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Biological activities of *Kalmegh* (*Andrographis paniculata* Nees) and its active principles-A review

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Kalmegh (*Andrographis paniculata* Nees) has wide range of medicinal and pharmacological applications. It is used in different traditional systems of medicine and exhibits anti-inflammatory, anti-HIV, antibacterial, antioxidant, antiparasitic, antispasmodic, antidiabetic, anticarcinogenic, antipyretic, hepatoprotective, nematocidal and various other activities. It is a potent scavenger of a variety of reactive oxygen species (ROS) including superoxide anion, hydroxyl radical, singlet oxygen, peroxynitrite and nitric oxide. Among several active chemical constituents, andrographolide, neoandrographolide and dehydroandrographolide are most important bioprotectants with wide range of therapeutic applications. Andrographolides significantly inhibit the expression of iNOS, COX-2, mRNA, protein, enzyme activity in RAW 264.7 macrophages that involves in anti-inflammatory activity. *Kalmegh* extract protects lipids, haemoglobin and red blood cells from lipid peroxidation. It prevents oxidative damage and inhibits binding to toxic metabolites to DNA. Safety evaluation studies indicate that *kalmegh* is well tolerated at very high dose without any toxic effects.

Keywords: *Andrographis paniculata, Kalmegh*, *Maha-tita*, *Bhui-neem*, Acanthaceae, Andrographolides, Biological activities, Toxicity.

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Introduction

Kalmegh (*Andrographis paniculata* Nees, family Acanthaceae) is commonly known as 'King of Bitters', *Maha-tita* or *Bhui-neem* as the plant, though much smaller in size, shows similar appearance and bitter taste as that of Neem (*Azadirachta indica* A. Juss.). It is distributed in tropical Asian countries, often in isolated patches. It can be found in a variety of habitats, e.g. plains, hill slopes, wastelands, farms, dry or wet lands, sea shores and even road sides. Native populations of plants are spread throughout South India and Sri Lanka which perhaps represent the centre of origin and diversity of the species. The herb is also available in northern parts of India, Java, Malaysia (including Penang, Malacca, Pangkor Island which is south of Penang and parts of Borneo), Indonesia, the West Indies (including Jamaica, Barbados and the Bahamas) and elsewhere in the Americas where it is probably an introduced species. The species also occurs in Hong Kong, Thailand, Brunei, and Singapore, etc. However, precise data are lacking on the introduction and naturalization of the species in these countries[1,2].

The genus *Andrographis* Wall. consists of 28 species of small annual herbs essentially distributed in tropical Asia. Only a few species are medicinal of which *A. paniculata* is the most popular. It has been used as medicinal herb for centuries in several traditional systems of medicine all over the world. It is extensively used in Ayurveda, Unani and Siddha medicines as home remedy for various diseases in Indian traditional system as well as in tribal medicine in India and some other countries for multiple clinical applications. The therapeutic value of *Kalmegh* is due to its mechanism of action by enzyme induction. It is an important cold property herb, used in fevers and to dispel toxins from the body. It is used to treat gastrointestinal tract and upper respiratory infections, fever, herpes, sore throat, hepatitis and a variety of other chronic and infectious diseases[1]. It exhibits antibacterial, antimalarial, filaricidal, antidiarrhoeal, cardiovascular activities, fertility effects and protection of liver and gallbladder. The herb and its isolates like andrographolide, neoandrograholide, dehydroandrographolide, isoandrographolide, etc. are reported to possess anti-inflammatory, hepato-protective, astringent, anodyne, tonic, alexipharmic

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and anti-pyretic properties and helps in arresting dysentery, cholera, diabetes, influenza, bronchitis, swellings and itches, piles and gonorrhoea[2]. Flavonoids present in plant showed potent inhibition of collagen, arachidonic acid, thrombin and platelet activation factor induced platelet aggregation. Furthermore, a diterpenoid demonstrated moderate vasorelaxing effect in isolated rat thoracic aorta[3]. The plant is used as an important ingredient in different medicinal formulations in national and international market. The present review deals with the recent research performed in the area of biological activities, phytochemistry, cultivation, structure activity relationship, pharmacological activities, clinical study, safety and doses of *kalmegh*.

It is an annual, profusely branched, erect herb, 0.5-1.0m in height with a tap root. Leaves are green, lanceolate, 3-7 cm × 1-2.3 cm in size, glabrous with slightly undulate margin, acuminate apex with a tapering base. Flowers are small and solitary; corolla whitish or light pink in colour with hairs. Fruit, a capsule, linear, oblong and acute at both ends; seeds numerous. It grows abundantly in South eastern Asia i.e. India, Sri Lanka, Pakistan and Indonesia, but cultivated extensively in China, Thailand, East and West Indies and Mauritius. It is generally found in all kinds of vegetative lands i.e. in pine, evergreen, deciduous forest areas, along roads and villages. It is easily cultivated from seeds on all types of soil[2].

The vernacular names of the species in various languages are: Beng.-*Kalmegh*, Eng.-The Creat; Guj.-*Kariyatu*; Hindi-*Kiraya*t; *Kalpanath*; Kan.-*Nelaberu*; Mal.-*Nelavepu*; *Kiriyattu*; Mar.-*Oli-kiryata*; Or.-*Bhuinimba*; Sans.-*Kalmegha*, *Bhunimba*, Tam.-*Nilavembu*, Tel.-*Nilavembu*

Cultivation

In India, it is cultivated as rainy season (Kharif) crop. The climatic requirement for the plant is hot and humid conditions with ample sunshine. *Kalmegh* can be cultivated on wide range of soils from loam to lateritic soils with moderate fertility. It can also be cultivated on shady wastelands. With the onset of monsoon, plant grows luxuriantly and starts flowering with the moderation in temperature after end of monsoon. The propagation is through shattered seeds in nature. Vegetative propagation is also possible in certain special cases through layering as each node is capable of producing enough roots. Seeds are small and remain dormant for 5-6 months. For raising crops in one hectare, three beds of 10 × 2 m size should be tilled, pulverized and leveled. Liberal use of organic manure in nursery is advised for raising healthy seedlings. The seeds (250-300g) are broadcast on each bed surface and they should be covered with thin layer of soil and compost mixture. Bed should be covered properly with suitable mulch and irrigated regularly with water fountain till seedlings emerge (6-7 days). Immediately after germination (70-80%), mulch is removed to avoid elongation of the seedlings. If possible seedlings should be raised in shade to protect them from heat. In well prepared and laid out beds, transplanting of seedlings is done when they are one month old at row spacing of 45-60 cm and plant spacing of 30-45 cm. Beds should be irrigated immediately after transplanting. It can be grown on poor to moderate fertile soil but application of 80 kg nitrogen and 40 kg phosphorus per hectare will increase the herb yield. Nitrogen dose can be split into two, which can be applied as basal and after 30-45 days of transplanting. In addition, 3-6 tonnes of well rotten farm yard manure are required for raising nursery. Maximum herb biomass can be obtained in 90-100 days beyond which leaves start shedding. The crop remains dormant in winter. At the time of flower initiation, the active principle andrographolide is high in leaves. Since the whole plant contains active principals, entire harvested material is dried in shade and powdered. A well maintained crop grown during monsoon season yields 3.5 to 4 tonnes/ha of dried herb[4-6].

Chemical constituents

Therapeutically important active principle of *kalmegh* found in aerial parts is andrographolide ($C_{20}H_{30}O_5$, mp 230-239ºC). It is colorless, crystalline, bitter in taste and known as diterpene lactone. Other reported compounds include 14-deoxy-11-oxoandrographolide ($C_{20}H_{28}O_5$, mp 98-100ºC);14-deoxy-11, 12- didehydroandrographolide/andrographlide D ($C_{20}H_{30}O_4$, mp 203-204ºC); 14-deoxy-andrographolide ($C_{20}H_{30}O_4$, mp 175ºC); non-bitter compound neoandrographolide ($C_{26}H_{40}O_8$, mp 167-168ºC); homoandrographolide ($C_{22}H_{32}O_9$, mp 115ºC); andrographosterol ($C_{23}H_{38}O$, mp 135ºC); andrographane ($C_{40}H_{82}$, mp 67-68ºC); andrographone ($C_{32}H_{64}O$, mp 85ºC); andrographosterin; andrograpanin; α-sitosterol; stigmasterol; apigenin- 7, 4'-di-O-methyl ether; 5-hydroxy 7, 8, 2', 3'-tetramethoxy flavone ($C_{19}H_{18}O_7$, mp 150-151ºC); monohydroxy trimethyl flavones; andrographin ($C_{18}H_{16}O_6$, mp 190-191ºC); dihydroxy-di-methoxyflavone; panicolin

($C_{17}H_4O_6$, mp 263-264ºC); andrographoneo; andrographoside; andropaniculosin A; andropani-culoside A[3,7,8]; andrograpanin; isoandrographolide and skullcaflavone[9-12]. Shen *et al*[13] reported six ent-labdane diterpenoids i.e. 3-O-beta-D-glucopyranosyl-14, 19-dideoxyandrographolide; 14-deoxy-17-hydroxyandrographolide; 19-O-[beta-D-apiofuranosyl (1->2)-beta-D-glucopyranoyl]-3,14-dideoxyandrographolide; 3-O-beta-D-glucopyranosyl-andrographolide; 12*S*-hydroxyandrographolide and andrographatoside from the aerial parts of plant. These compounds showed inhibitory activity against several bacterial and fungal strains[13]. Four xanthones 1,8-dihydroxy-3,7-dimethoxy-xanthone; 4,8-di-hydroxy-2,7-dimethoxy-xanthone;-1,2-dihydroxy-6,8-dimethoxy -xanthone and 3,7,8-trimethoxy-1-hydroxyxanthone are reported from the roots[14]. Structures of some major compounds are given in Plate 1.

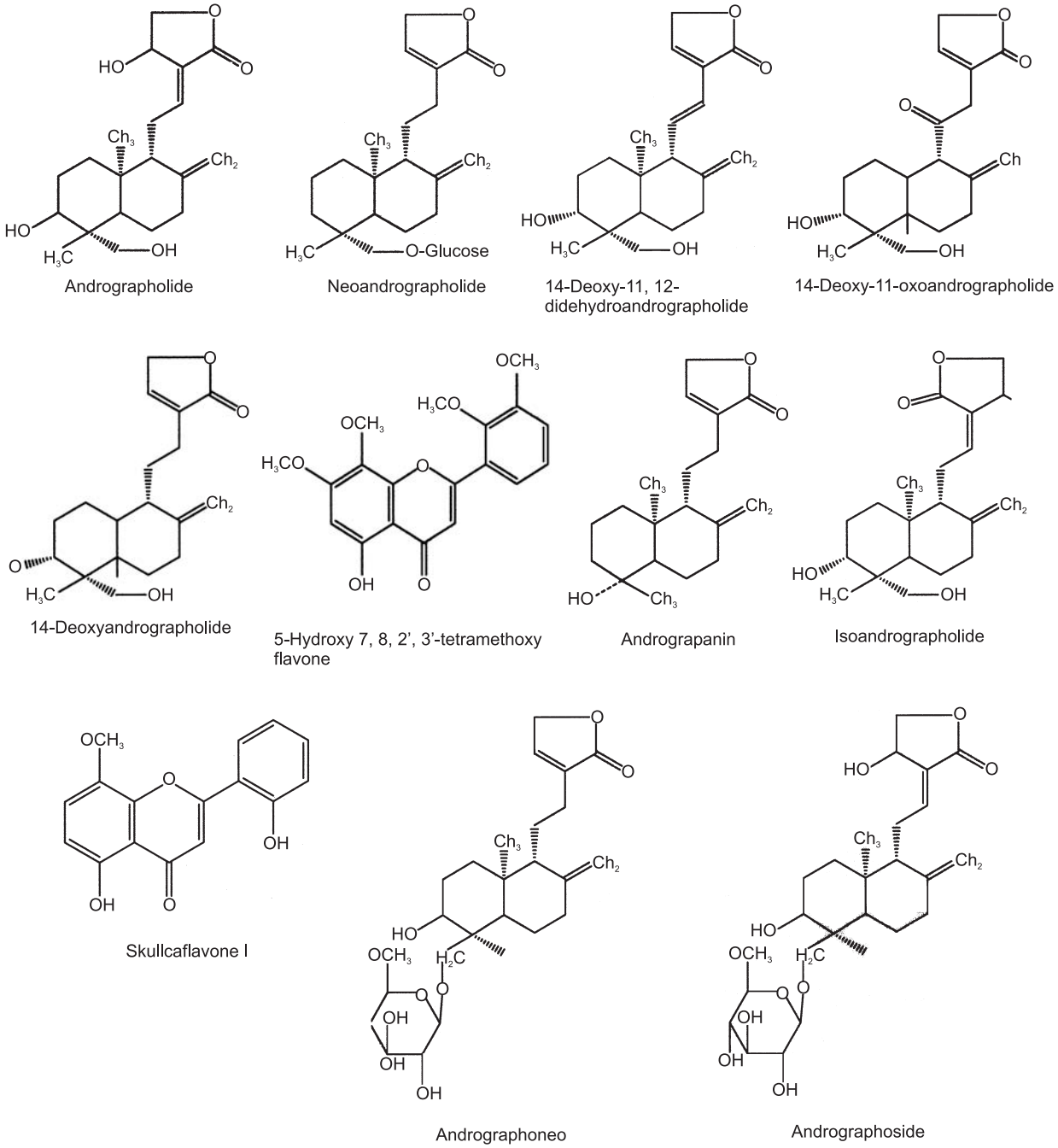

Plate 1: Chemical structures of some major compounds present in *Andrographis paniculata*

Pharmacological activities

Anti-inflammatory activity

It is reported that inflammation caused by histamine, dimethyl benzene and adrenaline was significantly reduced by dehydroandrographolide followed by neoandrographolide and andrographolide[15]. Yin and Guo reported that the anti-inflammatory action of dehydroandrographolide was due to its effect on increasing the synthesis and release of andrenocorticotrophic hormone (ACTH) of the pituitary gland of the brain[16]. ACTH signals the adrenal gland to make cortisol, a natural anti-inflammatory agent. Other research groups found andrographolide to inhibit edema by 60% at a concentration of 200 mg/kg body weight and 62.7% at 400 mg/kg body weight in three hours[17-19]. Liu *et al* found that oral administration of neoandrographolide (100-150 mg/kg) reduces the increased vascular permeability (induced by acetic acid) in mice[20,21]. Neoandrographolide at concentrations (30-90 μM) significantly (P<0.05) inhibited the productions of nitric oxide (NO) and prostaglandin E(2) in bacterial lipopolysaccharide (LPS) stimulated-murine macrophages without inducing cytotoxicity. Their study shows that the anti-inflammatory properties of neoandrographolide might result from the inhibition of iNOS and COX-2 expression through inhibiting p38 MAPKs activation. Sheeja *et al* reported that administration of methanolic extract of *kalmegh* produced complete inhibition of carageenan induced inflammation compared with control models[22]. Chiou *et al* reported significant effect of andrographolide on the expression of inducible NO synthase (iNOS), mRNA, protein, and enzyme activity in RAW 264.7 macrophages stimulated with lipopolysaccharide (LPS) plus interferon-gamma (IFN-gamma)[23]. In this condition, andrographolide (1-100 microM) inhibited NO production in a dose-dependent manner with an IC_{50} value of 17.4+/-1.1 μM. All above findings show that compounds and plant itself possesses significant anti-inflammatory effects. The anti-inflammatory activity of the aqueous extract was clinically proved by Tajuddin and Tariq[24].

Antioxidant activity

Verma and Vinayak studied the effect of the aqueous extract of *A. paniculata* on antioxidant defense system in lymphoma bearing AKR mice in liver[25]. Oral administration of the aqueous extract of plant in different doses caused a significant elevation of catalase, superoxide dismutase and glutathione-s-transferase activities. Trivedi *et al* studied the effect of andrographolide on the hepatocellular antioxidant defense system and lipid peroxidation of control mice, mice treated with hexachlorocyclohexane (BHC), andrographolide and BHC. Glutathione (GSH), glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GSH-Px), gamma-glutamyl transpeptidase (gamma-GTP), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) were studied by spectrophotometric methods[26]. The BHC experimental model forms an irreversible liver tumour in male mice. The activities of GSH, GR, GSH-Px, SOD and CAT showed significant ($P\leq 0.05$) increases, while gamma-GTP and GST showed significant decreases ($P\leq 0.05$) in andrographolide-supplemented mice as compared with BHC-treated mice. Sheeja *et al* explored the antioxidant and anti-inflammatory properties in methanolic extract of plant and found it to inhibit formation of oxygen derived free radicals such as superoxide (32%) hydroxyl radicals (80%) lipid peroxidation (80%) and nitric oxide (42.8%) *in vitro* system[22]. *In vivo* studies using BALB/c mice models showed significant inhibition in phorbol-12-myristate-13-acetate (PMA) induced superoxide (32.4%) and nitric oxide (65.3%) formation. Tripathi and Kamat examined aqueous extract for antioxidant activity using rat liver subcellular organelles as model systems and found that the extract shows potent antiradical agent against various pathophysiological oxidants[27].

Antidiabetic activity

Umamaheswari *et al* reported that oral administration of Ilogen-Excel (an Ayurvedic formulation fortified with this plant, 50 mg/kg and 100 mg/kg) for 60 days results in significant lowering of blood glucose and increasing levels of plasma insulin, hepatic glycogen and total hemoglobin[28]. It decreases the levels of glycosylated hemoglobin, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and vitamin E in diabetic rats. The ethanolic extract of whole plant has been found to be effective for antihyperglycaemic property and reduces oxidative stress in diabetic rats[29]. Reyes *et al* studied the effects of plant on estrous cyclicity of alloxan-induced diabetic rats and found that the antidiabetic potential of *kalmegh* could restore impaired estrous cycle in alloxan-induced diabetic rats[30]. Oral treatment

of andrographolide decreases the plasma glucose concentrations of streptozotocin-induced diabetic rats in a dose-dependent manner[31]. Andrographolide at the effective dose (1.5 mg/kg) significantly attenuated the increase of plasma glucose induced by an intravenous glucose challenge test in normal rats. Andrographolide can increase the glucose utilization to lower plasma glucose in diabetic rats lacking insulin. However, Borhanuddin *et al* reported the significant (P<0.001) hypoglycaemic effect of water extract (10 mg/kg body weight) on experimental rabbits)[32].

Antileishmanial activity

Mannosyl-fucosyl receptors on macrophages were used by Sinha *et al* to target antileishmanial drugs and andrographolide from *kalmegh*, encapsulated in mannosylated or fucosylated liposomes to treat the experimental leishmaniasis in the hamster model and found to be the most potent drug in reducing the parasitic burden in the spleen as well as in reducing the hepatic and renal toxicity[33].

Anti-diarrhoeal and intestinal effects

The plant powder can prevent or stop diarrhoea on animal model[34]. The components of plant like andrographolide and neoandrographolide showed similar activity to loperamide (Imodium), the most common antidiarrhoeal drug. In an experiment by Yin and Guo[16] acute bacterial diarrhoea in patients was treated with a total dose of 500 mg andrographolide divided over three dosing periods per day for six days (2.5 to 3.0 mg/kg of body weight). There were 66 cures of 80 patients treated at an 82.5% cure rate. Seven additional patients responded favorably to the treatment and only seven patients (8.8%) did not respond. The effectiveness of the treatment was confirmed by laboratory tests of stool samples. In another study, plant was used to treat 1,611 cases of bacterial dysentery and 955 cases of diarrhoea with overall effectiveness of 91.3%(Ref.15).

Anti-fertility activity

Dry leaf powder (105mg of powder/kg body weight) given to male rats each day for 60 days, stopped spermatogenesis (development and maturation of sperm cells)[35,36]. Studies done in cultured human placental tissue showed that andrographolide sodium succinate was effective in inhibiting human progesterone production. There were no detrimental effects on other normal human tissue, even at the highest doses tested. Study reveals that plant and its compounds are contraceptive in nature[16].

Antivenom activity

Plant extracts (7.2 mg/kg body weight) and partially purified fractions (2.4 mg/kg body weight) when orally administered to mice experimentally envenomed with rattlesnake venom s.c. injection (2.5-15 mg/kg body weight) showed potent neutralizing effect against the venom. The isolated fractions effectively inhibited the toxic effect of snake venoms *in vitro* than *in vivo*[37].

Anti-HIV activity

Andrographolide prevents transmission of the virus to other cells and stop the progress of the disease by modifying cellular signal transduction technology[38,39]. Bis-andrographolide ether; andrographolide; 14-deoxy-11, 12-didehydroandrographolide; andrograpanin; 14-deoxyandrographolide; 5-hydroxy-7,8-dimethoxyflavanone and 5-hydroxy-7,8-dimethoxyflavone were tested and found effective for anti-HIV and cytotoxic activity[40]. Anti-HIV activity has been evident by clinical trials reported by various authors[41,42]. Dehydroandrographolide succinic acid monoester (DASM) has been found to be an inhibitor against the HIV *in vitro*[43].

Anti-malarial, anti-filaricidal, anti-bacterial, cold and fever

Methanolic extract of *kalmegh* shows antimalarial activity against *Plasmodium berghei,* one of the parasites that transmit malaria. The extract considerably shows inhibition of multiplication of the parasites. The extract was also effective in killing filarial worms that obstruct lymph channels in the body, leading to gross swelling termed elephantiasis[44,45]. The chloroform extract showed complete parasite growth inhibition at as low as 0.05 mg/ml drug dose within 24h incubation period as compared to methanol extract of drug dose of 2.5 mg/ml but under incubation time of 48h of the same plant species[46]. Authors also found effect in *in vivo* study. Inhibition for anti-malarial activity towards *Plasmodium falciparum* (*in vitro*) using the lactate dehydrogenase (LDH) assay is also reported[47]. *In vitro* study by Dua *et al*[14] revealed that compound 1, 2-dihydroxy-6, 8-dimethoxy-xanthone possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with its IC_{50} value of 4 µg/ml. Xanthones bearing hydroxyl group at 2 position demonstrated most potent activity while xanthones with hydroxyl group at 1, 4 or 8 position possessed very low activity. *In vivo* anti-malarial sensitivity test of this compound on

Swiss Albino mice with *Plasmodium berghei* infection using Peters' 4-day test gave substantial reduction (62%) in parasitaemia after treating the mice with 30 mg/ kg dose. *In vitro* cytotoxicity against mammalian cells revealed that 1, 2-dihydroxy-6,8-dimethoxy-xanthone is non-cytotoxic with its IC_{50}>32 µg/ml. Ethanolic extracts of plant were examined with a salt aggregation test for their ability to modulate cell surface hydrophobicity of enterohaemorrhagic *Escherichia coli* strains, including *E. coli* O157:H7 and showed high bacteriostatic and bactericidal activities[48,49]. Studies on rats showed that andrographolide, neoandrographolide and dehydroandrographolide can lower the fever produced by different fever-inducing agents, such as bacterial endotoxins, pneumococcus, haemolytic streptococcus, typhoid, paratyphoid, and the chemical 2, 4-dinitrophenol. Caceres *et al* showed the prevention of the common cold with a herbal formulation, *Kang Jang* (standardized to 4% andrographolides) in a pilot double-blind study[50]. They reported that *Kang Jang* (dose of 200 mg/day and 1,200 mg/day) had a rate of incidence of colds of 30% reduction. Roxas and Jurenka reported the conventional treatment options of selected botanicals including *A. paniculata,* and nutritional considerations (vitamins A and C, zinc, high lactoferrin whey protein, N-acetylcysteine and DHEA) may help in the prevention and treatment of conditions of common cold and influenza viruses[51].

Antimicrobial and antifungal activity

Limsong *et al* in their findings suggested that ethanolic extract of plant inhibits adherence of *Streptococcus mutans* ATCC 25175 and *S. mutans* TPF-1 *in vitro* at the effective concentrations (0.5%). Singha *et al* reported the antimicrobial activity of aqueous extract whereas Qureshi *et al* reported antifungal activity in the sensitivity of the keratinophilic fungi on dry-weight method[52-54].

Cardiovascular benefits/vasorelaxation activity

Wang and Zhao studied on rabbits and reported that extracts of *kalmegh* in different solvents could increase the time taken for forming blood clots, thus decreasing the risk of subsequent closing of blood vessels (restenosis), seen after angioplasty procedures[55]. Zhao and Fang reported that plant powder given to dogs one hour after development of myocardial infarction decreased the damage that occurred to the heart muscle[35]. An added effect of the plant extract was that it activates fibrionolysis, the natural process in the body that dissolves clots. An extract of plant produced antihypertensive effects as it relaxed the smooth muscle in the walls of blood vessels and prevented the blood vessel from constricting and limiting blood flow to the heart brain, and other organs in the body[56]. The ethyl acetate fraction and andrographolide administered to Sprague-Dawley rats elicited no drop in mean arterial blood pressure (MAP), while water extract, semi purified n-butanol and aqueous fractions produced a significant fall in MAP in a dose-dependent manner without significant decrease in heart rate[57]. The ED_{50} values for water extract, semi purified n-butanol and aqueous fractions were 11.4, 5.0 and 8.6 mg/kg, respectively. Plant extracts and 14-deoxy-11, 12-didehydroandrographolide has shown hypotensive and vasorelaxation effects on conscious rats, their isolated aortas and right atria[58]. In experimental dogs, Guo *et al* reported the prominent effect of plant in alleviating the ischemia-reperfusion injury[59].

Hepatoprotective activity

Andrographolide was found to be more potent (0.75-12mg/kg) than silymarin, a standard hepatoprotective agent[60]. Shukla *et al* reported that andrographolide given to animals produces a significant increase in bile flow which facilitates the digestion[61]. Zheng found that the consumption of andrographolides appeared to accumulate in organs throughout the viscera[62]. After 48 hours, the concentration of labelled andrographolide was 20.9, 14.9, 11.1, 10.9; 8.6, 7.9, 5.6, 5.1, 5.1 and 3.2 % in brain, spleen, heart, lung, rectum, kidney, liver, uterus, ovary and intestine, respectively. Absorption and excretion was rapid i.e. 80% was removed within eight hours and 90% within 48 hrs via the kidney (urine) and G.I. tract. The researchers concluded that plant was a useful remedy for treatment of infective hepatitis. Andrographolide exhibits protective effects in carbon tetrachloride induced hepatopathy in rats. The hepatoprotective action of andrographolide is related to activity of certain metabolic enzymes. The inhibitory effect of plant extract and andrographolide on hepatic cytochrome P450s (CYPs) activities using rat and human liver microsomes has also been reported[63]. Hepatoprotective effect of andrographolide against hexachlorocyclohexane-induced oxidative injury in mice model has been established[27, 64].

Immunological benefits/anticancer activity

Andrographolide suppresses the adhesion of gastric cancer cells which express high level sialyl Lewis(X) to human vascular endothelial cells by blocking E-selectin expression[65]. The effect of ethanolic extract and andrographolide on cell-mediated immune responses in normal and tumour-bearing control animals was reported[66]. Authors observed that treatment with extract and andrographolide significantly elevated the production of interleukin-2 and interferon-gamma in normal and Ehrlich ascites carcinoma-bearing animals. The effects of two doses (50 and 100 mg/kg body weight/day for 14 days) of an 80% hydroalcohol extract of plant and butylated hydroxyanisole (BHA) were studied on Swiss albino mice (6-8 weeks old) and a significant increase in the levels of acid soluble sulphydryl (-SH) content, cytochrome P450, cytochrome P450 reductase, cytochrome b5 reductase, GST, DTD and SOD at both dose levels of extract treatment. The catalase, glutathione peroxidase and glutathione reductase showed significant increases only at the higher dose in the liver[67]. Plant with combination of other natural products may increase cytotoxic activity of Natural Killer Cells (NK) and Tumour Necrosis Factor alpha (TNF-alpha) while decreasing DNA damage in patients with late-stage cancer[68]. The anticancer activity has been reported in B16F0 melanoma syngenic and HT-29 xenograft models[69]. Jada *et al* reported the antitumour activity against a 2-cell line panel consisting of MCF-7 (breast cancer cell line) and HCT-116 (colon cancer cell line)[70]. Potent cell differentiation-inducing activity on leukemia cells has been observed by Matsuda *et al*[71]. It is reported that andrographolide is able to efficiently block T cell activation *in vitro* as well as *in vivo*, a feature that could be useful for interfering with detrimental T cell responses[72]. The plant has been reported as a potent stimulator of the immune system[73-75].

Structure activity relationship

The neoandrographolide might scavenge free radicals by donating the allylic hydrogen of the unsaturated lactone ring[76]. The stoichiometry of the reaction between neoandrographolide and superoxide radical generated from KO(2) in DMSO was reported to be 2 to 1. One major reaction product was isolated and determined to be a diacid formed by the opening of the lactone ring. The antiradical activity of neoandrographolide proceeded by hydrogen abstraction from carbon C-15.

Pharmacokinetic study

Validated analytical methods (HPLC, CE and GC-MS) for determining the amount of andro-grapholide in the blood plasma of rats and human volunteers following the oral administration of plant extract and *kalmegh* fixed combination *kang jang* tablets were developed and used for the pharmacokinetic study. Andrographolide was quickly and almost completely absorbed into the blood following the oral administration of plant extract at a dose of 20mg/kg body wt in rats. Its bio-availability, however, decreased four-fold when a 10-times-higher dose was used[73]. Since a large part (55%) of andrographolide is bound to plasma proteins and only a limited amount can enter the cells, the pharmacokinetics of same are described well by a one-compartment model. Renal excretion is not the main route for eliminating the compound. It is most likely intensely and dose dependently metabolized. The oral administration of four *kang jang* tablets (a single therapeutic dose, equal to 20mg of andrographolide) to humans, maximum plasma levels of approximately 393 ng/ml (approx. 1.12 µm) were reached after 1.5-2h. Half-life and mean residence times were 6.6 and 10.0h, respectively. The calculated steady state plasma concentration of compound for multiple doses of *kang jang* (after the normal therapeutic dose regimen, 3 × 4 tablets/day, about 1mg andrographolide/kg body weight/day) was approximately 660ng/ml (approx 1.9 µm), enough to reveal any anti-PAF effect, particularly after drug uptake when the concentration of andrographolide in blood is about 1342ng/ml (approx. 3.8µM, while for anti-PAF effect EC_{50} - 5 µm)[73].

Clinical studies

A phase I dose-escalating clinical trial of andrographolide has been conducted by Calabrese *et al* in 13 HIV positive patients and five HIV uninfected, healthy volunteers[41]. 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. Andrographolide inhibits HIV-induced cell cycle dysregulation, leading to a rise in CD4(+) lymphocyte levels in HIV-1 infected individuals[41]. Oral administration of *kalmegh* (500 mg twice daily) along with other nutraceuticals by 20 patients with late stage of cancer in various parts of body was evaluated. Total mercaptans and glutathione in plasma were taken and compared to levels measured 6 months later. Complete blood counts and chemistry panels

were routinely monitored. As of a mean of 6 months, 16/20 patients were still alive. The 16 survivors had significantly higher NK function than baseline ($P< 0.01$ for each) and TNF-alpha levels in all four cell populations studied ($P<0.01$ for each). Total mercaptans ($P <0.01$) and TNF-alpha receptor levels were significantly reduced ($P<0.01$)[68]. The andrographolide and its analogues exhibited *in vitro* antitumour activity with moderate to excellent growth inhibition against MCF-7 (breast) and HCT-116 (colon) cancer cells screened at National Cancer Institute (USA) on human tumour cell lines derived from nine cancer cell types[70].

Safety and doses

Guo *et al* reported that when 500 mg/kg of *kalmegh* was given daily for ten days to mice, there was no effect on growth, appetite, or stool production[77]. The animals were energetic and results of complete blood counts were normal. In rabbits given intravenous andrographolide (10mg/kg.), there were no abnormal cardiovascular responses. Liver enzyme tests and heart, liver, kidney and spleen were normal in these animals[77]. Mice that received oral plant extracts (10g/kg body weight) once a day for seven days were found safe and none of them died[78]. This very high amount did produce decreased activity and general lethargy. Heart, kidney, liver and spleen were found to be normal in these animals. In other tests for toxicity, rats or rabbits received 1g/kg of andrographolide or neoandrographolide orally for seven days. This amount also did not affect body weight, blood counts, liver or kidney function, or other important organs[16,36]. Singha *et al* observed that pretreatment of mice with plant, andrographolide and arabinogalactan proteins at 500mg/kg and 125mg/kg of body weight, respectively could minimize the toxicity in comparison to ethanol treated group as revealed by different enzymatic assay in liver and kidney tissues and the results were comparable with silymarin[79]. Hence, out of several ill-defined compounds present in plant, andrographolide and arabinogalactan proteins are the potential bioactive compounds responsible for protection against ethanol-induced toxicity. Four compounds were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride (a cleaning solvent), alcohol, or other toxic chemicals. These chemicals damaged the liver by causing lipid peroxidation[45]. This was a process whereby free radicals were produced by the chemical attack and destroyed cellular membranes that surrounded liver cells. When compounds of *kalmegh* were given to animals three days before the toxic chemicals, there was a significant protective effect in the liver. This effect was attributed to the antioxidant ability of the *kalmegh* compounds, which was effective as silymarin which is another very effective antioxidant derived from milk thistle.

Medicinal formulations

It is found in the Indian Pharmacopoeia and is prominent in several Ayurvedic formulations: andrographis 300 MG, andrographis standardized extract, andrographis 60VCaps, andrographis 400 MG, andrographis 200 MG, andrographis complex, *panchanga churna, kalamegharasa, kalmeghasav, kalmeghnavayas lauh, kalpataru ras, kalansunder ras*, Liv. 52, etc. are some of the herbal products in national and international market, having *kalmegh* as major ingredient[80-85].

Conclusion

Kalmegh has been used in Ayurveda, Unani and Siddha systems of medicines from ancient times. Literature survey shows wide spectrum of pharmacological activities of *kalmegh* either in the form of powder, extracts or in its isolated compounds with minimum side effects. Several products fortified with extract and isolated compounds have been launched in national and international markets for various diseases. Extensive work has been done on this plant, but still it requires more R&D work for drug development. Andrographolides are safe, non-toxic and strong natural antioxidant in comparison with other phyto-antioxidants.

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