Chapter-1

Introduction

INTRODUCTION

Andrographis paniculata (Burm. F.) Wall. Ex Nees (family: Acanthaceae) also known as *Kalmegh*, is extremely bitter in taste and it is often referred to as the "King of Bitters". This plant has been used as bitter tonic, stimulant and aperients in Ayurvedic and other traditionally known health care systems widely practiced in India and other Asiatic countries. In Traditional Chinese Medicine, *Andrographis paniculata* is indicated for conditions of "Heat", particularly in the lungs, throat and urinary tract, as well as for manifestations of "Fire Poison" on the skin, such as sores and carbuncles (Rege *et al.*, 1999; Sharma *et al.*, 1992; Bensky and Gamble, 1986). Amongst numerous plants of the *Andrographis* genus, *Andrographis paniculata* is the only one widely used for medicinal purposes, and it is also pre-clinically and clinically the most well studied one. Andrographolide is quantitatively the major bioactive secondary metabolite of the plant (Sharma *et al.*, 1992), and it is now often considered to be a structurally and functionally novel therapeutic lead potentially useful for treatments for inflammatory diseases and cancer (Hidalgo *et al.*, 2013; Panossian and Wikman, 2013; Jayakumar *et al.*, 2013; Lim *et al.*, 2012; Valdiani *et al.*, 2012; Saxena *et al.*, 2010; Chao and Lin, 2010; Mishra *et al.*, 2007). However, diverse types of medicinally used *Andrographis paniculata* extracts contain other structurally analogous labdane diterpenoids, oxygenated flavonoids and numerous other bioactive secondary plant metabolites. Some of the major medical conditions commonly treated with such extracts are diabetes, liver disorders, common cold, dyspepsia and other diseases of the gastrointestinal tract (Parixit *et al.*, 2012).

The World Health Organisation (WHO) monograph on *Andrographis paniculata* (Herba Andrographidis) mentioned its uses for prophylaxis and symptomatic treatments of upper respiratory tract infections, bronchitis, pharyngotonsillitis, lower urinary tract infections and acute diarrhoea are supported by clinical data (World Health Organisation, 2004). Since then several other clinical trials have not only continued to reaffirm such therapeutic benefits of diverse types of extracts of the plant, but also their therapeutic potentials for treatments of other diseases like rheumatoid arthritis (Hidalgo *et al.*, 2013; Burgos *et al.*, 2009), type-

2 diabetes (Agarwal *et al.*, 2005), and inflammatory bowel disease (Sandborn *et al.*, 2013; Xia *et al.*, 2004). Several recent reviews summarizing currently available information on medicinal phytochemistry and pre-clinical and clinical pharmacology on *Andrographis paniculata* have appeared during recent years (Valdiani *et al.*, 2012; Ghosh *et al.*, 2014; Subramanian *et al.*, 2012; Kumar *et al.*, 2014). Although as yet comparatively little attention has been paid to neuropsychopharmacology of andrographolide and *Andrographis paniculata*, recent preclinical observations made in our laboratories and elsewhere strongly suggest that they could as well be promising therapeutic leads potentially useful for prevention and cure of psychopathologies commonly associated with diabesity and diverse other chronic diseases with central sensitivity syndromes (Thakur *et al.*, 2014a; Thakur *et al.*, 2014b; Thakur *et al.*, 2014c).

Comorbidities of depression and anxiety are often encountered in patients suffering from, or prone to, diabetes (Bystritsky *et al.*, 2014), and central sensitivity syndromes (Yunus, 2009; Cryan and Dinan, 2012) almost always accompany all medical conditions for which traditionally known medicinal uses of *Andrographis paniculata* extracts are known since centuries. However, till recently only two preclinical reports on psychopharmacology of *Andrographis paniculata* extracts (Mandal *et al.*, 2001; Radhika *et al.*, 2012a) and one patent on potential uses of andrographolide against neurological disorders had appeared (Shaw *et al.*, 2011). However, the possibility that *Andrographis paniculata* extracts possess anti-stress or adaptogenic properties have often been pointed out by several modern scholars and researchers of traditionally known herbal remedies (Williamson, 2002; Govindarajan *et al.*, 2005; Thakur *et al.*, 2012a; Panossian and Wikman, 2013). They clearly revealed that like many other herbal adaptogens, the therapeutically interesting anti-depressants and anxiolytics-like and stress response-desensitizing effects of *Andrographis paniculata* extracts in animal models become detectable, or more prominent, after their daily oral doses only. Although a vast majority of preclinical reports on such extracts, or on pure andrographolide, have dealt mainly with their therapeutically interesting bioactivities observed through *in vitro*, in cellular, and other bioassay, or after their fairly high acute doses administered intraperitoneally or intravenously in

experimental animals, several reports on their therapeutically interesting pharmacological activities after their fairly low daily oral doses have also appeared. For example, it has been reported that fairly low daily oral doses (less than 10 mg/kg/day) of andrographolide or of *Andrographolide paniculata* extracts possess cardio-protective, anti-diabetic and anti-hyperlipidemic and gastro- and hepato-protective activities in experimental animals (Awang *et al.*, 2012; Nugroho *et al.*, 2012; Saranya *et al.*, 2011; Yang *et al.*, 2013a; Trivedi *et al.*, 2000; Chander *et al.*, 1995).

Several oral bioavailability studies conducted in experimental animals and volunteers have reaffirmed though, that very low, or undetectable, blood levels of andrographolide are observed even after it extremely high oral doses administered as pure compound or with *Andrographis paniculata* extracts (Yang *et al.*, 2013b; Zhou *et al.*, 2013; Guo *et al.*, 2012; Ye *et al.*, 2011; Panossian *et al.*, 2000). Although several other authors reporting neuro- or cerebro-protective activity of andrographolide in *ex vivo* or *in vitro* experimental models have often also suggested that andrographolide could as well cross the blood brain barrier (Chan *et al.*, 2010; Carretta *et al.*, 2009; Qin *et al.*, 2006; Burgos *et al.*, 2005; Wang *et al.*, 2004), it cannot be ignored that its biological half life is short and its blood levels are much lower than those necessary for observing its neuro-protective activity in cellular models.

In any case, available information on bioavailability of andrographolide and several other bioactive constituents of *Andrographis paniculata* extracts clearly reveal that more than 95% of their orally administered doses are extensively bio-transformed within the gastrointestinal tract (Guo *et al.*, 2012; Ye *et al.*, 2011; Panossian *et al.*, 2000), and that if orally absorbed andrographolide can alter diverse drug metabolizing activities in liver and other peripheral organs (Ooi *et al.*, 2011; Pan *et al.*, 2011; Chien *et al.*, 2010; Pekthong *et al.*, 2009; Pekthong *et al.*, 2008). Therefore, it can be expected that the long lasting and therapeutically interesting bioactivities of andrographolide observed in experimental animals after its oral administrations is due to its irreversible interactions with biologically important macromolecules within the gastrointestinal tract.

Therapeutically interesting preclinical information on medicinal phytochemistry and pharmacology of *Andrographis paniculata* extracts and andrographolide strongly suggest that they are promising therapeutic leads potentially useful for treatments of diverse spectrums of psychopathologies commonly encountered in almost all lifestyle associated chronic diseases (Kumar et al., 2014). Since their high efficacy and broad safety profiles have already been demonstrated (Bothiraja *et al.*, 2013; Chandrasekaran *et al.*, 2009; Allan *et al.*, 2009; Panossian *et al.*, 1999), appropriately controlled and properly designed clinical trials necessary for firmly establishing their psychotherapeutic potentials seems warrantable. Such efforts should eventually not only be useful for identifying validated novel pharmacological targets urgently needed for discovery and development drugs against neurological disorders of the 21st century, but also for better understanding of biological principles and processes involved in traditionally known widespread medicinal and health care uses of *Andrographis paniculata.*

Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. Metabolic syndrome is generally associated with diabetes mellitus, obesity, blood lipid disorders, inflammation and insulin resistance (Despres and Lemieux, 2006). The International Diabetes Federation has estimated that the worldwide prevalence of diabetes mellitus is expected to increase from 382 million people in 2013 to 592 million by 2035. There were 72.1 million people with diabetes mellitus in the South East Asia region in 2013 and this number is expected to increase to 123.0 million by 2035. India alone has 65.1 million people living with diabetes mellitus, this places India second to China with 98.41 million diabetic people (International Diabetes Federation, 2013). Diabetes has been associated with various disorders including brain disorders such as depression, anxiety and cognitive dysfunctions. Several co-morbid conditions have been described in rodent models of diabetes including depression and anxiety (Rowland *et al.*, 1989). The rate of Generalized Anxiety Disorders and subsyndromal anxiety are higher in diabetic patients (Grigsby *et al.*, 2002). Memory impairment associated with diabetes is also well reported. Chances of cognitive dysfunctions and

dementia are almost double in diabetic patients (Leibson *et al.*, 1997). Despite extensive efforts and considerable progress, proper pharmaco-therapeutic managements of comorbid mental health conditions often associated with almost all chronic health problems still remain to be one of the major challenges for medicine (Detweiler-Bedell *et al.*, 2008; Ban, 2001). It is now well established though, that diverse types of chronic mental as well as physical stress are the root causes of most such disorders, and that adaptation to stress-induced syndromes could be a feasible means for combating them.

Available therapy for neurological disorders is often associated with several undesirable side effects. Unfortunately, currently available psychoactive drugs do not meet the therapeutic demands of diabetic patients and many of them are even contraindicated for patients with diabetes. Therefore, the search of potential therapeutic candidate for the treatment of neurological disorders as well as management of diabetes associated neurological complications is still continuing. The aim of the study was to evaluate neuropsychopharmacological activities of standardised extract of *Andrographis paniculata* and isolated pure andrographolide in nondiabetic rodents and to explore its potential role in the management of comorbid neurological disorders generally associated with diabetes followed by elucidation of their mechanism(s) of action(s). Observed broad spectrum of activity profile of *Andrographis paniculata* and andrographolide strongly suggest that they could be promising therapeutic leads for prevention and cure of diverse mental health problems and other comorbidities commonly associated with, or caused by, diabetes.

The present study was designed to cover the following objectives:

- Pilot-cum-dose finding study with standardised extract of *Andrographis paniculata* and isolated pure andrographolide to establish three graded doses for general neuropharmacological screening and further studies.
- To evaluate standardised extract of *Andrographis paniculata* for anti-diabesity activity in type-2 diabetic rats and animal models of obesity.
- To evaluate the standardised extract of *Andrographis paniculata* for anti-depressant and anxiolytic activity in nondiabetic and diabetic rats.

- To evaluate the effect of standardised extract of *Andrographis paniculata* and andrographolide on learning and memory in nondiabetic and diabetic rats.
- To evaluate other neuropsychopharmacological effects with standardised extract of *Andrographis paniculata* and andrographolide in nondiabetic and diabetic rodents.
- To evaluate *Andrographis paniculata* and andrographolide for analgesic and anti-inflammatory activity in nondiabetic and diabetic rodents.
- To evaluate protective effects of *Andrographis paniculata* extract and pure andrographolide against chronic stress triggered pathologies in rats.
- Toxicity study with standardised extract of *Andrographis paniculata* and pure andrographolide in HL-60 cell line. Cytokines (TNF-α, IL-1β and IL-10) and toll-like receptors (TLR3, TLR7 and TLR8) expression study in HL-60 cell-line with andrographolide.