CHAPTER 2

LITERATURE REVIEW
2 LITERATURE REVIEW

2.1 General description of *Andrographis paniculata*

Genus Andrographis consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species have medicinal value, out of which *Andrographis paniculata* is the most popular. *Andrographis paniculata* (Burm.f.) Nees, a member of the family of Acanthaceae and commonly known as Kalmegh, is an herb used in traditional medicine in India and Southeast Asia. It is known as king of bitters in English, but is more commonly referred to simply as andrographis. *Andrographis paniculata* is an up to 1 meter tall annual shrub with lanceolate leaves and small white to pink, hairy flowers. It grows in moist deciduous forests and is also cultivated. The leaf is the main medicinal part, but the whole plant including the root has been used (WHO, 2002). However, literature reviews indicated that the genetic diversity and variation information of the *Andrographis paniculata* species and varieties are very limited.

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 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 ethanobotanical studies, biological activities, phytochemistry, cultivation, structure activity relationship, pharmacological activities, clinical study, safety and doses of *Andrographis paniculata*.

2.2 Ethanobotanical studies

*Andrographis paniculata* Nees is indigenously used in medicine particularly as bitter tonic and it is useful in curing fevers, dysentery and eliminating intestinal worms. The plant is used as stomachic, blood – purifier and liver tonic [42]-[44]. In a study in Madhya Pradesh, it was found that *Andrographis paniculata* is useful in snake bite, scorpion and centipede sting [45]. It is also useful in burning sensation, wounds, skin diseases and leprosy. Powdered plant mixed with *sarson* oil, applied in itching. The macerated leaves and juice together with certain spices, such as cardamom, clove and cinnamon, are made into pills and prescribed for relief from gripe and other stomach
ailments in infants and also used as domestic medicine for flatulence, diarrhoea of children and in jaundice [46],[47]. During the study in Kerla and Kolha tribals society of Orissa the herb is reported to possess astringent, anodyne, tonic and alexipharmic properties[48],[49]. Decoction or infusion of the leaves has been used with satisfactory results in sluggish liver, in fever, anemia and scabies. Leaves and roots are also used as febrifuge, tonic, stomachic, cholagogue and anthelmintic. A tincture of the root is tonic, stimulant and aperients [50].

Studies of whole plant of *Andrographis paniculata* in Bihar, eastern U.P.(certain tribes) and in Madhya Pradesh clearly indicate traditional use of this plant in spleen complaints, colic, strangulation of intestine, constipation, diarrhoea, cholera, pathesis, consumption and bite of rabid jackal[51]-[55]. Different parts of plant alone or with other Indian herbs are using in tropidity of liver, neuralgia and convalescence after fever in many societies of India and other Asian countries. The herb is the well-known drug *kalmegh* or 'green chiretta', and forms the principal ingredient of a reputed household medicine, used as a bitter tonic and febrifuge. The herb is reported to possess astringent, anodyne, tonic and alexipharmic properties and is helpful in dysentery, cholera, diabetes, consumption, influenza, bronchitis, swellings and itches, piles and gonorrhoea. It is also used as a cure for torpid liver and jaundice. It is also used as curative or preventive in snake venom poisoning.

### 2.3 Cultivation

*Andrographis paniculata* is cultivated and distributed widely in many countries in Asia such as India, China and Thailand which at present is facing threat of genetic depletion as a result of over-exploitation due to its wide popularity of medicinal uses in the primary health care system. 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 [56]. In a study it was found that this plant can also be cultivated on shady wastelands and 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 [57]. Effect of planting and harvesting dates on yield and quality of *Andrographis paniculata* was also reported [58]. 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.
The seeds are sown during May-June. The seedlings are transplanted at a distance of 60 cm x 30 cm in the last week of July. Two or three irrigations may be required during the dry periods. It flowers during Aug – Nov. Fertilizer requirements for this crop are – poultry manure or FYM @ 10 tons /ha, castor cake 2 tons/ha, 75 Kg N and 75 Kg P₂O₅. The plant is collected at maturity i.e. after complete flowering and fruiting. Depending upon area of cultivation harvesting is done in Oct. - Nov. However, in Andhra Pradesh, it is collected from wild populations from Nov-Jan. The whole plant is dried in shade by spreading on the floor for 7-8 days. During this period, it is protected from dew at nights [59]. The heavy demand of Andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant.

2.4 Physiological variation

Very few examples of Physiological variations are reported in plant *Andrographis paniculata*. Most of the Physiological variations depend on seasonal or developmental changes. An age related study reveals that there is a change in percentage of chlorophyll, carotenoid and anthocyanin content with increase in plants age [60]. Sabu, K. K. found about 33% change in chlorophyll content and 66% change in anthocyanins in *Andrographis paniculata* [61]. In a study of accelerators effects on plant it was found that concentration of chlorophyll content in *Andrographis paniculata* increases with use of Jasmonic acid (JA) and Gibberelic acid (GA3) as an elicitor, in variable concentration [62].

2.5 Phytochemical of *Andrographis paniculata*

The various phytochemical compounds have been detected in *Andrographis paniculata*. They are known to have beneficial importance in medicinal science. Phytochemical analysis of *Andrographis paniculata* shows presence of various types of chemical compounds like flavonoids, phenols, alkaloid, glycosides, saponins, tannins and amino acids. Work of Tang W. *et al.*, shows that phytochemical compounds have inherent ability to modify the bodies’ reaction to allergens, viruses and carcinogens[63]. Two flavonoids, identified as 5, 7, 2’, 3’- tetramethoxyflavonone and 5-hydroxy-7, 2’, 3’-trimethoxyflavone, as well as several other flavonoids were obtained from the whole plant [64]. Phenolic compounds are the largest group of phytochemical and accounts for most of the anti-oxidant activity in plants [65]. In medicine,
saponins are used in hyperglycemia, anti-oxidants, anti-cancer, weight loss, etc. [66]. Alkaloids have been used to treat diseases like malaria, painkillers and managing heart diseases [67]. Glycosides serve as defense mechanisms against predation by many micro-organisms, insects and herbivores. Gorter was the first to isolate the bitter water soluble diterpene lactone, Andrographolide (C$_{20}$H$_{30}$O$_5$) from the leaves of *Andrographis paniculata* [24]. Two other diterpenes, viz. DeoxyAndrographolide and Neoandrographolide with medicinal properties and clinical applications were further isolated by Bright *et al.*, [68]. It is reported to contain three active components diterpene lactones Andrographolide as the major compound, Deoxyandrographolide and Neoandrographolide. Recent studies by Pramanick *et al.* and Shen *et al.* has isolated new *ent*-labdane type diterpenoids from the leaves of *Andrographis paniculata* which are still undergoing various bioactivities testing’s [69,70].

**Table 2.1: Phytochemical content of *Andrographis paniculata* leaves:**

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<tr>
<th>Phytochemical content of <em>Andrographis paniculata</em> leaves (Shen et al., Chem &amp; Liang and Sharma et al.) [70]-[72].</th>
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<tr>
<td>1. Andrographolide</td>
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It was reported that the leaves contain the highest amount of Andrographolide being the most medicinally active phytochemical in the plant whereas the seeds contain the lowest. The levels of other medicinal phytochemical extracted from the leaves are low but may be active in nature [72].

2.6 Pharmacological properties of *Andrographis paniculata*

*Andrographis paniculata* has been widely studied in the last 50 years. Previous studies have focused on *Andrographis paniculata*'s pharmacological composition, efficacy, safety and mechanisms of action. Antioxidant activity, Anti-bacterial and anti-microbial activities, Anti-malarial activity, cold and fever, Anti-venom activity, Anti-diarrhoeal activity, Anticancer activity, Urinary tract infection, Antidiabetic activity, Cardiovascular activity, Immunomodulatory activity etc are some widely studied topics.
2.6.1 Antioxidant activity

Medicinal plants have been playing a vital role on the health and healing of man since down of human civilization. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. *Andrographis paniculata* is using as natural anti-oxidant in many European and Asian countries. Some very important research work has been done on its antioxidant activity. In a study of effect of the aqueous extract of *A. paniculata* on antioxidant defense system in lymphoma bearing AKR mice in liver it was found that oral administration of the aqueous extract of plant in different doses caused a significant elevation of catalase, superoxide dismutase and glutathione-s-transferase activities [73]. In a significant study the effect of Andrographolide on the hepatocellular antioxidant defense system and lipid per oxidation of control mice was studied and hepatoprotective effect of Andrographolide against hexa-chloro cyclohexane was also investigated for oxidative injury[74]. Antioxidant and anti-inflammatory properties in methanolic extract of plant was explored and it was found that it inhibit the formation of oxygen derived free radicals such as superoxide (32%) hydroxyl radicals (80%) lipid peroxidation (80%) and nitric oxide (42.8%) in vitro system [75]. During examination of aqueous extract for antioxidant activity using rat liver sub cellular organelles as model systems it was found that the extract shows potent antiradical agent against various pathophysiological oxidants [76].

It is believed by many in the anti-oxidant therapy that the administration of compounds with antioxidant properties perhaps acts as an effective drug therapy in the preventative and curative therapeutic agents of many diseases conditions. Anti-oxidants have an important role in the prevention or treatment of some diseases like mutagenesis, cancer, atherosclerosis, cardiovascular diseases, degenerative disorders and aging process [77], [78]. Natural anti-oxidant has been implicated in the pathogenesis of a wide variety of clinical disorders, such as degenerative diseases [79]. It may contribute as a factor in aging and progressive impairment of the immune function [80], [81]. It has also been reported that there may be a correlation between antioxidant capacity and phenolics content of drugs [82], [83]. The flavonoids are group of phenolic compounds having bioactive properties like free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [84], [85].
Free radicals are responsible for several disorders in human body [83], [86]. Oxidative process is one of the most important routes for producing free radicals in food, drug, and even in living systems. The free radicals in the human body have adverse effects on its immune system [87]. Consumption of natural oxidants as free radical scavengers may become necessary to improve the depleted immune system [86], [88] and [89]. It is reported that the antioxidant constituents of plant materials provide protection from coronary heart disease and cancer [90] and protect the body from damage caused by free radical induced oxidative stress [91], [92]. *Andrographis paniculata* has been studied for its anti-oxidants activities in relation to the antihepatotoxicity effect of the diterpenoid components of the herb and positive results were obtained. When comparison between normal and diabetic rats was investigated, results of diabetic rats were also encouraging [93], [96]. However, only few reports on the antioxidant activities of *Andrographis paniculata* with comparison of synthetic anti-oxidant are available.

### 2.6.2 Anti-microbial activities

Microbial infections are the world’s leading killing diseases. In recent years, drug resistance to human pathogenic microbes has been commonly reported all over the world. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide so that antimicrobial drug therapy becomes effective, safe and affordable. There are several reports on antimicrobial activity of different herbal extracts. It was reported that the ethanol extract of *Andrographis paniculata* leaves inhibited the growth of Escherichia coli and Staphylococcus aureus in vitro. [97]. 50% methanol extract of *Andrographis paniculata* leaves were found to inhibit growth of *Proteus vulgaris* in vitro [98]. However, there was no in vitro activity observed when the dried powder of the leaves of *Andrographis paniculata* was tested against *E.coli, Staphylococcus aureus, Salmonella typhi* or *Shigella* species based on three assays conducted [99]. Other research group found that Andrographolide and arabinogalactan proteins from *Andrographis paniculata* showed significant anti-microbial activity which may be due to their combined effects in the aqueous extract [100]. Limsong *et al* in their findings suggested that ethanolic extract of plant inhibits adherence of *Streptococcus mutans* and *S. mutans in vitro* at the effective concentrations (0.5%) [101]. Antimicrobial activity of aqueous extract of *Andrographis paniculata* was reported by Singha *et al.* [102]. Antifungal activity in the sensitivity of the keratinophilic fungi on dry-weight method was also reported [103]. In a study in vitro screening of five local medicinal
plants for antibacterial activity using disc diffusion method, researchers found that water extract of *Andrographis paniculata* posses’ potential antibacterial activity towards both gram positive and gram-negative microorganisms [104].

### 2.6.3 Hepatoprotective activity

Hepatoprotective activity of *Andrographis paniculata* was reported by many researchers. In a comparative study Andrographolide was found to be more potent (0.75-12mg/kg) than silymarin, a standard hepatoprotective agent [26]. It was reported that Andrographolide given to animals produces a significant increase in bile flow which facilitates the digestion [105]. After a study of consumption of Andrographolides accumulation in different organs like brain, spleen, heart, lung, kidney, liver, intestine etc. researchers found that the 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. They concluded that plant was a useful remedy for treatment of infective hepatitis [106]. 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 [107].

Hepatoprotective effect of Andrographolide against hexachlorocyclohexane-induced oxidative injury in mice model has been established by Triphathi et al., and Trivedi et al.,[74], [95]. In a comparative study with the known hepatoprotective agent silymarin demonstrated that Andrographolide exhibited a lower protective potential than Andrographiside and NeoAndrographolide, which were as effective as silymarin in the formation of the degradation products of lipid peroxidation and release of glutamic-pyruvate transaminase (GPT) and alkaline phosphatase[93]. The *Andrographis paniculata* leaves extract protected carbontetrachloride-induced hepatic toxicity better than Andrographolide [27]. Studies have demonstrated the *in vivo* hepatoprotective effect of Andrographolide against carbontetrachloride-induced liver damage and galactosamine or paracetamol-induced hepatotoxicity in rats [21], [108]. The significant hepatoprotective activity of aqueous extract of *Andrographis paniculata* was reported on hexachlorocyclohexane (BHC) induced severe liver damage in Swiss male mice by estimating serum ALT & AST and parameters such as alkaline phosphatase, g-Glutamyl transpeptidase, glutathione and lipid peroxidase[109].

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2.6.4 Anti-fertility

Use of *Andrographis paniculata* as natural medicine is increasing day by day hence its adverse effects on human body if any also is the topic of interest. Regarding this *Andrographis paniculata* have been tested for anti-fertility also. Male albino rats fed at a dose of 20mg/day of dried leaf powder of *Andrographis paniculata* for 60 days resulted in the cessation of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells in the epididymis seminal vesicle, ventral prostate and coagulating gland demonstrated the antispermatogenic and antiandrogenic effect of the plant [110]. Female mice on *Andrographis paniculata* at 2gm/kg daily for a period of 6 weeks were not pregnant compared to the majority of the control female mice (95.2%) without *Andrographis paniculata* were pregnant indicated its potent antifertility effect indicating a precaution against use during pregnancy. Extra contraception is necessary as *Andrographis paniculata* has not been proven as an absolute contraceptive [111].

2.6.5 Anti-malarial activity, cold and fever

The leaves of *Andrographis paniculata* have been widely used for relieving fever and lowering body temperatures by the Asian communities for many years. Misra et al reported that methanolic extract of *kalmegh* shows antimalarial activity against *Plasmodium berghei*, one of the parasites that transmit malaria [112]. The extract considerably shows inhibition of multiplication of the parasites. Inhibition for anti-malarial activity towards *Plasmodium falciparum (in vitro)* using the lactate dehydrogenase (LDH) assay is also reported [113]. *In vitro* studies it was revealed that compound 1, 2-dihydroxy-6, 8-dimethoxyxanthone possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with its IC50 value of 4 g/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 vitro* and *in vivo* studies of *Andrographis paniculata* demonstrated the anti-malarial properties which can be further researched into the development of future potent anti-malarial drugs [114], [115].

Prevention of the common cold with a herbal formulation, *Kang Jang* (standardized to 4% Andrographolides) in a pilot double-blind study was also reported. It was found that *Kang Jang* (dose of 200 mg/day and 1,200 mg/day) had a rate of incidence of colds of 30% reduction [116]. In a study of conventional treatment options of selected botanicals including *A. paniculata*, and nutritional considerations (vitamins A and C, zinc, high lactoferrin protein, N-acetylcysteine and
DHEA etc.) it was found that its help in the prevention and treatment of conditions of common cold and influenza viruses[117]. In another study by Thamlikitkul's research team 152 adult patients with pharyngotonsilitis were enrolled in a randomized double-blind study to assess the efficacy of *Andrographis paniculata*. The patients were randomized to receive either paracetamol or 3 g/day of *Andrographis paniculata* or 6 g/day of *Andrographis paniculata* for 7 days. The efficacy of paracetamol or high dose Andrographolide was significantly more than that of low dose Andrographolide at day 3 in terms of relief of fever and sore throat. The same clinical improvement was noted on day seven [118].

### 2.6.6 Anti-pyretic activity

Some reports on Anti-pyretic activity of *Andrographis paniculata* are available. In an investigation the intragastric administration of the ethanol extract of *Andrographis paniculata* leaves at 500mg/kg to rats on yeast-induced pyrexia was found as effective as 200mg/kg body weight of aspirin (at equipotency dosage) with no toxicity observed at doses up to 600mg/kg body weight [119]. Intragastric administration of the major active component Andrographolide at 100mg/kg body weight to mice decreased brewer’s yeast-induced pyrexia [120]. Similar study was conducted on deoxyAndrographolide, Andrographolide, or 11, 12-didehydro-14- deoxyAndrographolide at 100mg/kg body weight to mice, rats or rabbits were found to reduce pyrexia induced by 2, 4-dinitrophenol or endotoxins [121], [122].

### 2.6.7 Anti-HIV activity

When Anti-HIV activity of *Andrographis paniculata* was tested it was found that Andrographolide prevents transmission of the virus to other cells and stop the progress of the disease by modifying cellular signal transduction technology [123], [124]. 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[125]. Anti-HIV activity has been evident by clinical trials reported by Calabrese et al., [126] DehydroAndrographolide succinic acid monoester (DASM) has been found to be an inhibitor against the HIV *in vitro* [127]. Andrographolide esters have been found to be inhibitors against the HIV virus *in vitro*. It was non-toxic to the H9 cell at concentrations of 1.6 - 3.1 mcg/ml. It was also inhibitory to two other strains of HIV-1 and a strain
of HIV-2 [128]. Andrographolide administered at 5, 10 and 20 mg/kg bodyweight for 3 weeks for each. It have been found significant rise in the mean of CD4(+) lymphocyte level of HIV subject Andrographolide may inhibit HIV- induced cell cycle deregulation leading to a rise in CD4(+) lymphocyte levels [129].

2.6.8 Anti-venom activity

*Andrographis paniculata* is indicated as an herbal medicine for snake bites in traditional medicine therapy. Intra-peritoneal injection of an ethanol extract of the leaves (25g/kg body weight) to mice poisoned with cobra venom markedly delayed the occurrence of respiratory failure and death. This extract also induced contractions in guinea-pig ileum at concentrations of 2mg/ml, and these contractions were enhanced by physostigmine and blocked by atropine, but were unchanged by antihistamines. Study indicated that the extracts do not modify the activity of the nicotinic receptors but possibly mediated by producing significant muscarinic activity, accounting for its anti-venom effects [130].

2.6.9 Anti-diarrhoeal activity

The components of plant *Andrographis paniculata* like Andrographolide and neoAndrographolide showed anti-diarrhoeal activity to loperamide (Imodium), the most common antidiarrhoeal drug. It was found that ethanol, chloroform or 1-butanol extracts of *Andrographis paniculata* leaves at 300mg/ml inhibited the E. coli enterotoxin-induced secretory response diarrhoeal syndrome in rabbit but the aqueous extract was ineffective. Andrographolide and Neoandrographolide exhibited potent antisecretory activity *in vivo* against E. coli enterotoxininduced diarrhoea at 1mg of equivalent potency as loperamide when tested against heat labile E. coli enterotoxin-induced diarrhoea by acting through the stimulation of adenylate cyclase[131]. In a clinical study acute bacterial diarrhoea in 1,611 patients was treated with Andrographolide,955 cases of diarrhea responded favorably to the treatment and effectiveness was 91.3%, and the effectiveness of the treatment was confirmed by laboratory tests of stool samples [132].

2.6.10 Anticancer activity

Andrographolide has been shown to inhibit human breast, liver and prostate cancer cells. Oncologists at the Rosell Park Cancer Institute are currently studying the potential use of
Andrographolide as a therapeutic agent in several prostate cancer cell lines. Unlike cytotoxic anticancer drugs, Andrographolide is rapidly metabolized when taken orally [133]. Antitumour activity against a 2-cell line panel consisting of MCF-7 (breast cancer cell line) and HCT-116 (colon cancer cell line) was also reported [134]. Study of Singh et al., using different cancer cell lines on the modulatory influence of Andrographis paniculata (Burm. f.) Nees on mouse hepatic and extrahepatic carcinogen metabolizing and anti-oxidant status also indicated that it has chemoprotective potential against chemotoxicity including carcinogenicity [135]. Results of the study of late cancer stage indicated that a combination of vitamin, mineral, antioxidants and immuneenhancing natural products and Andrographis paniculata at 500 mg twice daily was quite promising as an anti-cancer agent .They use this combination on the increased tumor necrosis factor alpha (TNF-alpha) and natural killer cell (NK) function in late stage cancers [136]. Andrographis paniculata plant extract contains diterpenes, flavonoids and stigmasterols with Andrographolide as the major active diterpenoid which have shown to possess potent cytotoxic activity against KB (human epidermoid carcinoma) and P388 (Mouse leukaemic lymphoblastic parental tumour cell line [137].

2.6.11 Urinary tract infection

Testing of Andrographis paniculata for Urinary tract infection is under trial but in tribes area its use is very common .Study on fifty patients post Extracorporeal Shock Wave Lithotripsy (ESWL) pyuria and hematuria found that Andrographis paniculata tablets at an oral dose of 250mg reduced symptoms in ESWL urinary tract infection[138].

2.6.12 Antidiabetic activity

Diabetes is a condition strongly related to oxidative stress. Complications of the diabetes such as neuropathy, retinopathy, nephropathy and multi-organ atherosclerosis are believed to be implicated by oxygen free radicals namely superoxide, hydrogen peroxides (H₂O₂) and hydroxyl radicals (OH) [139]. Study on blood glucose clearly indicates 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 [140]. A group of researchers 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 [141]. Significant ($P<0.001$) hypoglycaemic effect of water extract (10 mg/kg body weight) on experimental rabbits was also reported [142]. Oral treatment of Andrographolide decreases the plasma glucose concentrations of streptozotocin-induced diabetic rats in a dose-dependent manner [143].

2.6.13 Cardiovascular activity
The published studies from China indicate that extracts of the herb have successfully inhibited the thickening of arterial walls of experimental animals. Damage to the heart was also induced. An added effect of the plant extract was that it activates fibrinolysis, 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 [144]. Separate studies of Zhang et al show that Andrographolide inhibits aortic smooth muscle cell proliferation in vitro. The potential exists for use of Andrographolide in many types of patients with cardiac risk. 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 [145]. It was reported that AND increased the radioactive glucose uptake in cultured myoblast C2C12 cells and the uptake may reduce glucose in blood flow [146]. In a clinical study workers found a hypotensive activity of aqueous extract of AP in rats and they suggested that the aqueous extract of AP lowers the systolic blood pressure of spontaneously hypertensive rats possibly by reducing circulating angiotensinconverting enzyme in the plasma as well as by reducing free radical levels in the kidneys [147].

2.6.14 Immunomodulatory activity
Andrographolide has been reported to have both immuno-stimulant and suppressant activities. The immunomodulatory property of a diterpene lactone Andrographolide was reported to be associated with enhancement of proliferation of human peripheral blood lymphocytes as well as the production of key cytokines and the expression of immune activation markers (such as INF-γ, neopterin and β-2-microglobulin) in whole blood cells in culture in vitro [148]. The extract and purified Andrographolide was reported to stimulate an innate immune response in mice, measured
by macrophage migration index, phagocytosis of [14 C] leucine-labelled E.coli, and proliferation of splenic lymphocytes stimulated with Andrographis paniculata extract [149]. Immunostimulatory activity of Andrographolide reported in vitro in PHA stimulated HPBLs (human peripheral blood lymphocytes) by increased proliferation of lymphocytes and production of IL-2. reported that Andrographolide inhibited the production of TNF-α and IL-12 in macrophages stimulated by lipopolysaccharide [150], [151]. The plant has been reported as a potent stimulator of the immune system [152]. 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 [153].

2.7 Pharmacokinetic study of Andrographis paniculata

Pharmacokinetics is the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body. Andrographolide is highly bio available in human and animals. Analyst used validated analytical methods (HPLC, capillary electrophoresis and GC-MS) to determine the amount of Andrographolide in the blood plasma of rats and human volunteers. Oral administration of Andrographis paniculata extract and fixed combination tablets were developed and used for the pharmacokinetic study. They found that Andrographolide was quickly and almost entirely absorbed into the blood [154]. Burgos et al., used male rats to evaluate the possible testicular toxicity of Andrographis paniculata dried extract for 60 days. No toxicity was found after the rats were treated with 20, 200 and 1000 mg/kg bodyweight for 60 days by evaluation of reproductive organ weight, testicular histology, ultra structural analysis of Leydig cells and testosterone level. They suggested that Andrographis paniculata extract had no subchronic testicular toxicity effect in male rats [155].

No toxicity was observed when humans had been treated in a dose of 1200 mg daily for 4 days. 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 [156]. In another study, it was found that after 28 hours of administration, 20.9% of labelled Andrographolide was accumulated in the brain; 14.9% in the spleen; 11.1% in the heart;
10.9% in the lung; 8.6% in the rectum; 7.9% in the kidney; 5.6% in the liver; 5.1% in the uterus; 5.1% in the ovary and 3.2% in the intestine [157]. In a study in Thailand it was reported that high amount of Andrographolide decrease activity and caused general lethargy, but the mouse heart, kidney, liver and spleen were found to be normal. When extract of 20 g leaves with 600 ml water administered at the rate of 10 ml/kg bodyweight in rats and rabbits, it did not produce abnormalities [158].

### 2.8 General characterization of Andrographolide

In 1896 Boorsma first isolated Andrographolide from *Andrographis paniculata* and Gorter identified Andrographolide as a lactone in 1911[24]. It is a colourless or light yellow crystal compound with a very bitter taste. Andrographolide’s chemical name is 3α, 14, 15, 18-tetrahydroxy-5β, 9βH, 10α-labda-8(20), 12-dien-16-oic γ-lactone. Its molecular formula and weight are C_{20}H_{30}O_{5} and 350.4 (C 68.54%, H 8.63%, O 22.83%) respectively. A colourless plate from ethanol or methanol with a melting range from 205-235.3°C has been reported. It is also reported that its UV absorption maxima in methanol or ethanol is 222-224 nm [159]-[161]). Its structure was analyzed by X-ray, 1H, 13 C-NMR and ESI-MS. It does not mix well with water, but is soluble in acetone, chloroform, ether and hot ethanol. Rajani et al. reported a simple and rapid method for isolation of Andrographolide from the leaf of *Andrographis paniculata*. They extracted it from the leaf powder using 1:1 mixture of dichloromethane and methanol in cold condition and then isolated Andrographolide directly from the extract by re-crystallization. They confirmed the compound through IR, UV, MS, melting point and co-chromatography with a reference standard on TLC. The purity of the compound was also checked by TLC, UV absorption spectrum, HPLC, LCMS and differential scanning calorimetry (DSC), the DSC gave the melting point of Andrographolide as 235.3°C[159]-[163].Crystalline Andrographolide was reported to be highly stable even at 70°C (75% relative humidity) over a period of 3 months [164].

### 2.9 Safety and doses of *Andrographis paniculata*

*Andrographis paniculata* has been used in Ayurveda, Unani and Siddha systems of medicines from ancient times. Literature survey shows wide spectrum of pharmacological activities of *Andrographis paniculata* either in the form of powder, extracts or in its isolated compounds with minimum side effects. *Andrographis paniculata* is sold commercially as a medicine in China,
India, Thailand and Malaysia. Several products fortified with extract and isolated compounds have been launched in national and international markets for various diseases. Andrographolide are safe, nontoxic and strong natural antioxidant in comparison with other phyto-antioxidants. It is contraindicated in pregnancy due to the suggestion that it has abortifacient activity. It is not recommended to be injected in various routes of administration to avoid adverse effects. Ingestion of large doses of *Andrographis paniculata* orally have been reported to cause gastric discomfort, vomiting and loss of appetite as associated adverse reactions or side effects of the extremely bitter taste of Andrographolide. Thin-layer chromatography, ultraviolet spectrophotometry, liquid chromatography and volumetric and colorimetric techniques are used in a variety of laboratory methodologies to ensure a standardized level of Andrographolide [165]-[168]. Andrographolide is used as standard to analyze *Andrographis paniculata*. Literature survey indicates that very less work has been done on pharmaceutical potential of *Andrographis paniculata* hence this research topic “Chemical Study of *Andrographis paniculata* for pharmaceutical Potential” has been selected.

There is a wide variation in the amount and type of chemical constituents in samples of different places, in samples that differ in method and time of collection. Any change in chemical constituents during seasonal variation and different stages of life cycle of plants will be studied. Location variation of the plant is to be studied. Besides this it will also be studied that same plant of *Andrographis paniculata* will continue to grow in Dehradun for the second year or not. If the plants continue to grow then what would be the change in its main chemical constituent “Andrographolide” in comparison to the one year old plants, if any. Study of antioxidant activities of *Andrographis paniculata* with comparison of synthetic anti-oxidant will also be the part of study. Thus the potency, quality and purity of drugs have to be evaluated. Extensive work has been done on this plant, but still it requires more R&D work for pharmaceutical industries and drug development.