EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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EMA/HMPC/320433/2012 *Corr*[1]
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)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Andrographis paniculata* Nees, folium
Herbal preparation(s)	
Pharmaceutical form(s)	
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[1] Minor additional information on plant parts/extracts included on page 10, 18, 20 and 24.

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Table of contents

1. Introduction

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

- Herbal substance(s)

Andrographidis paniculatae folium consists of the leaves of *Andrographis paniculata* Nees (Acanthaceae). It is not covered by any pharmacopoeial monograph officially in use in the European Union (EU).

Other herbal substances have been described:

- Andrographidis herba consisting of the dried aerial parts of *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae) is described in a WHO monograph (2004) and in the Pharmacopoeia of the People's Republic of China (PPRC 2005);

- the entire dried plant of *Andrographis paniculata* Nees, referred to as Creyat, Kiryat and Kreat, is described in The British Pharmaceutical Codex (BPC 1934);

- the whole herb is listed in the Indian Materia Medica (1976).

Major constituents

The major constituents are diterpene lactones (free and in glycosidic forms) including andrographolide, deoxyandrographolide, 11,12-didehydro-14-deoxyandrographolide, neoandrographolide, andrographiside, deoxyandrographiside and andropanoside (WHO 2004).

Of these diterpene lactones, andrographolide is considered to be the most biologically active constituent. Therefore, most of the quantification of *Andrographis paniculata* extracts is exclusively based on the content of andrographolide (Naik and Hule 2009).

- Herbal preparation(s)

Preparations described in the literature:

- Extract of leaves of *Andrographis paniculata* (about 17:1) extraction solvent methanol (Saxena *et al.* 2010)
- Extract of leaves and aerial parts of *Andrographis paniculata* (10:1) extraction solvent 75% ethanol (Burgos *et al*. 2009)
- Extract of leaves of *Andrographis paniculata* extraction solvent: the ethanol/water (90 /10 v/v); 8 - 10% andrographolide content (Tang *et al*. 2011).

Combination products containing *Andrographis paniculata* extract described in the literature:

- One capsule comprises 85 mg of the standardised extract of *Andrographis paniculata* (plant part not specified) equivalent to 5 mg of andrographolides, as well as 10 mg extract of *Eleutherococcus senticosus* (plant part not specified) extract equivalent to 120 mg of the crude drug per tablet (Gabrielian *et al*. 2002, Spasov *et al*. 2004, Melchior *et al*. 2000);

- A combination product (370 mg tablet) of *Andrographis paniculata*Herba Nees special extract (50 mg) standardized for content of adrographolide 4 mg, *Eleutherococcus senticosus* special extract *(*10 mg) standardized for content of Eleutherosid (E >0.8mg), *Schizandra chinensis* special extract (100 mg) standardized for content of Schisandrins (>0.8 mg), and *Glycyrrhiza glabra* L. extract (10 mg) standardized for the content of Glycyrrhizin (> 0.6 mg) (Amaryan *et al.* 2003);

- A liquid extract (about 1 in 2) of the whole plant prepared with boiling water and addition of different further constituents, like 2% of fennel oil, 2% of ajowa oil, and 55 - 60% ethanol and then adjusted to a 5% content of andrographolides (Hagers Handbuch der Pharmazeutischen Praxis 1972, Reynolds Martindale the extra Pharmacopoeia 1982).

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:	
Croatia	☐ 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 authorised 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
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 using 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 2.2.). *Andrographis paniculata* is described in "Indigenous drugs of India", a book published in 1896 (Dey and 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 of medicinal use in the EU could be confirmed from the literature or based on the regulatory status overview, according to available information.

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

The WHO monograph on Andrographidis herba (2004) lists three categories of medicinal uses, depending on the nature of the supportive data.
- the uses supported by clinical data are: 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;
- the uses described in pharmacopoeias are: treatment of bacillary dysentery, bronchitis, carbuncles, colitis, coughs, dyspepsia, fevers, hepatitis, malaria, mouth ulcers, sores, tuberculosis and venomous snake bites;
- the uses described in folk medicine, not supported by experimental data, are: 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. It is a predominant constituent of at least 26 Ayurvedic formulations used to treat liver disorders (Naik and Hule 2009, Ganguly *et al*. 2000, Varma *et al*. 2011).

It 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).

The medical use of *Andrographis paniculata* against sore throat has a long history 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 includes 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

The following herbal preparations and products are described in the literature:

- In Indian Materia Medica (1976)

Dried leaves – about 10 grains (with 20 grains of black–pepper).

(1 grain=64.79891 milligrams)

Succus (concentrated expressed juice) of fresh leaves and stalks, 1 in 4 of the drug; dose: 10 - 60 minims.

Compound infusion (1 in 20) containing orange peel and coriander, each 1 - 4 of the drug; dose 1 to 2 ounces.

Compound tincture (3 in 20) containing myrrh and aloes, each 1 - 6 of the drug; dose: 1 - 4 drachms

Compound pill or tablet containing cumin, aniseed, cloves and greater cardamoms, all in equal parts, mixed in juice of Kalmegh; dose: 2 - 5 grains.

Infusion *Andrographis*: dose ½ to 1 fl. oz.

Tinctura *Andrographis*: dose ½ to 1 fl. drachm.

Kalmegh Resin: dose ½ to 2 grains.

- In the WHO monograph (2004)

Crude drug, capsules, tablets and pills.

Posology, unless otherwise indicated:

For pyrexia: a decoction from 3 g crude drug, twice daily. For the common cold: 1.5 - 3 g powdered crude drug three times daily, after meals and at bedtime. For diarrhoea: a decoction from 3 - 9 g crude drug as a single dose as needed, or two tablets of 500 mg four times daily, after meals and at bedtime

- In the Hagers Handbuch (Kern 1972) and The Martindale (Reynolds 1982)

A liquid extract (about 1 in 2) of the whole plant prepared with boiling water and addition of different further constituents, like 2% of fennel oil, 2% of ajowa oil, and 55 - 60% ethanol. The extract is adjusted to a content of 0.5% of andrographolides. Dose: 0.5 – 1 ml.

- In the publication by Saxena *et al.* (2010)

A special extract of Andrographidis paniculatae folium was investigated by Saxena *et al*. (2010) in the indication of uncomplicated upper respiratory tract infection where the daily dose was one capsule twice a day with glass of water after breakfast and dinner. Each capsule contains 100 mg of the extract. The preparation of this very special extract is described as follows: 300 kg of the coarse ground leaves were extracted in a reflux process with 1,200 l of methanol over 3 h. Next 1,000 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.

- In the publication by Burgos *et al.* (2009)

A clinical study in the indication of rheumatoid arthritis was conducted by Burgos *et al*. (2009) with the extract obtained from leaves and aerial parts of *Andrographis paniculata* (extraction solvent: 75% ethanol, ratio of herbal drug to extract 10:1). The posology was 100 mg of extract three times a day.

- In the publication by Tang *et al.* (2011)

An ethanol/water (90/10 v/v) extract of *Andrographis paniculata*, folium. One capsule contains 200 mg *Andrographis paniculata* extract with 8 - 10% andrographolide content. The daily dose was 600 – 1,200 mg of the extract in the indication of ulcerative colitis in a clinical study performed by Tang *et al*. (2011).

Assessor's conclusion

There is no monocomponent herbal preparation for which 15 years of medicinal use in the EU could be confirmed from the literature or based on the regulatory status 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

Twenty-five µg/ml of an ethanolic extract from *Andrographis paniculata* aerial parts and 5 µg/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 immediate-early 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 µg/ml (Lin *et al*. 2008).

Antimicrobial effect

Non-polar (dichloromethane) and polar (methanolic 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: 1,000, 500 and 250 µg/disc respectively. The extracts showed antibacterial activities against both Gram-positive and Gram-negative bacterial strains tested. Highest antibacterial activity was exerted by the aqueous extract against *M. luteus* at 1,000 µg/disc concentration with a 23.17 ± 0.76 mm of inhibition zone. This value is 14.00 ± 00 mm for gentamicin and 21.67 ± 0.52 mm for tetracycline at 30 µg/disc concentration. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) observed were between 150 - 300 µg/ml and 250 to 400 µg/ml respectively, depending on microorganism and the type of extract. Time-kill experiments indicated that *Andrographis paniculata* extracts have bactericidal characteristic against most of the Gram-positive bacteria and bacteriostatic activity against both Gram-negative and Gram-positive bacteria. However, they were ineffective at low concentrations against *S. saprophyticus, B. anthracis, M. luteus*, *S. pyogenes* and *P. aeruginosa* (Sule *et al*. 2011).

The antimicrobial activity of an aqueous extract, andrographolides and arabinogalactan proteins from air-dried herb of *Andrographis paniculata* were evaluated. The aqueous extract showed significant antimicrobial activity against *Bacillus 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).

An ethanol extract of the aerial part of *Andrographis paniculata* was evaluated for antimicrobial activity against 11 bacterial strains by determining MIC and zone of inhibition. MIC values were compared with control and zone of inhibition values were compared with standard ciprofloxacin in concentration 100 and 200 µg/ml. The results revealed that the ethanol extract (100 – 1,000 µl/disc) inhibited bacterial

growth of both Gram-negative and Gram-positive bacteria (similar inhibition zones in case of higher concentrations) (Mishra US *et al*. 2009).

The study of Murugan *et al*. reported the antibiofilm activity of different extracts of *Andrographis paniculata* leaves 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 *Andrographis 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 a study on antimalarial effect, based on its ethnomedicinal use. It was screened *in vitro* for antimalarial activity towards *Plasmodium falciparum* using the lactate dehydrogenase (LDH) assay. The crude methanolic extract of *Andrographis paniculata* leaves inhibited the chloroquine sensitive strain Gombak A with a 50% inhibitory concentration (IC_{50}) of 45 mg/ml) but it seemed to be less effective towards the resistant strain D10 of *Plasmodium falciparum* (IC_{50} = 65 mg/ml) (Najila *et al*. 2002).

The ethanol and methanol extracts of *Andrographis paniculata* leaves and roots were evaluated for their effects on growth, development and reproduction of malarial vector *Anopheles stephensi* Liston. After 8 days of treatment with ethanol and methanol extracts, 88.60 and 85.25% of the larvae treated at 35 ppm failed to emerge. 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 *A. stephensi* (Kuppusamy and Murugan 2010).

Deltamethrin (a synthetic pyrethroid) and different solvent extracts of *Andrographis paniculata* (plant part not specified in the abstract) were evaluated under laboratory conditions for larvicidal activity against the malarial vector *Anopheles stephensi* Liston. The ethanolic extract, with lethal concentration LC_{50} 35.47 and LC_{90} 47.43 after 24 h and LC_{50} 25.22 and LC_{90} 46.32 ppm after 48 h of exposure 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 24 h and 0.0055 and 0.0085 ppm respectively after 48 h 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 than that when evaluated alone, indicating synergistic activity (Chenniappan and Kadarkarai 2008).

Bacillus thuringiensis var *israelensis* (Bti) and ethanolic extracts (Ee) of *Andrographis paniculata* leaves and roots exhibited both larvicidal as well as pupicidal activity against *Anopheles stephensi* Liston. For Ee, lethal concentrations LC_{50} 36.56 and LC_{90} 53.04 ppm after 24 h and LC_{50} 22.28 and LC_{90} 51.26 ppm after 48 h were recorded for fourth instars larvae, respectively. LC_{50} and LC_{90} for Bti were 0.0967 and 0.1003 ppm after 24 h and 0.2944 and 0.4158 ppm after 48 h of exposure for fourth instars 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. The larvicidal activity was 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 was 51.6 fold more toxic at the LC_{90} for larvicidal activity than Bti alone; this high level of activity resulted from synergism between the Bti and Ee (Chenniappan *et al*. 2011).

A methanolic extract of *Andrographis paniculata* aerial parts was tested *in vitro* on choloroquine sensitive (MRC-pf-20) and resistant (MRC-pf-303) strains of *Plasmodium falciparum* for its antimalarial activity. The IC_{50} of *Andrographis paniculata* was found 7.2 µg/ml (Mishra *et al*. 2009).

Antioxidant effect

A methanolic extract of the dried whole plant 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 (NO) (42.8%) in *in vitro* system. The extract also showed significant inhibition in PMA-induced superoxide (32.4%) and NO (65.3%) formation in *in vivo* studies using BALB/c mice models (Sheeja *et al*. 2006).

An aqueous extract of *Andrographis paniculata* (plant part not specified) was examined for antioxidant activity using rat liver subcellular organelles as model systems. The antimutagenic activity of *Andrographis paniculata* was examined following inhibition in AAPH-induced strand breaks in plasmid pBR322 DNA. The aqueous extract (5 - 200 µg/ml) was a potent scavenger of DPPH, ABTS radicals, exemplified by ESR signals, O-2(-), OH and H2O2, displayed reducing power, FRAP potentials to reduce Fe (III) -> Fe (II) and it had considerable amount of phenolics/flavonoids contents, established indexes for antioxidant action. The observed antioxidant effect might be primarily due to scavenging of reactive oxygen species (ROS). It was confirmed *ex vivo* following inhibition in peroxidation, restoration in superoxide dismutase (SOD) enzyme, SOD band intensity and protein degradation in *Andrographis paniculata* fed liver homogenate. Based on these results, it was concluded that the aqueous extract of *Andrographis paniculata* might be a potent antiradical agent against various pathophysiological oxidants (Tripathi and Kamat 2007).

Immunomodulatory effect

Ethanol extract and purified diterpene andrographolides of *Andrographis paniculata* (plant part not specified) induced significant stimulation of antibody and delayed type hypersensitivity response to sheep red blood cells in mice. The extract also stimulated nonspecific immune response of the animals measured in terms of macrophage migration index phagocytosis of ^{14}C-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 ethanol extract, suggesting that substance(s) other than andrographolide present in the extract contribute to the immunostimulation (Puri *et al*. 1993).

Anti-inflammatory effect

The anti-inflammatory activity of a 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 anti-inflammatory drug in this study. The chloroform extract of *Andrographis paniculata* stem showed statistically significant effect in 6th hour at a dose of 200 mg/kg and the results were comparable with ibuprofen (10 mg/kg) ($t=64.06$, $p<0.001$) (Radhika *et al*. 2009).

The suppressive effects of *Andrographis paniculata* leaves on NO production in mouse peritoneal macrophages elicited by Bacillus Calmette-Guerin (BCG) and stimulated by lipopolysaccharide (LPS) were investigated *in vitro* and *ex vivo*. Incubation of BCG-induced macrophages with the methanol extract of *Andrographis paniculata* leaves reduced LPS-stimulated NO production. The diterpene lactones andrographolide and neoandrographolide were isolated as active components from the extract. They 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/day and LPS-stimulated NO

production was examined. However, andrographolide did not reduce NO production on oral administration at the same doses (Batkhuu *et al*. 2002).

The effects of ethyl acetate extract from *Andrographis paniculata* (plant part not specified) on the level of inflammatory mediators were examined using a nuclear factor-kappa B (NF-$_K$B) driven luciferase assay. The results showed that *Andrographis paniculata* significantly inhibited NF-$_K$B luciferase activity and tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-2 (MIP-2) and NO secretions from LPS/interferon-gamma (IFN-γ) 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 (AP3) and 6.25 (AP4) mg/kg body weight/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/kg body weight PDTC and 1 h later, all of the mice were injected with 15 mg/kg body weight 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-α, IL-12p40, MIP-2 or NO in serum or 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. These results suggest that *Andrographis paniculata* can inhibit the production of inflammatory mediators and alleviate acute hazards at its optimal dosages (Chao *et al*. 2011).

The effects of a methanolic extract of *Andrographis paniculata* leaves on inhibition of LPS-induced mediators [NO, prostaglandin E_2 (PGE_2) IL-1β and IL-6] and calcimycin-induced mediators [leukotriene B4 (LTB4), thromboxane B2 (TXB2) and histamine] were studied in diverse cell based models. The extract illustrated significant alleviation of pro-inflammatory, inflammatory and allergic mediators. However, no inhibition was observed against histamine release. The results showed that the extract was fairly potent in attenuating the inflammation by inhibiting pro-inflammatory (NO, IL-1β and IL-6), inflammatory (PGE_2 and TXB2) and allergic (LTB4) mediators. The extract showed a 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. The extract significantly inhibited PGE_2 levels at the highest concentration of 50 µg/ml, where a maximum inhibition of 53% was attained. The extract inhibited (5 µg/ml) LPS-induced IL-1β 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 dose-dependent reduction in the levels of IL-6 was observed 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 (5 µM) calcimycin-induced LTB4 production in HL-60 cells was studied using various concentrations. The *Andrographis paniculata* extract displayed 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 (5 µM) to augment the production of TXB2. Pretreatment of the 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).

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).

A dichloromethane fraction of a methanol extract of *Andrographis paniculata* whole plant 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. All the three molecules showed enhanced proliferation and IL-2 induction in HPBLs (Kumar *et al*. 2004).

Chemopreventive effect

The effects of two doses (50 and 100 mg/kg body weight/day for 14 days) of an 80% hydroalcohol extract of *Andrographis paniculata* leaves and stems and butylated hydroxyanisole (BHA) were examined on drug metabolising enzymes, antioxidant enzymes, glutathione (GSH) content, LDH and lipid peroxidation in the liver of Swiss albino mice (6 to 8 weeks-old). The effects of the extract and of BHA were also examined on lung, kidney and forestomach for the activities of glutathione S-transferase (GST), DT-diaphorase (DTD), SOD and catalase (CAT). 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 CAT, glutathione peroxidase (GSH-Px) 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 CAT in liver; while LDH and MDA levels were reduced significantly compared with their control values. In the lung, SOD, CAT and DTD, in the kidney, CAT, 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 CAT 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 (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 *Andrographis paniculata* (air-dried whole plant) extract prepared with 70% ethanol (10 mg/dose/animal) intraperitoneal (i.p.) and ANDLE (500 µg/dose/animal, i.p.) 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 *Andrographis 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 *Andrographis 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 h of CTX administration (24.25 ± 0.2 g/l) was found to be reduced by the treatment with *Andrographis paniculata* (14.19 ± 0.5 g/l) and ANDLE (15.79 ± 0.4 g/l). A decreased level of reduced 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 *Andrographis 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 nmol/mg protein). Production of the proinflammatory cytokine, TNF-α, which was elevated during CTX administration, was found to be

inhibited by *Andrographis paniculata* and ANDLE treatment. The lowered level of IL-2 and IFN-γ during CTX treatment was elevated by the administration of *Andrographis 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 *Andrographis paniculata* whole plant (200 mg/kg, p.o.)-treated. The nephrotoxic model was induced by gentamicin (80 mg/kg, i.p.). Blood samples were examined for serum creatinine, serum urea, and blood urea nitrogen after the 10 days of treatment. The aqueous extract of *Andrographis 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 ANDLE, an aqueous extract of *Andrographis paniculata* aerial parts (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 i.p. administered vehicle, nicotine (1 mg/kg body weight/day), nicotine + ANDLE (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 a period of 7 days. The significantly increased levels of lipid peroxidation, protein oxidation and the decreased antioxidant enzyme status were noted in the nicotine-treated group as compared to the vehicle-treated group. ANDLE, AE-AP and vit.E significantly reduced the lipid peroxidation, protein oxidation and increased the antioxidant enzyme status. According to the authors, *Andrographis 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).

Spasmolytic effect

The possible blockade of voltage-operated calcium channels (VOCs) by an *Andrographis paniculata* herb 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 blocked by 0.2 and 0.4 mg/ml *Andrographis paniculata*. In other experiments, the maximum contractile response induced by norepinephrine was not antagonised by 0.2, 0.4 or 0.8 mg/ml *Andrographis paniculata*. The possible blockade of Ca^{2+} entry by *Andrographis paniculata* was evaluated with labelled $^{45}Ca^{2+}$-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/ml *Andrographis paniculata*. These results suggest that *Andrographis paniculata* selectively blocks VOCs, hence inhibiting the $^{45}Ca^{2+}$ influx (Burgos *et al*. 2000).

Uterorelaxant effect

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

Antithrombotic effect

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

***In vivo* studies**

Antidiabetic effect

Oral administration of a crude ethanolic extract of *Andrographis paniculata* aerial parts at different doses (0.1, 0.2, and 0.4 g/kg body weight) significantly reduced the fasting serum glucose level in streptozotocin (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/kg 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/kg body weight), metformin (0.5 g/kg body weight), and vehicle were given twice daily to diabetic rats for 14 days. On day 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-day treatment, whereas this level was significantly decreased ($P < 0.05$) in the vehicle-treated group. The activity of hepatic glucose-6-phosphatase 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 an ethanol extract of *Andrographis paniculata* leaves on alpha-glucosidase inhibition was investigated in both normal and STZ-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), 1,000 (D3) mg/kg ethanol extract of *Andrographis paniculata*, vehicle (control), and acarbose 10 mg/kg, respectively. Blood samples were analysed 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 demonstrated that 500 mg/kg and 1,000 mg/kg ethanol extract of *Andrographis paniculata* reduce and prolong 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 PBG suppressive effect after exogenous glucose load in both normal and STZ-induced diabetic rats (Subramanian and Asmawi 2006).

An ethanolic extract of *Andrographis paniculata* leaves 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 ± 0.28 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 *Andrographis paniculata* extract significantly ($P < 0.05$) reduced PBG and AUC in diabetic rats when challenged with oral administration of starch and sucrose. Further, andrographolide also caused a significant ($P < 0.05$) reduction in PBG and AUC in diabetic rats. Hence alpha-glucosidase

inhibition may possibly be one of the mechanisms for the *Andrographis paniculata* extract to exert antidiabetic activity. The authors indicated that *Andrographis paniculata* 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 STZ-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 body weight) or the extract (400 mg/kg body weight), twice a day for 14 days. At the end of the 14 day period, the extract, like metformin, significantly increased body weight ($P < 0.01$) and reduced fasting serum glucose in diabetic rats ($P < 0.001$) when compared with vehicle, but had no effect on body weight 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 GSH concentrations were significantly decreased ($P < 0.005$) in vehicle-treated diabetic rats. Liver and kidney TBARS 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 TBARS level ($P < 0.005$) in normal rats. Hepatic SOD, CAT and 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 authors concluded the results show that oxidative stress is evident in STZ-diabetic rats and indicate that the ethanolic extract of *Andrographis paniculata* not only possesses an antihyperglycaemic property, but may also reduce oxidative stress in diabetic rats (Zhang and Tan 2000b).

Momordica charantia fruit juice or *Andrographis paniculata* leaves decoction was orally administered to alloxan-induced diabetic rats. Rats that were treated with *Momordica charantia* and *Andrographis paniculata* had higher body weight compared with diabetic positive control ($P < 0.01$) from day 22 to day 27 (D27) but exhibited lower body weight 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 longer mean estrous cycles (8 days) compared to *Momordica charantia* and *Andrographis paniculata*-treated diabetic rats (5 days; $P < 0.05$) (Reyes *et al*. 2006).

Antioxidant effect

The effect of the aqueous extract of *Andrographis paniculata* leaves on antioxidant defense system in liver was investigated in lymphoma bearing AKR mice. Oral administration of the aqueous extract of *Andrographis paniculata* in different doses (10, 20 and 30 mg per mouse oraly) caused an elevation of CAT, SOD and GST activities. A decrease in LDH activity to about 27% was observed after treatment with *Andrographis paniculata* (Verma and Vinayak 2008).

In the study of Lin *et al*., extracts prepared from *Andrographis paniculata* whole plant (cultivated) and their active constituent ANDLE were evaluated for antioxidant, anti-oedema and analgesic activities on rats using different experimental settings. The results showed that the aqueous *Andrographis paniculata* extract (AP-H_2O) exhibited a greater antioxidant activity than the ethanol *Andrographis paniculata* 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 ANDLE, but not AP-EtOH showed anti-oedema and analgesic activities. In phytochemical analysis, AP-H_2O showed a higher concentration of total flavanoid but a lower phenol content than AP-EtOH (Lin *et al*. 2009).

Oral treatment of rats with 1 g/kg body weight of the methanol extract of *Andrographis paniculata* leaves (for 14 days followed by carbon tetrachloride (CCl_4) administration) preserved CAT and 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).

Immunomodulatory effect

Effects of an *Andrographis paniculata* whole plant extract (extraction solvent: ethanol 70%; the yield of the extract was 14%) and its major component, andrographolide (ANDLE), on cell-mediated immune responses were studied in metastatic tumour bearing animals. The *Andrographis paniculata* extract and ANDLE were given i.p. at a concentration of 10 mg/dose/animal and 500 µg/dose/animal, respectively. Natural Killer (NK) cell-mediated target cell lysis was enhanced by the administration of *Andrographis paniculata* extract (45% cell lysis) and ANDLE (40.2% cell lysis) on the 5^{th} day after tumour induction when compared to untreated metastatic tumour bearing animals in which maximum target cell lysis was observed on 11^{th} day (11.4%). Antibody-dependent cell-mediated cytotoxicity (ADCC) was also enhanced by treatment with 5 doses of extract (10 mg/dose/animal) (42% cell lysis) and ANDLE (500 µg/dose/animal) (40.2%) in comparison with the untreated case (11%). Similarly, the extract (25%) and ANDLE (22%) showed higher ADCC activity than the control (14%) and treatment of extract and ANDLE resulted in significant increase in serum IL-2 and TIMP-1 levels. Furthermore, the levels of proinflammatory cytokines such as IL-1β, IL-6, GM-CSF and TNF-α were effectively reduced by the administration with 5 doses of extract (10 mg/dose/animal) and ANDLE (500 µg/dose/animal) in metastatic tumour 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), omeprazole (positive control, 20 mg/kg), aqueous leaf extracts (APLAE, 250 and 500 mg/kg) and ethanol leaf extracts (APLEE, 250 and 500 mg/kg) 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 analysed 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).

In a follow-up study, the gastroprotective effect of an hydroalcoholic extract of *Andrographis paniculata* aerial parts (HAEAP) was evaluated in male albino wistar rats. Rats were pretreated with HAEAP (100, 200, 500 mg/kg body weight 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, 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 body weight 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 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).

Anti-ulcer activity of an hydroalcoholic extract of *Andrographis paniculata* aerial parts (HAEAP) was evaluated in male albino Wistar rats. Rats were divided into 5 groups. Group 1 treated with water served as control. Group 2 were administered cysteamine to induce ulcer (i.p., 420 mg/kg body weight, as a single dose). Group 3 were pretreated with HAEAP (orally, 200 mg/kg body weight, for 30 days) before ulcer induction. Group 4 were pretreated with ranitidine (30 mg/kg body weight) before ulcer induction and served as standard drug reference. Group 5 were treated with HAEAP only. Ulcer index, TBARS, mucin, GSH-Px 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 minimised in rats treated with HAEAP. Mucin content was found to be preserved in rats treated with the extract. GSH/GSSG ratio and GSH-Px 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 pretreated 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 control 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).

Effect on the central nervous system

Psychopharmacological studies were conducted on a methanol extract of *Andrographis paniculata* herb. 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 (Mandal *et al*. 2001).

Hepatoprotective effect

Administration of an alcohol extract of *Andrographis paniculata* (plant part not specified in the abstract) (25 mg/kg) and two of its constituent diterpenes, ANDLE and neoandrographolide (6 mg/kg/day for two weeks) showed significant antihepatotoxic action against *Plasmodium berghei* K173-induced hepatic damage in *Mastomys natalensis*. The increased levels of serum lipoprotein-X, alkaline phosphatase (ALP), ALT, AST and bilirubin were markedly reduced by *Andrographis paniculata*

and its diterpenes. In the liver, these preparations decreased the levels of lipid peroxidation products and facilitated the recovery of SOD and glycogen. The protective effects of ANDLE were comparable to those of neoandrographolide (Chander *et al*. 1995).

The hepatoprotective effect of *Andrographis paniculata* and its constituent, ANDLE, was studied using ethanol as the hepatotoxin. Acute and subacute hepatotoxicities were induced in rats by varying doses of ethanol (2 - 6 g/kg) and time of treatment (0 - 21 days). Serum transaminases (AST and ALT) and histopathology changes in the livers were used to monitor the hepatoprotective activity. Single dose pretreatment of ANDLE (20, 50, 100, 200 mg/kg, i.p.) and aqueous extract of *Andrographis paniculata* leaves (300, 500, 800, 1,000 mg/kg, p.o.) 48 h and 4 h respectively, before ethanol administration, reduced the toxicity induced by ethanol (4 g/kg, p.o.). Similar results were obtained with 7 days pretreatment of ANDLE (100 mg/kg/day, i.p.) and aqueous extract of *Andrographis paniculata* leaves (500 mg/kg/day, p.o.). Since hepatic alcohol dehydrogenase activity was unaffected by these pretreatments, it is suggested that hepatoprotective mechanisms of ANDLE and *Andrographis paniculata* may not involve the metabolism of ethanol (Pramyothin *et al*. 1994).

The ability of the extracts of *Andrographis paniculata* aerial parts to protect against acute hepatotoxicity induced by paracetamol (150 mg/kg) was studied in Swiss albino mice. The oral administration of *Andrographis paniculata* extracts (70% ethanol dry extract; yield: 5.8% w/w; 100 - 200 mg/kg) resulted in a significant ($p<0.001$) dose-dependent protection against paracetamol-induced hepatotoxicity as assessed in terms of biochemical and histopathological parameters. The paracetamol induced indeed elevated levels of serum marker enzymes such as serum GPT, serum GOT, ALP, and bilirubin in peripheral blood serum and also distorted hepatic tissue architecture along with increased levels of lipid peroxides and reduction of SOD, CAT, reduced GSH and GSH-Px in liver tissue. Administration of the extract after paracetamol intoxication restored the levels of serum marker enzymes to control (untreated) levels (Nagalekshmi *et al*. 2011).

In an *in vivo* study, CCl_4 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 GSH ($P<0.05$). Parallel to these changes, an enhanced hepatic damage was observed which was characterised by a sharp increase in serum transaminases (e. g. ALT, AST and LDH) ($P<0.05$). 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 an ethanolic (80 %) extract of the dried aerial parts of *Andrographis paniculata* ($P<0.05$). The ability of *Andrographis paniculata* to scavenge the 2,2-Diphenyl-2-picrylhydrazyl radical was determined through its EC_{50} value. The EC_{50} value of *Andrographis paniculata* was 583.60 ± 4.25 µg/ml. In addition, *Andrographis paniculata* was found to contain 65.37 ± 1.20 mg/g total phenolics expressed as gallic acid equivalent (Koh *et al*. 2011).

Cardiovascular effects

The cardiovascular activities of crude water extract (WE) of *Andrographis paniculata* aerial parts, its three semi-purified ethyl acetate (FA), n-butanol (FB) and aqueous (FC) fractions and of ANDLE were elucidated in anaesthetised Sprague Dawley rats. FA and ANDLE 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 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 (ACE), because was not affected by propranolol, atropine and captopril, respectively. 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* (plant part not specified in the abstract) was studied using chronic 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 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. These results suggest that the aqueous extract of *Andrographis 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 mechanisms of hypotensive action seem to be different in WKY rats (Zhang and Tan 1996).

The effects of *Andrographis paniculata* (AP) (plant part not specified in the abstract) and fish oil (FO, ω3-polyunsaturated fatty acids over 70%) on atherosclerotic stenosis and restenosis after experimental angioplasty and the relevant mechanisms were studied *in vivo*. Preliminary results showed that AP can significantly alleviate atherosclerotic iliac artery stenosis induced by both de-endothelialisation 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 AP or FO, especially the former alleviated this reduction. Refined extract of *Andrographis paniculata* 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 AP for 2 weeks. *Andrographis paniculata* significantly inhibited cell growth or DNA synthesis in a dose-dependent manner. The authors concluded that because of the mechanisms described above, AP can alleviate atherosclerotic artery stenosis induced by both de-endothelialisation and HCD, as well as lower restenosis rate after experimental angioplasty. The effects of AP are superior to those of FO (Wang and Zhao 1994).

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% aqueous extracts (1:20) of *Andrographis paniculata* leaves, 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 wounds dressed with an *Andrographis 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% *Andrographis paniculata* extract. Histologically, wounds dressed with *Andrographis 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% *Andrographis 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 an *Andrographis paniculata* extract (APE) during the first 19 days of pregnancy in doses of 200, 600, and 2,000 mg/kg did not exhibit any effect on the elevated level of progesterone in the blood plasma of rats (Panossian *et al*. 1999).

Anticancer effect

The effects of an ethanolic extract of the air-dried whole plant of *Andrographis paniculata* (APE) and its isolated compound andrographolide (ANDLE) were studied on cell-mediated immune responses in normal and tumour-bearing control animals. Treatment with APE and ANDLE arm enhanced NK cell activity in normal (APE, 46.82% cell lysis; ANDLE, 40.79% cell lysis) and tumour-bearing animals (APE, 48.66% cell lysis; ANDLE, 42.19% cell lysis) on the fifth day, and it was observed earlier than in tumour-bearing 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 tumour-bearing animals (APE, 47.39% cell lysis; ANDLE, 41.48% cell lysis on day 11) compared to untreated tumour-bearing control animals (maximum of 11.76% cell lysis on day 17). Enhancement of antibody-dependent complement-mediated cytotoxicity was also observed after the administration of APE and ANDLE in normal as well as tumour-bearing animals. APE and ANDLE administration could enhance the mitogen-induced proliferation of splenocyte, thymocyte, and bone marrow cells. Moreover, treatment of APE and ANDLE elevated the production of IL-2 and INF-γ in normal and Ehrlich ascites carcinoma-bearing animals (Sheeja and Kuttan 2007).

Sheeya *et al.* studied the anti-angiogenic activity of an extract of *Andrographis paniculata* whole plant (APE) and its major component andrographolide (ANDLE) using both *in vitro* and *in vivo* models. I.p. 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-1β, IL-6, TNF-α and GM-CSF and the most potent angiogenic factor VEGF in angiogenesis induced animals. Treatment of APE and ANDLE significantly reduced these 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 lowered by the administration of APE and ANDLE. Anti-angiogenic 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 tumour specific angiogenesis by regulating the production of various pro- and anti-angiogenic factors such as proinflammatory cytokines NO, VEGF, IL-2 and TIMP-1 (Sheeja *et al*. 2007).

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 (ANDLE) and 14-deoxy-11, 12-didehydroandrographolide (DIAP) in rat plasma after an oral dose of a methanol extract (1 g/kg body weight) of *Andrographis paniculata* leaves. An increase in plasma concentration of ANDLE and DIAP was observed from 30 min to 3 h after oral administration of the extract. The maximum plasma concentrations of ANDLE and DIAP were 1.42 ± 0.09 µg/ml and 1.31 ± 0.04 µg/ml, respectively (Akowuah *et al*. 2009).

In the study by Panossian *et al.*, andrographolide (ANDLE) was quickly and almost completely absorbed into the blood following the oral administration of an extract from *Andrographis paniculata* herb at a dose of 20 mg/kg body weight in rats. Its bio-availability, however, decreased four-fold when

a 10-times-higher dose was used. Since a large part (55%) of ANDLE is bound to plasma proteins and only a limited amount can enter the cells, the pharmacokinetics of ANDLE are described well by a one-compartment model. Renal excretion is not the main route for eliminating ANDLE. It is most likely intensely and dose-dependently metabolised (Panossian *et al*. 2000).

Pharmacokinetic and pharmacodynamic interactions

Effect on the CYP isoenzymes

Effects of the administration of *Andrographis paniculata* (aerial parts) crude aqueous or ethanolic extracts on cytochrome P450 (CYP450) enzymes were studied in 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 CYP450 enzymes of which CYP1A and CYP2B are the responsive P450 isoforms (Jarukamjorn *et al*. 2006).

The 2008 publication by Jarukamjorn is a review of the effects exhibited by *Andrographis paniculata* on hepatic CYP1A enzymes and their mechanisms. It reports on investigations showing that the crude extract of *Andrographis paniculata* (plant part not specified) increased hepatic CYP1A enzymes including ethoxyresorufin and methoxyresorufin activities. This corresponds with the inductive effects conveyed by ANDLE. Synergistic induction of CYP1A1 by co-treatment with ANDLE and a typical CYP1A inducer as well as a robust increase of CYP1A1 by ANDLE in which the induction was blocked by an aryl hydrocarbon receptor (AhR) antagonist resveratrol, affirmed participation of AhR-mediated transcription activation on ANDLE-induced CYP1A1 expression (Jarukamjorn 2008).

The inhibitory effect of an extract (extraction solvent: 60% ethanol v/v; yield of extract: 10% w/w from dried starting material) from *Andrographis paniculata* leaves (APE) and ANDLE on hepatic cytochrome P450s 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 ANDLE (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 µM. ANDLE was found to be a weak inhibitor of rat CYP2E1 with a K_i of 61.1 µM but did not affect human CYP2E1. APE can cause drug-drug interactions in humans through CYP3A and 2C9 inhibition (Pekthong *et al*. 2008).

The ability of a 60% ethanolic extract from *Andrographis paniculata* leaves (APE) and andrographolide (ANDLE), 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 ANDLE equivalents) and at 2.5 g/kg/day (i.e. 25 mg/kg/day ANDLE equivalents) and ANDLE at dose levels of 5 and 25 mg/kg/day significantly decreased CYP2C11 activity. In primary cultures of rat and human hepatocytes, treatment with ANDLE 50 µM and APE-containing 50 µM ANDLE also resulted in significant decreases in CYP2C expression and activity. In addition, in human

hepatocytes, treatment with APE and ANDLE 50 µM resulted in a decrease in CYP3A expression and activity (Pekthong *et al*. 2009).

The effects of andrographolide (ANDLE), the major diterpenoid constituent of *Andrographis paniculata*, on the expression of enzymes of the cytochrome P450 family, including CYP1A1, CYP1A2 and CYP1B1, as well as on AhR expression in primary cultures of mouse hepatocytes were investigated in comparison with the effects of typical CYP1A inducers, including benz[a]anthracene, β-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. ANDLE 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 or AhR. ANDLE 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 (ANDLE) and 14-Deoxy-11, 12-Didehydroandrographolide (DIAP) 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™ Assays. The LanthaScreen® TR-FRET Pregnane X Receptor (PXR) Competitive Binding Assay was used to determine if the compounds are potential PXR-ligands; this assay uses a human PXR ligand-binding domain (LBD) tagged with GST. Both diterpenoids inhibited the mRNA and protein expressions of CYP1A2, CYP2D6, and CYP3A4. 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 DIAP 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 CYP450 enzyme activities *in vitro*, an *Andrographis paniculata* aerial parts ethanolic extract was tested on the activities of CYP2C9, CYP2D6 and CYP3A4. The abilities to inhibit CYP450 enzyme activities were analysed 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 characterised with an IC_{50} value of 442 ± 4 5 µg/ml. The *A. paniculata* 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 metabolised by CYP2C9, CYP2D6 and CYP3A4 respectively (Hanapi *et al*. 2010).

The effects of *Andrographis paniculata* whole plant extracts (APE) and andrographolide (ANDLE) were investigated on the catalytic activity of three human cDNA-expressed CYP450 enzymes: CYP2C9, CYP2D6 and CYP3A4. *In vitro* probe-based HPLC 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 APE and ANDLE. The results indicated that *Andrographis paniculata* 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 µ/ml). ANDLE was found to exclusively but weakly inhibit CYP3A4 activity (Pan *et al*. 2011).

The effects of an *Andrographis paniculata* stem and leave parts extract (APE) and its major component andrographolide (ANDLE) on the pharmacokinetics of theophylline, a typical substrate of CYP450 1A2 enzyme, were investigated in rats. After APE or ANDLE 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 was reduced in both ANDLE and APE pretreated groups at low-dose theophylline administration (1 mg/kg). The elimination half-life and mean residence time of theophylline were shortened by 14% and 17% respectively, in the ANDLE pretreated group when high-dose theophylline (5 mg/kg) was given. However, theophylline accumulated in rats of the group with APE pretreatment. These results suggest that some other components contained in the 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 preparation containing a standardised fixed combination of extracts from *Andrographis paniculata* (plant part not specified) 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 the preparation at a dose of 17 mg/kg of the active principle ANDLE (a daily dose ~17-fold higher than that recommended for humans); the control group received similar treatment with appropriate volumes of water only. Sixty min after the final daily administration of the preparation 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 administration of the preparation and warfarin did not produce significant effects on the pharmacokinetics of warfarin, and practically no effect on its pharmacodynamics (Hovhannisyan *et al*. 2006).

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

Acute toxicity

In an acute oral toxicity study, healthy female albino Wistar rats (8 – 12 weeks) were treated at 5,000 mg/kg of a special extract of *Andrographis paniculata* leaves (DER: about 17:1; extraction solvent: methanol) and observed for signs of toxicity for 14 days. The extract 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 CNS 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. The extract-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. The extract at 5,000 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 the 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

Akbarsha and Murugaian investigated in male albino rats if ANDLE is responsible for the antispermatogenic effect of the *Andrographis paniculata* leaves extract. The compound was administered to 3-month-old male Wistar albino rats at two dose levels, 25 mg and 50 mg/kg body weight respectively, 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 ANDLE could affect spermatogenesis by preventing cytokinesis of the dividing spermatogenic cell lines. The multinucleate giant cells were 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 were also apparent. These results suggest that ANDLE has a toxic effect on the male reproductive system (Akbarsha and Murugaian 2000).

Administration of 50 mg/kg ANDLE orally to male mice once daily for 2, 4, 6 or 8 weeks had no significant effects on sperm morphology and motility (Sattayasai *et al.* 2010).

Andrographis leaves

Dried leaves powder of *Andrographis paniculata*, when fed orally to male albino rats, at a dose level of 20 mg powder per day for 60 days, resulted in cessation of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells and regressive and/or degenerative changes in the epididymis, seminal vesicle, ventral prostate and coagulating gland. There was reduction in the weight and fluid content of the accessory glands. The treatment also resulted in accumulation of glycogen and cholesterol in the testis, and increased activities of LDH in testis and ALP in testis and ventral prostate (Akbarsha *et al*. 1990).Andrographis extract

An *Andrographis paniculata* herb extract possessed no acute (> 17 g/kg, LD_{50}) or subchronic toxicity. The possible testicular toxicity of this *Andrographis paniculata* standardised extract (extraction solvent: ethanol 70%; DER: 5:1, with a minimum of 5.6% of ANDLE content) was evaluated in male Sprague Dawley rats for 60 days. No testicular toxicity was found with the treatment of 20, 200 and 1,000 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. The authors concluded that this *Andrographis paniculata* extract did not produce subchronic testicular toxicity effect in male rats (Burgos *et al*. 1997).

The possible effect of an ethanolic extract of the whole plant , the majority of which was leaves, of *Andrographis paniculata* (standardised to ≥ 10% ANDLE) on male fertility in albino Wistar rats was evaluated, by orally administering 0, 20, 200, and 1,000 mg/kg 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, the authors concluded that the no-observed adverse effect level of this extract of *Andrographis paniculata* (≥ 10% ANDLE) was found to be more than 1,000 mg/kg per day (Allan *et al*. 2009).

Genotoxicity

The genotoxicity of a special extract of *Andrographis paniculata* leaves (DER: about 17:1; extraction solvent: methanol) was assessed in three different *in vitro* tests: Ames test, chromosome aberration (CA) test, and micronucleus (MN) test. The Ames test was performed at 5,000 µg/ml, 1,581 µg/ml, 500 µg/ml, 158 µg/ml, 50 µg/ml and 16 µg/ml. The clastogenicity tests were performed at 80 µg/ml, 26.6 µg/ml, 8.8 µg/ml for short-term treatment without S9, at 345 µg/ml, 115 µg/ml, 38.3 µg/ml for short-term treatment with S9, and at 46 µg/ml, 15.3 µg/ml and 5.1 µg/ml for long-term without S9 using DMSO as a vehicle control. The results of the Ames test confirmed that the special extract did not induce mutations both in the presence and absence of S9 in *Salmonella typhimurium* mutant strains TA98 and TAMix. In the CA and MN tests, the extract did not induce clastogenicity 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 various activities, conducted with different extracts of *Andrographis paniculata* (plant material being exclusively the leaves or a mixture of leaves and other plant part such as the roots). Antidiabetic, antioxidant, anti-inflammatory, antinociceptive, anti-allergic, immunomodulatory, gastroprotective, CNS, hepatoprotective, neuroprotective, antithrombotic, cardiovascular, choleretic, wound healing, anticancer effects were assessed in *in vivo* animal experiments. Although there is a clear effect on some CYP isoenzymes, the available acute and genotoxicity data show no toxicity of a special extract of *Andrographis*. The reproductive toxicity data are contradictory. There is a large variety of non-clinical 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

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

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 randomised, double-blind placebo-controlled clinical study was conducted to evaluate the efficacy of a preparation containing of a special extract of *Andrographis paniculata* leaves (DER: about 17:1; extraction solvent: methanol) 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 randomised in two groups which received either the preparation (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 the preparation-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 the preparation 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).

Pharyngotonsillitis

One hundred and fifty-two adult patients with pharyngotonsillitis were enrolled in a randomised, double-blind study to assess the efficacy of *Andrographis paniculata*. The patients were randomised to receive either paracetamol or 3 g/day of *Andrographis paniculata* dried leaves (containing at least 6% total lactones calculated as ANDLE) or 6 g/day of *Andrographis paniculata* dried 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% in each group (Thamlikitkul *et al.* 1991).

Rheumatoid arthritis

A prospective, randomised, double-blind and placebo-controlled study in patients with rheumatoid arthritis (RA) was performed. Tablets made of an extract of *Andrographis paniculata* leaves and aerial parts (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; other clinical parameters were evaluated using ACR and EULAR. In addition, Health Assessment Quality (HAQ) and Short Form Health Survey (SF36) standardised health questionnaires were used. 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), 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 the extract of leaves of *Andrographis paniculata* (extraction solvent: ethanol/water (90/10 v/v); 8 - 10% ANDLE content) 1,200 mg/day compared with 4,500 mg/day of slow-release mesalazine granules in patients with mild-to-moderately active 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 extract-treated patients, and 16% and 82% of mesalazine-treated patients. By colonoscopy, remission and response were seen in 28% and 74% of extract-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).

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

No studies are available.

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 (Gabrielian *et al*. 2002, Melchior *et al*. 2000) and common cold (Caceres *et al*. 1997, Caceres *et al*. 1999, Melchior *et al*. 1997, Spasov *et al*. 2004, Hancke *et al*. 1995). Most studies were performed with a fixed-combination product. Because the aim was to establish a monograph on Andrographidis paniculatae folium as a single ingredient, the results of these studies are not included in this assessment report.

The randomised, double-blind placebo-controlled clinical study by Saxena *et al.* evaluated the efficacy of an Andrographidis paniculatae folium methanolic extract in upper respiratory tract infection, using a visual analogue scale (Saxena *et al*. 2010). Tamlikitkul *et al.* assessed the efficacy of dried *Andrographis* leaves in pharyngotonsillitis in a randomised double-blind study (Thamlikitkul *et al.* 1991). These two studies with monocomponent preparations are not sufficient to prepare a well-established medicinal use monograph for Andrographidis paniculatae folium. In the case of the study by Saxena *et al.*, the lack of objective endpoints and, in the study by Tamlikitkul *et al.*, the absence of a placebo group make the assessment of the efficacy difficult.

In the study with patients with RA by Burgos *et al*. (2009), *Andrographis* leaves and aerial parts were administered. The efficacy in ulcerative colitis of an Andrographidis paniculatae folium extract was assessed in the study by Tang *et al.*, however without placebo control (2011).

In conclusion, a well-established use monograph cannot be adopted on *Andrographis paniculata* Nees, folium.

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 body weight for 3 weeks, escalating to 10 mg/kg body weight for 3 weeks, and to 20 mg/kg body weight 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. The authors concluded that 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 randomised, double-blind, placebo controlled clinical study the monopreparation of a special extract of leaves of *Andrographis paniculata* (DER: about 17:1; extraction solvent: methanol) 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 the preparation-treated group) and urticaria, all other effects stopped spontaneously without any medical aid (Saxena *et al*. 2010).

5.2. Patient exposure

About 1,200 patients were involved in clinical trials with preparations containing *Andrographis paniculata* as a single component or in combination with other substances.

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 and lactation has not been studied.

5.6. Overall conclusions on clinical safety

Products containing *Andrographis paniculata* have been found safe during the clinical studies in adults.

The safety of products containing *Andrographis paniculata* has not been evaluated in children and adolescents.

The safety of use during pregnancy and lactation has not been studied.

6. Overall conclusions

Due to the lack of appropriate studies, the requirements for well-established use are not fulfilled.

There is no monocomponent herbal preparation for which 15 years of medicinal use in the EU could be confirmed from the literature or based on the regulatory status 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.

Annex

List of references