 600, and 2000 mg/kg (doses with 1 and 2 magnitude higher than the therapeutic dose in humans) does not exhibit any effect on the elevated level of progesterone in the blood plasma of rats (Panossian et al. 1999).

Effect on sexual functions

Effects of andrographolide on sexual functions, vascular reactivity and serum testosterone level in experimental animals were observed. The suspension of andrographolide in 5% DMSO was administered orally at the dose of 50 mg/kg to male ICR mice. The female mice involved in mating were made receptive by hormonal treatment. The mating behaviors, mounting latency and mounting frequency, were determined and compared with the standard reference drug sildenafil citrate. Administration of andrographolide significantly decreased the mounting latency at 120 and 180 min and increased mounting frequency at 180 min after treatment. In endothelium-intact rat aortic strips, norepineprine-induced contraction was reduced by preincubation with andrographolide. Administration of 50 mg/kg andrographolide orally to male mice once daily for 2, 4, 6 or 8 weeks had no significant effects on sperm morphology and motility. Interestingly, at week 4, serum testosterone level in mice treated with andrographolide was significantly increased when compared to the control. Thus, the effects of andrographolide on vascular response to norepinephrine and testosterone level observed in this study might be contributed to the sexual enhancing properties observed (Sattayasai et al. 2010).

Anticancer effect

The effects of *Andrographis paniculata* extract (APE) and its isolated compound andrographolide (ANDLE) were studied on cell-mediated immune responses in normal and tumor-bearing control animals. Treatment with APE and ANDLE significantly arm enhanced natural killer cell activity in normal (APE, 46.82% cell lysis; ANDLE, 40.79% cell lysis) and tumor-bearing animals (APE, 48.66% cell lysis; ANDLE, 42.19% cell lysis) on the fifth day, and it was observed earlier than in tumor-beating control animals (12.89% cell lysis on day 9). Antibody-dependent cellular cytotoxicity was also increased in APE (45.17% cell lysis on day 11) as well as ANDLE (39.92% cell lysis on day 11)-treated normal and tumor-bearing animals (APE, 47.39% cell lysis; ANDLE, 41.48% cell lysis on day 11) compared to untreated tumor-bearing control animals (maximum of 11.76% cell lysis on day 17). An early enhancement of antibody-dependent complement-mediated cytotoxicity was also observed by the administration of APE and ANDLE in normal as well as tumor-bearing animals. APE and ANDLE administration could significantly enhance the mitogen-induced proliferation of splenocyte, thymocyte, and bone marrow cells. Moreover, treatment of APE and ANDLE significantly elevated the production of interleukin-2 and interferon-gamma in normal and Ehrlich ascites carcinoma-bearing animals (Sheeja and Kuttan 2007).

Sheeya et al. studied the antiangiogenic activity of *Andrographis paniculata* extract (APE) and its major component andrographolide (ANDLE) using both in vitro and in vivo models. Intraperitoneal administration of APE and ANDLE 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-1 beta, IL-6, TNF-alpha and GM-CSF and the most potent angiogenic factor VEGF in angiogenesis induced animals. Treatment of APE and ANDLE significantly reduced this elevated levels. Moreover, VEGF mRNA level in B16F-10 cell line showed a reduced level of expression in the presence of APE and ANDLE. Serum NO level which was increased in B16F-10 melanoma injected control animals was also found to be significantly lowered by the administration of APE and ANDLE. Antiangiogenic factors such as TIMP-1 and IL-2 level was elevated in APE and ANDLE

treated angiogenesis induced animals. In the rat aortic ring assay APE and ANDLE inhibited the microvessel outgrowth at non toxic concentrations. These results demonstrate that APE and ANDLE 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 (Sheeja et al. 2007).

Pharmacokinetic and pharmacodynamic interactions

Effect on the CYP isoenzymes

Effects of *Andrographis paniculata* crude aqueous or ethanolic extract on cytochrome P450 (CYP) enzymes were studied 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 showed 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 affect hepatic cytochrome P450 enzymes of which CYP1A and CYP2B are the responsive P450 isoforms (Jarukamjorn et al. 2006). The crude extract of *Andrographis paniculata* increased hepatic CYP1A enzymes including ethoxyresorufin and methoxyresorufin activities. This corresponds with the inductive effects conveyed by andrographolide. Synergistic induction of CYP1A1 by co-treatment with andrographolide and a typical CYP1A inducer as well as a robust increase of CYP1A1 by andrographolide in which the induction was blocked by an AhR (arylhydrocarbon receptor) antagonist resveratrol, affirmed participation of AhR-mediated transcription activation on andrographolide-induced CYP1A1 expression (Jarukamjorn 2008).

The inhibitory effect of *Andrographis paniculata* extract (APE) and andrographolide (ANDon hepatic cytochrome P450s (CYPs) activities was examined using rat and human liver microsomes. For this purpose, CYPIA2-dependent ethoxyresorufin-O-deethylation, CYP2B1-dependent benzyloxyresorufin-O-dealkylation, CYP2B6-dependent bupropion hydroxylation, CYP2C-dependent tolbutamide hydroxylation, CYP2E1-dependent p-nitrophenol hydroxylation and CYP3A-dependent testosterone 6 beta-hydroxylation activities, were determined in the presence and absence of APE or AND (0-200 µM). APE inhibited ethoxyresorufin-O-deethylation activity in rat and human liver microsomes, with apparent K_i values of 8.85 and 24.46 µM, respictively. In each case, the mode of inhibition was noncompetitive. APE also inhibited tolbutamide hydroxylation both in rat and human microsomes with apparent K_i values of 8.21 and 7.51 µM, respectively and the mode of inhibition was mixed type. In addition, APE showed a competitive, inhibition only on CYP3A4 in human microsomes with K_i of 25.43 µ M. AND was found to be a weak inhibitor of rat CYP2E1 with a K_i of 61.1µM but did not affect human CYP2E1APE can cause drug-drug interactions in humans through CYP3A and 2C9 inhibition (Pekthong et al. 2008).

The ability of a 60% ethanolic *Andrographis paniculata* extract (APE) and andrographolide (AND), to modulate hepatic CYP expression was examined *in vivo* in rats and *in vitro* in rat and human hepatocyte cultures. After *in vivo* administration, APE at dose levels of 0.5 g/kg/day (i.e. 5 mg/kg/day AND equivalents) and at 2.5 g/kg/day (i.e. 25 mg/kg/day AND equivalents) and AND at dose levels of 5 and 25 mg/kg/day significantly decreased CYP2C11 activity. In primary cultures of rat and human hepatocytes, treatment with AND 50 mu M and APE-containing 50 mu M AND also resulted in significant decreases in CYP2C expression and activity. In addition, in human hepatocytes, treatment with APE and AND 50 mu M resulted in a decrease in CYP3A expression and activity (Pekthong et al. 2009).

The effects of andrographolide, the major diterpenoid constituent of *Andrographis paniculata*, on the expression of cytochrome P450 superfamily 1 members, including CYP1A1, CYP1A2, and CYP1B1, as well as on aryl hydrocarbon receptor (AhR) expression in primary cultures of mouse hepatocytes were investigated in comparison with the effects of typical CYP1A inducers, including benz[a]anthracene, beta-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Andrographolide significantly induced the expression of CYP1A1 and CYP1A2 mRNAs in a concentration-dependent manner, as did the typical CYP1A inducers, but did not induce that of CYP1B1 orAhR. Interestingly, andrographolide plus the typical CYP1A inducers synergistically induced CYP1A1 expression, and the synergism was blocked by an AhR antagonist, resveratrol. The CYP1A1 enzyme activity showed a similar pattern of induction (Jaruchotikamol et al. 2007).

Andrographolide and 14-deoxy-11,12-Didehydroandrographolide were evaluated for their effects on CYP1A2, CYP2D6 and CYP3A4 expressions in HepG2 cells. Quantitative RT-PCR and Western blot analysis were used to assess the mRNA and protein expression of the three CYPs. CYP3A4 enzyme activity was evaluated using P450-Glo (TM) Assays. The LanthaScreen (R) TR-FRET PXR (SXR) Competitive Binding Assay was used to determine if the compounds are potential PXR-ligands. Both diterpenoids inhibited the mRNA and protein expressions of CYP1A2, CYP2D6, and CYP3A4. Interestingly, the lowest concentration of both diterpenoids produced a more than 50% reduction in the mRNA and protein expression of CYP3A4 and this reduction was consistent with the enzyme activity. Further experiments revealed that both diterpenoids were also capable of attenuating the ability of dexamethasone to induce CYP3A4 expression, and 14-Deoxy-11,12-Didehydroandrographolide tended to bind to the PXR-LBD site in a concentration-dependent manner (Ooi et al. 2011).

In a study investigating the effects of selected Malaysian medicinal plant extracts towards human recombinant cytochrome P450 (CYP450) enzyme activities in vitro, *Andrographis paniculata* was also tested on the three main CYP450 enzyme activities of CYP2C9, CYP2D6 and CYP3A4. The abilities of the to inhibit human cytochrome P450 enzyme activities were analyzed using a luminescent assay. Andrographis paniculata showed negligible inhibition activity against CYP2C9. On the metabolism mediated by CYP2D6, inhibitory activity of *Andrographis paniculata* can be characterized with an IC(50) value of 442 +/- 4 5 µg mL(-1). *Andrographis paniculata* ethanolic extract gave the lowest IC_{50} value towards CYP3A4 with an apparent IC(50) value of 27 6 +/- 3 7 µg/mL). Sulfaphenazole, quinidine and ketoconazole were used as positive controls for CYP2C9, CYP2D6 and CYP3A4 respectively The findings suggest that *Andrographis paniculata* may contribute to herb-drug interactions if they are administered concomitantly with drugs metabolized by CYP2C9, CYP2D6 and CYP3A4 respectively (Hanapi et al. 2010).

The effects of *Andrographis paniculata* extracts and andrographolide were investigated on the catalytic activity of three human cDNA-expressed cytochrome P450 enzymes: CYP2C9, CYP2D6 and CYP3A4. *In vitro* probe-based high performance liquid chromatography assays were developed to determine CYP2C9-dependent tolbutamide methylhydroxylation, CYP2D6-dependent dextromethorphan O-demethylation and CYP3A4-dependent testosterone 6 beta-hydroxylation activities in the presence and absence of AP extracts and andrographolide. The results indicated that AP ethanol and methanol extracts inhibited CYP activities more potently than aqueous and hexane extracts across the three isoforms. Potent inhibitory effects were observed on CYP3A4 and CYP2C9 activities (K (i) values below 20 mu g/ml). Andrographolide was found to exclusively but weakly inhibit CYP3A4 activity (Pan et al. 2011).

The effects of *Andrographis paniculata* extract (APE) and its major component, andrographolide (AG), on the pharmacokinetics of theophylline, a typical substrate of cytochrome P450 1A2 enzyme, were investigated in rats. After APE or AG pretreatment for 3 days, on the fourth day rats were administered

theophylline via femoral vein cannula. The blood theophylline levels were monitored by microdialysis sampling combined with HPLC-UV. The results indicated that the clearance of theophylline was significantly increased and the area under concentration-time curve (AUC) was reduced in both AG and APE pretreated groups at low-dose theophylline administration (1 mg/kg). The elimination half-life (t(1/2 beta)) and mean residence time (MRT) of theophylline were shortened by 14% and 17%, respectively, in the AG pretreated group when high-dose theophylline (5 mg/kg) was given. However, theophylline accumulated in rat of the group with APE pretreatment. These results suggest that some other herbal components contained in APE may interact with theophylline and retard its elimination when theophylline was administered at a high dose (Chien et al. 2010).

Interaction with warfarin

Effects of concomitant treatment of rats with Kan Jang (a standardized fixed combination of extracts from *Andrographis paniculata* and *Eleutherococcus senticosus*) on the pharmacological effects of warfarin were studied by Hovhannisyan et al.. Each day for 5 days a group of animals was treated orally with an aqueous solution of Kan Jang at a dose of 17 mg/kg of the active principle andrographolide (a daily dose some 17-fold higher than that recommended for humans): the control group received similar treatment with appropriate volumes of water only. Sixty minutes after the final daily administration of Kan Jang or water, an aqueous solution of warfarin (0.2 mg/ml) was given to each animal at a dose of 2 mg/kg. From each group, 6 animals were sacrificed at 0, 2, 4, 6, 8, 12, 24, 30 and 48 h after warfarin administration and blood samples taken. The concentration of warfarin in blood plasma was measured by capillary electrophoresis using 50 mM borate buffer (pH 9.3) as mobile phase with simultaneous detection of warfarin at 208.1 and 307.5 nm. Prothrombin time in blood plasma was measured using thromboplastin reagent. The concomitant application of Kan Jang and warfarin did not produce significant effects on the pharmacokinetics of warfarin, and practically no effect on its pharmacodynamics (Hovhannisyan et al. 2006).

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

An HPLC-UV method was used to determine the content of andrographolide (AP) and 14-deoxy-11,12-didehydroandrographolide (DIAP) in rat plasma after oral dose of methanol extract (1 g/kg body weight) of *Andrographis paniculata* leaf. An increase in plasma concentration of AP and DIAP was observed from 30 min to 3 h after oral administration of the extract. The maximum plasma concentrations of AP and DIAP were 1.42 +/- 0.09 mu g/ml and 1.31 +/- 0.04 mu g/ml, respectively (Akowuah et al. 2009).

Validated analytical methods (HPLC, CE and CC-MS) for determining the amount of andrographolide (AND) in the blood plasma of rats following the oral administration of Andrographis paniculata extract (APE) was developed and used for the pharmacokinetic study. Andrographolide was quickly and almost completely absorbed into the blood following the oral administration of APE at a dose of 20 mg/kg body wt. in rats. Its bio-availability, however, decreased four-fold when a 10-times-higher dose was used. Since a large part (55 %) of AND is bound to plasma proteins and only a limited amount can enter the cells, the pharmacokinetics of AND are described well by a one-compartment model. Renal excretion is not the main route for eliminating AND. It is most likely intensely and dose dependently metabolized (Panossian et al. 2000).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Acute toxicity

Additionally in acute oral toxicity study, healthy female albino Wistar rats (8–12 weeks) were treated at 5000 mg/kg of KalmCold (TM) and observed for signs of toxicity for 14 days. KalmCold ™, suspended in carboxymethyl cellulose (1%), was administered by oral gavage in a sequential manner. On the day of dosing, all the animals were observed for mortality and clinical signs for first 10 min, 30 min, 1 h, 2 h, 4 h and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs, for 14 days. Cage side observations included changes in the skin, fur, eyes and mucous membrane. It also included respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavioral pattern. Attention was directed to the observation of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma. The body weight of rats was recorded and weekly body weight gain was calculated. Macroscopic examination was performed on animals found dead and animals sacrificed at the end of the study period of 14 days. KalmCold TM-treated rats survived till the end of the study period and did not show any treatment-related adverse clinical signs immediately following dosing and during the observation period of 14 days. KalmCold® at 5000 mg/kg did not reveal any major adverse effect on the body weight gain except for one female rat, which showed reduced body weight gain during the second week of 14-day observation period. Overall, the percent body weight gain during the complete 14-day observation period was found to be normal in all the treated animals. On necropsy, no major gross pathological changes were observed in any of the treated rats. (Chandrasekaran et al. 2009).

Reproductive toxicity

Andrographolide

The study of Akbarsha and Murugaian was undertaken to investigate whether andrographolide, one of the major constituents of this plant, is responsible for the antispermatogenic effect of the *Andrographis paniculata* leaf extract on male albino rats, The compound was administered to S-month-old male Wistar albino rats at two dose levels, for 48 days, Fertility tests, analysis of the counts, motility and abnormalities of the cauda epididymidal spermatozoa, and histopathological evaluation of the testis were carried out. The results showed that sperm counts decreased, the spermatozoa were not motile, and several of them possessed abnormalities. The seminiferous epithelium was thoroughly disrupted and in the seminiferous tubules, fully differentiated spermatozoa were far too limited; cells in the divisional stages were prevalent; multinucleate giant cells were abundant and Leydig cells appeared intact. It is inferred that andrographolide could affect spermatogenesis by preventing cytokinesis of the dividing spermatogenic cell lines. The multinucleate giant cells are comparable to the symplasts generated by cytochalasin-D and ursolic acid due to action at stages V-VII of the spermatogenic cycle, Sertoli cell damage and spermatotoxic effects are also apparent. Thus, the study points to a male reproductive toxic effect of this compound (Akbarsha and Murugaian 2000).

Andrographis extract

Andrographis paniculata dried extract posesses no acute (> 17 g/kg, LD_{50}) or subchronic toxicity. The possible testicular toxicity of *Andrographis paniculata* standardized dried extract was evaluated in male Sprague Dawley rats for 60 days. No testicular toxicity was found with the treatment of 20, 200 and 1000 mg/kg during 60 days as evaluated by reproductive organ weight, testicular histology, ultrastructural analysis of Leydig cells and testosterone levels after 60 days of treatment. It is

concluded that *Andrographis paniculata* dried extract did not produce subchronic testicular toxicity effect in male rats (Burgos et al. 1997).

The possible effect of extract of *Andrographis paniculata* extract standardized to >= 10% andrographolide, the main bioactive component, on male fertility in albino Wistar rats was evaluated, by orally administering 0, 20, 200, and 1000 mg/kg of body weight per day, for 65 days prior to mating and 21 days during mating. The treated groups showed no signs of dose-dependent toxicity. The body weight gain and feed consumption were not affected at any of the dose levels. The testosterone levels and fertility indices in treatment groups were found to be comparable with that of the control indicating no effect on fertility. Total sperm count and sperm motility were not affected. The testes and epididymides did not show any gross and histopathological changes. Based on these findings, it can be concluded that the no-observed adverse effect level of extract of *A paniculata* (>= 10% andrographolide) was found to be more than 1000 mg/kg per day (Allan et al. 2009).

Genotoxicity

The genotoxicity of a special extract of *A. paniculata* (KalmCold (TM)) was assessed through three different *in vitro* tests: Ames, chromosome aberration (CA), and micronucleus (MN). Ames test was performed at 5000 mu g/ml, 1581 mu g/ml, 500 mu g/ml, 158 mu g/ml, 50 mu g/ml, 16 mu g/ml, while the clastogenicity tests were performed at 80 mu g/ml. 26.6 mu g/ml, 8.8 mu g/ml for short-term treatment without S9; 345 mu g/ml, 115 mu g/ml, 38.3 mu g/ml for short-term treatment with S9: and 46 mu g/ml, 15.3 mu g/ml, 5.1 mu g/ml for long-term without S9 using DMSO as a vehicle control. Results of Ames test confirmed that KalmCold (TM) did not induce mutations both in the presence and absence of S9 in Salmonella typhimurium mutant strains TA98 and TAMix. In CA and MN, KalmCold (TM) did not induce clastagenicity in CHO-K1 cells *in vitro* (Chandrasekaran et al. 2009).

3.4. Overall conclusions on non-clinical data

Several *in vitro* experiments have been published on the different activities (antiviral, antimicrobial, antimalarial, antioxidant, immunomodulatory, anti-inflammatory, vasorelaxing, cytotoxic and antitumour, anti-apoptotic, chemopreventive, hepatoprotective, spasmolytic, uterorelaxant, antithrombotic) of different extracts of Andrographis paniculata. Antidiabetic, antioxidant, anti-inflammatory, antinociceptive, antiallergic, immunomodulatory, gastroprotective, CNS, hepatoprotective, neuroprotective, antithrombotic, cardiovascular, choleretic, wound healing, antifeedenat, repellent, larvicidal, ovicidal, anticancer effects were assessed in *in vivo* animal expertiments. Although there is a clear effect on some CYP isoenzymes, the available acute and reproductive toxicity and genotoxicity data support the safety of Andrographis. There is a large variety of preclinical data on Andrographis, and this is in line with the versatile traditional application of the plant.

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Effect on the NO and IL-6 levels

Familial Mediterranean fever (FMF) is a recessively inherited inflammatory disorder, characterised by recurrent attacks of fever and serositis. Since nitric oxide (NO) is an important mediator of inflammation, the production of NO (assessed as the accumulation of nitrate and nitrite and measured

by capillary electrophoresis) in blood plasma of FMF patients during acute attacks (active) and attack-free periods (inactive) of the disease has been determined and compared with NO levels found in healthy volunteers (control group C). Thirty-six FMF patients were involved in a placebo-controlled double-blind study (group A received the drug, group B the placebo) of the effects of a multicomponent herbal preparation (combination of extracts of Andrographis paniculata Herba Nees, Eleutherococcus senticosus Maxim., Schizandra chinensis Bail., and Glycyrrhiza glabra L.) which is applied to relieve the severity and longevity of FMF attacks on NO blood levels. Thirty-two FMF patients (group D) being permanently treated with colchicine were also examined with respect to their NO blood level. No significant differences were found between the NO levels in blood of inactive FMF patients and those of control group C, or between inactive colchicine-treated group D patients and inactive patients of groups A and B, a finding which is atypical for chronic inflammatory disorders. Significantly lower plasma NO levels were found in active FMF patients in groups A and B compared with inactive patients in those groups ($p = 0.031$ and 0.036, respectively) and with patients of group D and the control group C ($p = 0.0235$ and 0.0453, respectively). The decrease of NO in blood of FMF patients may trigger the generation of fever by initiating the production of pro-inflammatory IL-6. Plasma NO levels in inactive FMF patients were significantly increased during attack-free periods following treatment with the herbal preparation. The preparation has a normalising effect both on NO and IL-6 blood levels in FMF patients during attacks, demonstrating a relationship between the beneficial effect of the herbal peparation in reducing the severity of inflammatory attacks in FMF patients and the increase in NO blood levels (Panossian et al. 2003).

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Validated analytical methods (HPLC, CE and CC-MS) for determining the amount of andrographolide (AND) in the blood plasma of human volunteers following the oral administration of Andrographis paniculata fixed combination Kan Jang tablets was developed and used for the pharmacokinetic study. Following the oral administration of four Kan Jang tablets (a single therapeutic dose, equal to 20 mg of AND) to humans, maximum plasma levels of approximately 393 ng/ml (approx. 1.12 muM) were reached after 1.5-2 hours, as quantified using a UV diode-array detection method. Half-life and mean residence times were 6.6 and 10.0 hours, respectively. AND pharmacokinetics in humans are explained well by an open two-compartment model. The calculated steady state plasma concentration of AND for multiple doses of Kan Jang (after the normal therapeutic dose regimen, 3 x 4 tablets/day, about 1 mg AND/kg body wt./day) was approximately 660 ng/ml (approx. 1.9 muM), and after drug uptake the concentration of AND in blood was about 1342 ng/ml (approx. 3.8 muM) (Panossian et al. 2000).

4.2. Clinical Efficacy

4.2.1. Dose response studies

No data

4.2.2. Clinical studies (case studies and clinical trials)

Upper respiratory tract infection

A randomized, double blind placebo controlled clinical study was conducted to evaluate the efficacy of ***KalmCold™***, an ***extract of Andrographis paniculata***, in patients with uncomplicated upper respiratory tract infection (URTI). The assessment involved quantification of symptom scores by Visual Analogue Scale. Nine self evaluated symptoms of cough, expectoration, nasal discharge, headache, fever, sore throat, earache, malaise/fatigue and sleep disturbance were scored. A total of 223 patients

of both sexes were randomized in two groups which received either KalmCold(TM) (200 mg/day) or placebo in a double blind manner. In both the treatments, mean scores of all symptoms showed a decreasing trend from day 1 to day 3 but from day 3 to day 5 most of the symptoms in placebo treated group either remained unchanged (cough, headache and earache) or got aggravated (sore throat and sleep disturbance) whereas in KalmCold(TM) treated group all symptoms showed a decreasing trend. Within groups, mean scores of symptoms in both the groups decreased significantly (p 0.05). The comparison of overall efficacy of KalmCold(TM) over placebo was found to be significant (p <= 0.05) and it was 2.1 times (52.7%) higher than placebo (Saxena et al. 2010).

A double blind, placebo-controlled, parallel-group clinical study was carried out to evaluate the effect of an *Andrographis paniculata* extract ***SHA-10 fixed combination***, Kan Jang, in the treatment of acute upper respiratory tract infections, including sinusitis. Ninety-five individuals in the treatment group and 90 individuals in the placebo group completed the study according to the protocol. The medication was taken for 5 days. Temperature, headache, muscle aches, throat symptoms, cough, nasal symptoms, general malaise and eye symptoms were taken as outcome measures with given scores. The total score analysis showed a highly significant improvement in the verum group versus the placebo. This result applied to the group as a whole and to the sinusitis subgroups. The individual symptoms of headache and nasal and throat symptoms together with general malaise showed the most significant improvement while cough and eye symptoms did not differ significantly between the groups. Temperature was moderately reduced in the verum group. It can be concluded that Kan Jang has a positive effect in the treatment of acute upper respiratory tract infections and also relieves the inflammatory symptoms of sinusitis. The study drug was well tolerated (Gabrielian et al. 2002).

Two randomized double-blind, placebo-controlled parallel group clinical trials were performed to investigate the effect of a standardized extract (***SHA-10***) of Andrographis paniculata ***fixed combination*** (Kan jang) in the treatment of uncomplicated upper-respiratory tract infections. 46 patients in the pilot study and 179 patients in the phase III study completed the study according to the protocol. Medication was taken three times daily for a minimum of 3 days and a maximum of 8 days for the pilot study, and for exactly three days in the phase III study. The primary outcome measures in the patients self-evaluation were: related to pain in the muscle, cough, throat symptoms, headache, nasal symptoms and eye symptoms and temperature. The physician's fixed score diagnosis was based mainly on sign/symptoms: ears, nose, oral cavity, lymph glands-tonsils and eyes. The total symptom score showed a tendency toward improvement in the pilot study (p = 0.08), while both the total symptom score and total diagnosis score showed highly significant improvement (p less than or equal to 0.0006 resp. 0.003) in the verum group as compared with the placebo. In both studies throat symptoms/signs, were found to show the most significant improvement (Melchior et al. 2000).

Common cold

In a placebo-controlled double-blind study, the therapeutic effect of *Kan Jang* ***SHA-10 fixed combination*** tablets made from *Andrographis paniculata* dried extract was tested in patients with common colds. The patients were divided in two groups, in which group 1 (n=33) received 1200 mg of Andrographis paniculata and group 2 (n=28) a placebo (P). On day 3-4 after treatment the possible effect of Kan Jang tablets on selected symptoms and clinical signs of common cold was evaluated. A significant reduction in clinical symptoms at day 4 of administration of the Kan Jang tablets was observed. A better efficacy against the placebo is discussed. The differences in the total 'sumscores' of clinical and symptomatic findings indicate that the Kan Jang treated group did far better than the placebo group. It was concluded that Kan Jang in a dose of 1200 mg daily has the capacity to significantly shorten the course/duration of the disease and therefore is indicated for an enhanced resistance to common cold (Hancke et al. 1995).

A three-arm study comparing the efficacy of ***SHA-10 fixed combination*** containing standardized Andrographis paniculata, with Immunal, a preparation containing Echinacea purpurea (L.) extract, in uncomplicated common colds was carried out in 130 children aged between 4 and 11 years over a period of 10 days. The study was designed as an adjuvant treatment of Kan Jang and Immunal with a standard treatment. The patients were assigned to one of the three groups. In control group C; 39 patients received only standard treatment. Kan Jang and Immunal were used as an adjuvant to this therapy in the other two groups. Adjuvant group A; 53 patients treated with Kan Jang tablets concomitant to standard treatment, and adjuvant control group B; 41 patients treated with concomitant Immunal. It was found that the adjuvant treatment with Kan Jang, was significantly more effective than Immunal, when started at an early stage of uncomplicated common colds. The symptoms of the disease were less severe in the Kan Jang group. The effect of Kan Jang was particularly pronounced in two objective parameters, amount of nasal secretion g/day and nasal congestion. Kan Jang also accelerated the recovery time, whereas Immunal did not show the same efficacy. The use of standard medication was significantly less in the Kan Jang adjuvant group than in either the Immunal or standard treatment group. Kan Jang treatment was well tolerated and no side effects or adverse reactions were reported (Spasov et al. 2004).

In a controlled, double-blind study performed with ***SHA-10 fixed combination*** (Kan Jang) tablets as a pilot trial at the Health Center, Hallehalsan, during the autumn of 1992, conducted in 50 patients, both subjective symptoms as well as duration of the symptoms of common cold were significantly reduced. From the results evaluated it is quite clear that Kanjang(R) tablets decrease the subjective symptoms of common cold as well as shortening of the period of sick leave significantly (Melchior et al. 1997).

The objective of the study of Caceres et al. was to measure the effectiveness of Andrographis paniculata ***SHA-10 fixed combination*** extract in reducing the prevalence and intensity of symptoms and signs of common cold as compared with a placebo. A group of 158 adult patients of both sexes completed the randomized double blind study in Valdivia, Chile. The patients were divided in two equal size groups, one of which received Andrographis paniculata dried extract (1200 mg/day) and the other a placebo during a period of 5 days. Evaluations for efficacy were performed by the patient at day 0,2, and 4 of the treatment; each completed a self-evaluation (VAS) sheet with the following parameters: headache, tiredness, earache, sleeplessness, sore throat, nasal secretion, phlegm, frequency and intensity of cough. In order to quantify the magnitude of the reduction in the prevalence and intensity of the signs and symptoms of common cold, the risk (Odds Ratio = OR) was calculated using a logistic regression model. At day 2 of treatment a significant decrease in the intensity of the symptoms of tiredness (OR = 1.28; 95 % CI 1.07-1.53), sleeplessness (OR = 1.71; 95 % CI 1.38-2.11), sore throat (OR = 2.3; 95 % CI 1.69-3.14) and nasal secretion (OR = 2.51; 95 % CI 1.82-3.46) was observed in the Andrographis SHA-10 group as compared with the placebo group. At day 4, a significant decrease in the intensity of all symptoms was observed for the Andrographis paniculata group. The higher OR values were for the following parameters: sore throat (OR = 3.59; 95 % CI 2.04-5.35), nasal secretion (OR = 3.27; 95 % CI 2.31-4.62) and earache (OR = 3.11; 95 % CI 2.01-4.80) for Andrographis paniculata treatment over placebo, respectively. It is concluded that Andrographis paniculata had a high degree of effectiveness in reducing the prevalence and intensity of the symptoms in uncomplicated common cold beginning at day two of treatment. No adverse effects were observed or reported (Caceres et al. 1999).

In a randomized placebo-controlled double blind study, the possible preventive effect against common colds of ***SHA-10 fixed combination*** (Kan Jang) tablets containing Andrographis paniculata dried extract was tudied during the winter season. The study was carried in a rural school. The students were divided in two groups, of which Group 1 (n=54), received 2 tablets of Kan Jang per day and Group 2 (n=53), 2 tablets of a placebo (P) per day during three months. The individuals were

evaluated weekly by a clinician who diagnosed the presence or absence of common colds during the three months. The analysis of the occurrence of colds revealed that the administration of Kan Jang after the first month did not produced any significant difference. 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 rate of incidence of colds among the students treated with Kan Jang was 30% (16/54) compared to 62% (33/53). The relative risk of catching a cold was therefore 2.1 (1.32-3.33, 95% confidence interval) times lower for the Kan Jang group. The attributable protective effect of Kan Jang was 33% (Caceres et al. 1997).

Pharyngotonsillitis

152 adult patients with pharyngotonsillitis were enrolled in the randomized double blind study to assess the efficacy of Andrographis paniculata. The patients were randomized to receive either paracetamol or 3 g/day of *Andrographis paniculata* dry leaves (containing at least 6% total lactones calculated as andrographolide) or 6 g/day of *Andrographis paniculata* dry leaves for 7 days. The baseline characteristics of the patients among the three groups were not different. The efficacy of paracetamol or high dose Andrographis paniculata was significantly more than that of low dose *Andrographis paniculata* at day 3 in terms of the relief of fever and sore throat. The clinical effects were not different at day 7. Minimal and self limiting side effects were found in about 20 per cent in each group (Thamlikitkul, 1991).

Rheumatoid arthritis

A prospective, randomized, double blind, and placebo-controlled study in patients with rheumatoid arthritis (RA) was performed. Tablets (***Paractin®***) made of an extract of *A. paniculata* (30% total andrographolides) were administered three times a day for 14 weeks, after a 2-week washout period to 60 patients with active RA. The primary outcomes were pain intensity measured using a horizontal visual analog pain scale (VAPS). In addition, ACR, EULAR, and SF36 clinical parameters were recorded. The intensity of joint pain decreased in the active vs placebo group at the end of treatment, although these differences were not statistically significant. A significant diminishing for week in tender joint -0.13 95% confidence interval (CI; -0.22 to 0.06; p = 0.001), number of swollen joints -0.15 95%CI (-0.29 to -0.02; p = 0.02), total grade of swollen joint -0.27 95%CI (-0.48 to -0.07; p = 0.010), number of tender joints -0.25 95%CI (-0.48 to -0.02; p = 0.033), total grade of swollen joints -0.27 95%CI (-0.48 to -0.07; p = 0.01), total grade of tender joints -0.47 95%CI (-0.77 to -0.17; p = 0.002) and HAQ -0.52 95%CI (-0.82 to -0.21; p < 0.001) and SF36 0.02 95%CI (0.01 to 0.02; p < 0.001) health questionnaires was observed within the group with the active drug. Moreover, it was associated to a reduction of rheumatoid factor, IgA, and C4 (Burgos et al. 2009).

Ulcerative colitis

A randomised, double-blind, multicentre, 8-week parallel group study was conducted using ***HMPL-004*** 1200 mg/day compared with 4500 mg/day of slow release mesalazine (mesalamine) granules in patients with mild-to-moderately active ulcerative colitis. to determine the efficacy and safety of HMPL-004 in patients with mild-to-moderate ulcerative colitis. Disease activity was assessed at baseline and every 2 weeks for clinical response, and at baseline and 8 weeks by colonoscopy. One hundred and twenty patients at five centres in China were randomised and dosed. Clinical remission and response were seen in 21% and 76% of HMPL-004-treated patients, and 16% and 82% of mesalazine-treated patients. By colonoscopy, remission and response were seen in 28% and 74% of HMPL-004-treated patients and 24% and 71% of mesalazine-treated patients, respectively. There was no significant difference between the two treatment groups (Tang et al. 2011).

Familial Mediterranean fever

A double blind, randomized, placebo controlled pilot study of a standardized ***fixed combination*** of Andrographis paniculata Nees., Eleutherococcus senticosus Maxim., Schizandra chinensis Bail., and Glycyrrhiza glabra L. special extracts standardized for the content of Andrographolide (4 mg/tablet), Eleuteroside E, Schisandrins and Glycyrrhizin, was carried out in two parallel groups of patients. The study was conducted in 24 (3-15 years of both genders) patients with Familial Mediterranean Fever (FMF), 14 were treated with tablets of series A (verum) and 10 patients received series B product (placebo). The study medication was taken three times of four tablets daily for 1month. Daily dose of the andrographolide - 48 mg. The primary outcome measures in physician's evaluation were related to duration, frequency and severity of attacks in FMF patients (attacks characteristics score). The patient's self-evaluation was based mainly on symptoms - abdominal, chest pains, temperature, arthritis, myalgia, erysipelas-like erythema. All of 3 features (duration, frequency, severity of attacks) showed significant improvement in the verum group as compared with the placebo. In both clinical and self evaluation the severity of attacks was found to show the most significant improvement in the verum group (Amaryan et al. 2003).

Effect of semen quality

The safety of different doses of ***SHA-10 fixed combination*** (Kan Jang®) compared to two extensively used medicinal plants, *Valeriana officinalis* and *Panax ginseng* in the form of standardized extracts, has been examined. A phase I clinical study was designed to evaluate the effect on semen quality of healthy males in terms of spermatogenesis and quality of semen. The results of the study revealed no significant negative effect of Kan Jang on male semen quality and fertility, but rather a positive trend with respect to the number of spermatozoids in the whole ejaculate, the percentage of active (normokinetic) forms of spermatozoids, and fertility indexes, together with a decrease in the percentage of inactive (diskinetic) forms of spermatozoids. In the group receiving ginseng, no significant negative effects on the fertility parameters were revealed and there was a clear decrease in the percentage of diskinetic forms of spermatozoids. Subjects receiving valerian showed a temporary increase in the percentage of normokinetic spermatozoids and a decrease in diskinetic forms, but these changes had no effect on fertility indices. The results indicate that Kan Jang, ginseng and valerian are safe with respect to effects on human male sterility when administered at dose levels corresponding to approximately 3 times the human daily dose (Mkrtchyan et al. 2005).

4.2.3. Clinical studies in special populations (e.g. elderly and children)

No data.

4.3. Overall conclusions on clinical pharmacology and efficacy

The efficacy of *Andrographis* was assessed in the treatment of respiratory diseases, including upper respiratory tract infections ((Saxena et al. 2010), (Gabrielian et al. 2002), (Melchior et al. 2000)), common cold ((Caceres et al. 1997), (Caceres et al. 1999), (Melchior et al. 1997), (Spasov et al. 2004), (Hancke et al. 1995) and pharyngotonsillitis (Thamlikitkul, 1991). In most of the studies the composite Kan Jang® (SHA-10) preparation was applied. The randomized, double blind placebo controlled clinical study of *Saxena et al.* evaluated the efficacy of a monopreparation (KalmCold™) in upper respiratory tract infection using a visual analogue scale (Saxena et al. 2010), *Tamlikitkul et al.* assessed the efficacy of dry Andrographis leaves in pharyngotonsillitis in a randomized double blind study (Thamlikitkul, 1991). These two studies with monocomponent preparations are not sufficient to grant a well-established use monograph for *Andrographidis folium*. In case of the study of *Saxena et al.* the lack of objective end points, in study carried out by *Tamlikitkul et al.* the lack of placebo group makes the assessment of efficacy difficult.

In the study with patients with rheumatioid arthritis (Burgos et al. 2009) Andrographis leaf and aerial parts were applied, the efficacy in ulcerative colitis was assessed in a study without placebo control (Tang et al. 2011).

In conclusion, a well-established monograph cannot be granted to *Andrographis paniculata*, leaf, and the efficacy is not plausible because of the lack of evidence of traditional application.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

A phase I dose-escalating clinical trial of andrographolide from Andrographis paniculata was conducted in 13 HIV positive patients and five HIV uninfected, healthy volunteers. The objectives were primarily to assess safety and tolerability and secondarily to assess effects on plasma virion HIV-1 RNA levels and CD4(+) lymphocyte levels. No subjects used 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. All adverse events had resolved by the end of observation. A significant rise in the mean CD4(+) lymphocyte level of HIV subjects occurred after administration of 10 mg/kg andrographolide (from a baseline of 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 (Calabrese et al. 2000).

In a randomized, double-blind, placebo controlled clinical study the monopreparation KalmColdgroup had total of six patients (6/112) suffering from minor adverse effects, one patient each with vomiting, epistaxis, urticaria and three with diarrhoea. Of the three with diarrhoea, in addition one each had nausea or lethargy. The placebo group had three patients (3/110) with adverse effects, one each with diarrhoea, vomiting (both mild in severity) and moderate rigor. The adverse effects between two groups were found to be same (Z=0.63, p>0.05). In eight patients the effects were mild and isolated, and in one patient the effect was moderate and isolated. Except for vomiting (patient in KalmCold group) and urticaria, all other effects stopped spontaneously without any medical aid (Saxena et al., 2010).

5.2. Patient exposure

<Rapporteur to include text>

5.3. Adverse events and serious adverse events and deaths

None reported.

5.4. Laboratory findings

No data

5.5. Safety in special populations and situations

The safety during pregnancy, lactation has not been studied.

5.6. Overall conclusions on clinical safety

<Rapporteur to include text>

6. Overall conclusions

Due to the lack of appropriate studies, the requirements for well-established use is not fulfilled. The evidence of traditional use (long-standing use of preparation(s) with specific posology) is considered insufficient.

Annex

List of references