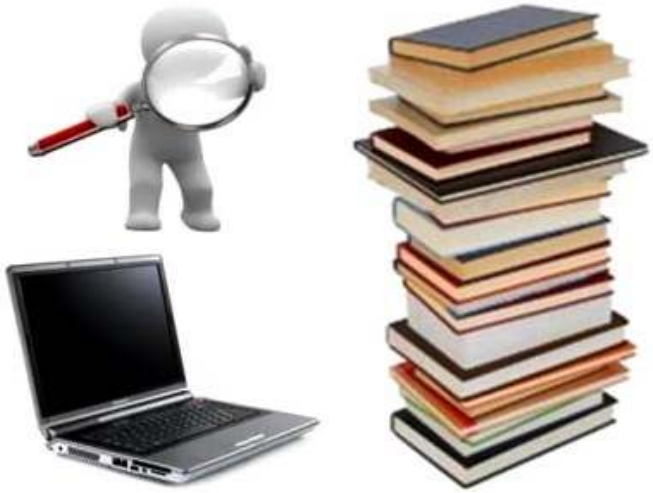
Chapter-2



Literature Review

LITERATURE REVIEW

2.1. *Andrographis paniculata*

Andrographis paniculata (Burm. F.) Wall. Ex Nees is a traditionally known medicinal plant of Acanthaceae family, and andrographolide is quantitatively the major bioactive secondary metabolite of the plant identified to date. Besides being well known as an Ayurvedic herb, *Andrographis paniculata* is also medicinally used in the traditionally known medical systems of China and Thailand. *Andrographis paniculata* is also known as Cheonshimryeon in Korea and Chuan Xin Lian in China. Extracts of this plant parts and isolated andrographolides have been used to pharmacologically and experimentally verify its traditional usage for rheumatoid arthritis, inflammation, cold, fever and diarrhea (Burgos *et al.*, 2009; Chandrasekaran *et al.*, 2010; Chandrasekaran *et al.*, 2011; Shen *et al.*, 2013).

Extensive efforts made during past few decades have identified not only broad spectrums of therapeutically interesting pharmacological properties of diverse types of *Andrographis paniculata* extracts, but also of andrographolide and other structurally unique bioactive constituents of such extracts (Chao and Lin, 2010; Kumar *et al.*, 2012; Mishra *et al.*, 2007; Subramanian *et al.*, 2012; Valdiani *et al.*, 2012; Jayakumar *et al.*, 2013). Amongst them the ones dealing with anticancer and anti-inflammatory activities of extracts rich in andrographolide, or of pure andrographolide, have attracted the most attention of modern drug discoverers (Hidalgo *et al.* 2013; Lim *et al.* 2012). Although the numbers of clinical reports revealing, or reconfirming, therapeutic potentials of *Andrographis paniculata* extracts for treatment of inflammatory disorders have continued to increase during recent years.

In the Ayurvedic system of medicine currently widely practiced in Indian, *Andrographis paniculata* is often used in combination with other herbs and health care procedures for helping patients suffering from diverse spectrums of organ pathologies and mental health problems. It has been estimated that *Andrographis paniculata* is used in more than 50% of herbal compositions commercialized in India for hepatic disorders (Govindarajan *et al.*, 2005). Some

marketed formulations are listed in **Table 2.1**. Modern Ayurvedic scholars often classify *Andrographis paniculata* as *Rasayana* herb useful for maintaining stomach integrity and regulating energy metabolism and immune functions (Govindarajan *et al.*, 2005; Thakur *et al.*, 2012a; Williamson, 2002). Although many *Rasayana* herbs are now pharmacologically classified as herbal adaptogens, so far only some scattered information on adaptogenic or anti-stress activity of *Andrographis paniculata* extracts and their bioactive constituents have appeared.

2.1.1. Vernacular names

Hindi: Kirayat, Mahatit

Sanskrit: Kalamegha, Bhunimba

English: Green chirayta, King of bitters

Tamil: Nilavembu

2.1.2. Other names: Bidara, Carmantina, Carmantine, Chiretta, Chirette Verte, Chuan Xin Lian, Chuanxinlian, Chuan Xin Lin, Gubak, Herba Andrographidis, Indian Echinacea, Kariyat, Kirta, Poogiphalam, Roi des Amers, Sadilata, Sambilata, Shivaphala and Yavatikta.

Figure 2.1: *Andrographis paniculata* (Burm. F.) Wall. Ex Nees

2.1.3. Taxonomy

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Super division: Spermatophyta (Seed plants)

Division: Angiosperma

Class: Dicotyledonae

Sub class: Gamopetalae

Series: Bicarpellatae

Order: Personales

Tribe: Justicieae

Family: Acanthaceae

Genus: *Andrographis*

Species: *paniculata*

2.1.4. Morphological outline

- Height of 30-110 cm in moist shady places.
- Leaves are simple, opposite, lanceolate, glabrous, 2-12 cm long, 1-3 cm wide with margin acute.
- The flowers possess botanical features of calyx 5-particle, small, linear, corolla tube narrow about 6 mm long, two stamens.
- Seeds are very small, sub-quadrate.

2.1.5. Botanical description

Andrographis paniculata is an annual, branched, herbaceous plant erecting to a height of 30-110 cm in moist shady places with stem acutely quadrangular, much branched, easily broken fragile texture stem. Leaves are simple, opposite, lanceolate, glabrous, 2-12 cm long; 1-3 cm wide with margin acute and entire or slightly undulated and upper leaves often bractiform with short petiole **(Figure 2.1)**. Inflorescence of the plant is characterized as patent, terminal and axillary in

panicle, 10-30 mm long; bract small; pedicel short. The flowers possess botanical features of calyx 5-particle, small, linear; corolla tube narrow, about 6 mm long; limb longer than the tube, bilabiate; upper lip oblong, white with a yellowish top; lower lip broadly cuneate, 3-lobed, white with violet markings; stamens 2, inserted in the throat and far exerted; anther basally bearded. Superior ovary, 2-celled; style far exserted. Capsule of the plant is erect, linear-oblong, 1-2 cm long and 2-5 mm wide, compressed, longitudinally furrowed on broad faces, acute at both ends, thinly glandular-hairy. Seeds are very small, subquadrate (Mishra *et al.*, 2007). It grows abundantly in southeastern Asia: India, Sri Lanka, Pakistan and Indonesia but it is cultivated extensively in China and Thailand, the East and West Indies and Mauritius. *Andrographis paniculata* is normally grown from seeds ubiquitously in its native areas where it grows in pine, evergreen and deciduous forest areas, and along roads and in villages. In India, it is cultivated during rainy phase of summer season crop. Any soil having fair amount of organic matter is suitable for commercial cultivation of this crop. About 400 g seed are sufficient for one hectare. The plants at flowering stage (90-120 days after sowing) are cut at the base leaving 10-15 cm stem for plant for regeneration. About 50-60 days after first harvest, final harvest is performed. In Indian condition, the yield varies between 2000-2500 kg dry herbs per hectare.

2.1.6. Phytochemistry

Andrographolide diterpinoids and 2'-oxygenated flavonoids are common chemotaxonomic markers of the Andrographis genus to which *Andrographis paniculata* belongs to (Koteswara Rao *et al.*, 2004; Pramanick *et al.*, 2007). Amongst more than 40 plants of this family, *Andrographis paniculata* is phytochemically as well as pharmacologically the most well studied one (Parixit *et al.*, 2012). A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolated from *Andrographis paniculata.* The most bitter compounds among them are andrographolide, neoandrographolide, isoandrographanolide, 14-deoxy 11,12-didehydroandrographolide and andrograpanin. Other phytochemicals amassed by the plant are 14-deoxyandrographolide, andrographiside, deoxyandrographiside,

homoandrographolide, andrographan, andrographon, andro-graphosterin, andrographidine G, stigmasterol, flavonoids, xanthones, phenol carboxylic acids and other antibacterial components (Hapuarachchi *et al.*, 2013; Siripong *et al.*, 1992; Subramanian *et al.*, 2012). The leaves of *Andrographis paniculata* contain the highest amount of andrographolide (2.35%), while the roots (0.52%) and stem (0.35%) contain less amount of andrographolide in 110 days old harvested *Andrographis paniculata* plant (Pandey and Mandal, 2010). Andrographolide has highly bitter taste, is colourless crystalline in appearance and possess a 'lactone function' (Sharma *et al.*, 1992).

Although flavonoids and other structurally diverse bioactive phytochemicals are encountered in medicinally used extracts of the plant, by far a vast majority of preclinical reports on such extracts concentrate mainly on their contents of andrographolide like labdane diterpinoids only. Structures of some the quantitatively major diterpinoids and their glycosides commonly encountered in such extracts are shown in **Figure 2.2**. The relative contents of such bitter tasting molecules vary considerably in different parts of the plant, whereupon the content of andrographolide seems to be highest in its leaves (Jarukamjorn and Nemoto, 2008; Sharma *et al.* 1992).

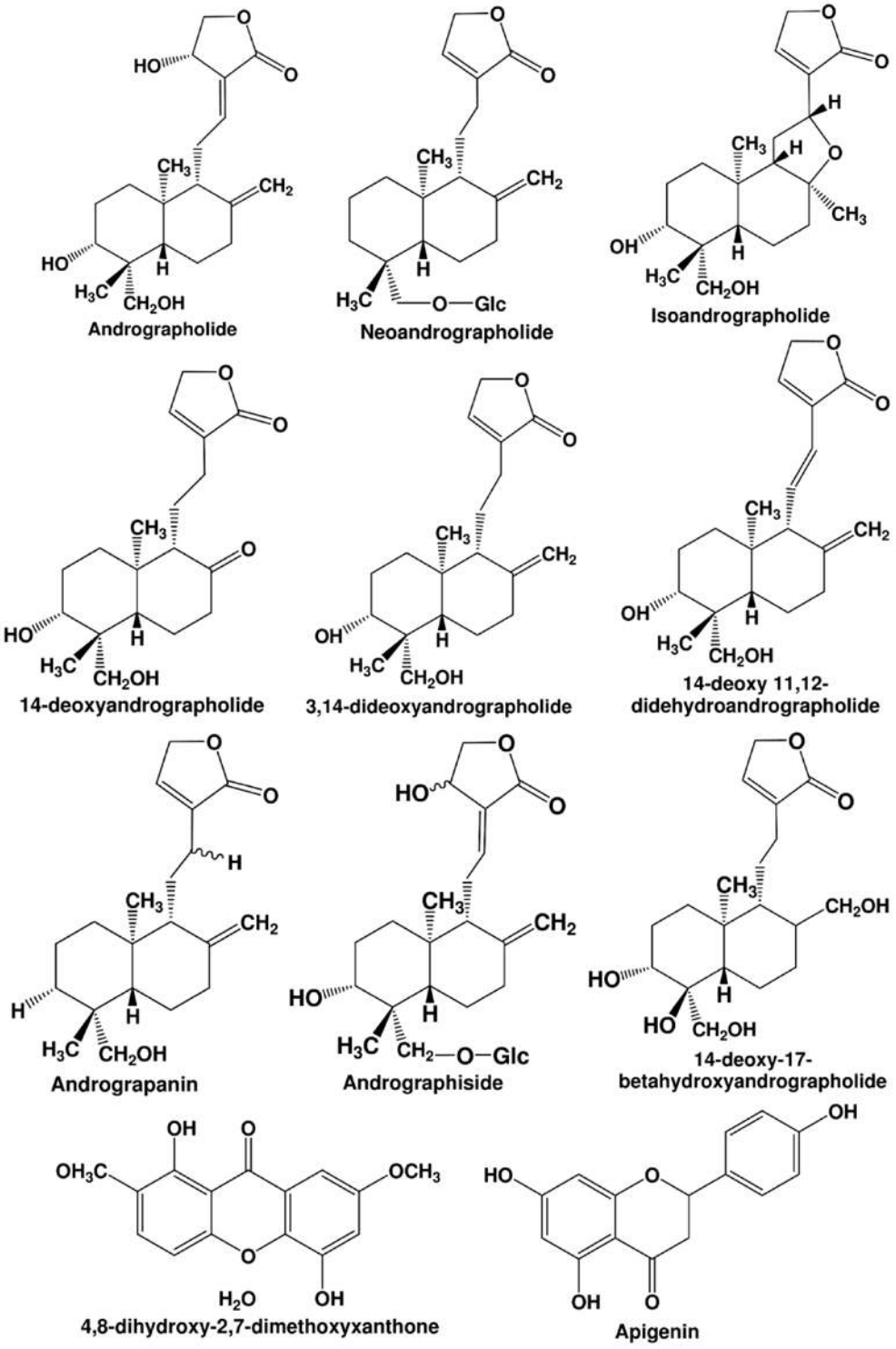


Figure 2.2: Major phytochemical constituents from *Andrographis paniculata*

2.1.7. Pharmacology

Available preclinical information on pure andrographolide and diverse types of *Andrographis paniculata* extracts (Parixit *et al.*, 2012) strongly suggests that most probably andrographolide is the quantitatively major, but not the only, bioactive constituent of the plant. However, as yet little concentrated efforts have been made to define the roles of flavonoids and other structurally diverse bioactive constituents of the plant in the clinically observed efficacy of *Andrographis paniculata* extracts have yet been made, and therapeutic relevance of the experimentally observed brain function modulating effects of *Andrographis paniculata* extracts, or of andrographolide (Mandal *et al.*, 2001; Radhika *et al.*, 2012a) still remain at the best speculative only. Several recent observations made in cellular and other *in vitro*, or *ex vivo*, models strongly suggest that andrographolide is a neuro- or cerebro-protective agent, and that it could as well cross the blood brain barrier (Burgos *et al.*, 2005; Carretta *et al.*, 2009; Chan *et al.*, 2010; Qin *et al.*, 2006; Wang *et al.*, 2004). After oral administration of andrographolide, several metabolites were found in blood, urine, bile and the contents of the small intestine and stomach of rats, which may be responsible for neuroprotective effect against cerebral ischaemia (Chan *et al.*, 2010). The bioavailability of andrographolide and metabolites in brain may be due to crossing of brain-blood barrier (He *et al.*, 2003). It must be noted though, that oral bioavailability of andrographolide is poor (<3 %), and that after oral administration it is extensively bio-transformed to other molecules within the gastrointestinal tract itself (Guo *et al.*, 2012; Panossian *et al.*, 2000; Ye *et al.*, 2011). Therefore, yet no very definitive statements on the role of andrographolide in the observed brain function modulating effects of the plant can yet be made.

Similar, or analogous, is also the situation for many other therapeutically interesting pharmacological activities reported for diverse types of *Andrographis paniculata* extracts. Some such major ones reported during recent decades are summarized in **Table 2.2**. Although most such therapeutically interesting bioactivities have also been reported for pure andrographolide, yet little

concentrated effort have been made to verify the possibility whether other bioactive constituents of *Andrographis paniculata* extracts modulates the efficacy and safety of andrographolide or not. Efforts to clarify the situation is not only necessary for appropriate analytical standardisation of *Andrographis paniculata* extracts for therapeutic purposes, but also proper standardisation of plant collection and processing procedures necessary for obtaining safe and more sustainable medicinal products from this wildly growing and well known medicinal plant.

Andrographolide was first isolated in crystalline form during 1911 (Gorter, 1911), and the very first report suggesting that it is a bioactive constituent of *Andrographis paniculata* appeared during 1951 (Chakravarti and Chakravarti, 1951). Since then diverse of other structurally analogous diterpene lactones and their glycosides have been isolated from different parts of the plants, and several of them have also been reported to possess diverse therapeutically interesting bioactivities potentially useful for treatments of inflammatory disorders. Some such therapeutically interesting bioactivities of andrographolide and other diterpenes isolated from *Andrographis paniculata* are summarized in **Table 2.3**. It must be noted though, that apart from these diterpenes the medicinally used *Andrographis paniculata* extracts also contain diverse other constituents with antiviral, bactericidal, anti-oxidative and other therapeutically interesting bioactivities (Parixit *et al.*, 2012), and reports on other secondary metabolites of the plant and their therapeutically interesting bioactivities still continue to appear (Hapuarachchi *et al.*, 2013; Radhika *et al.*, 2012b; Wu *et al.*, 2008; Xu *et al.*, 2010).

Andrographis paniculata has a broad range of pharmacological activities, such as antihyperglycaemic activity, hypolipidemic activity, antihyperlipidemic activity and anti-oxidant activity (Nugroho *et al.*, 2012; Subramanian *et al.*, 2008; Yang *et al.*, 2013a; Zhang and Tan, 2000), cardiovascular activity (Awang *et al.*, 2012; Thisoda *et al.*, 2006; Zhang and Tan, 1997), hepatoprotective activity (Nagalekshmi *et al.*, 2011; Chander *et al.*, 1995), gastroprotective activity (Sandborn *et al.*, 2013; Saranya *et al.*, 2011), neuroprotective activity (Chan *et*

al., 2010), antidiarrhoeal activity (Gupta, 1993), immunostimulatory activity (Calabrese *et al.*, 2000; Iruretagoyena *et al.*, 2005), antimalarial activity (Mishra *et al.*, 2009; Najib Nik *et al.*, 1999), antiviral activity (Wiart *et al.*, 2005), anticancer activity (Li *et al.*, 2007; Sheeja *et al.*, 2007; Sheeja and Kuttan, 2006), anti-inflammatory activity (Chandrasekara *et al.*, 2011; Liu *et al.*, 2007; Parichatikanond *et al.*, 2010; Pramanick *et al.*, 2007; Sheeja *et al.*, 2006; Xia *et al.*, 2004; Yan *et al.*, 2008) and protective effects in oxidative stress in brain associated with nicotine-induced toxicity (Das *et al.*, 2009). It was demonstrated that the extract having some pharmacological activities related to central nervous system as indicated by its potentiating hypnotic and sedative activity (Mandal *et al.*, 2001; Thakur *et al.*, 2012b; Thakur *et al.*, 2014a), and immunostimulant, cerebroprotective and nootropic activities in normal and type-2 diabetic rats (Radhika *et al.*, 2012a). Literature survey also revealed that this medicinal plant was proven in preclinical and clinical studies for the prevention and treatment of common cold (Saxena *et al.*, 2010), and upper respiratory tract infections (Coon and Ernst, 2004). Psychopharmacological studies were conducted with an extract of *Andrographis paniculata*. The extract produced a prolongation of the pentobarbiotone-induced sleeping time and lowered the body temperature in experimental animal after single intraperitoneal injection. The extract also exhibited significant motor in-coordination and muscle relaxant activity. These finding reveal a potent brain function altering activities of *Andrographis paniculata* in rodent models and further detailed investigations are necessary (Mandal *et al.*, 2001).

2.1.8. Safety profile and drug interactions

In traditionally known medicinal systems of China, India, Thailand and many other Asiatic countries *Andrographis paniculata* has since long been known to be a safe and effective medicinal plant. Although a systematic review on safety and efficacy of diverse types of its extracts (Coon and Ernst, 2004) currently widely used for treatments of upper respiratory tract infections did identify a few mild and infrequent occurrence of adverse events, in general the extracts were evaluated as a safe and effective remedies. A recent review critically analyzing

available information on safety and efficacy of the plant have pointed out some potential health hazards that might eventually arise from its uncontrolled widespread uses (Valdiani *et al.*, 2012). Oral administration of *Andrographis paniculata* extract (up to 1000 mg/kg of body weight per day) for 65 days prior to mating and 21 days during mating, did not reveal any signs of its dose-dependent toxicity on reproduction and fertility (Allan *et al.*, 2009), and it was found to be safe during pregnancy in doses up to 2000 mg/kg (Panossian *et al.*, 1999). A series of *in vitro* toxicology studies conducted with a well-standardised extract of the plant did not reveal any genotoxicity potential of the extract (Chandrasekaran *et al.*, 2009). A recent international pharmacovigilance report reveals though, that *Andrographis paniculata* derived drugs can sometimes cause hypersensitivity reaction in HIV positive patients (Farah *et al.*, 2008). Since andrographolide and diverse other bioactive secondary metabolites of *Andrographis paniculata* possess diverse spectrums of immune function modulating activities, further more detailed studies will be necessary for proper assessment of the safety profile of its medicinally used extracts or of pure andrographolide in immune suppressed patients. It must be noted though, that even fairly high oral daily oral doses of pure andrographolide (up to 500 mg/kg/day for 3 weeks) is well tolerated by laboratory rodents (Bothiraja *et al.*, 2012), and that its reported pharmacologically active oral doses administered with *Andrographis paniculata* extracts are several folds lower.

The extract of *Andrographis paniculata* including andrographolide and its analogues have been reported to exhibit a marked effect on hepatic biotransformation enzymes, i.e., anilinehydroxylase, N- and O-demethylase (Choudhury and Poddar, 1984), alanine aminotransferase and aspartate aminotransferase (Trivedi and Rawal, 2000), including phase II enzymes i.e. glutathione S-transferase and DT-diaphorase (Singh *et al.*, 2001). *Andrographis paniculata* extract and its active constituents also demonstrated interaction with CYP1A1 and CYP1A2 substrates (Jaruchotikamol *et al.*, 2007; Jarukamjorn *et al.*, 2006). In a study, pre-treatment of andrographolide shown to increase the elimination of theophylline, and chronic use of *Andrographis paniculata* extract elevated the concentration of theophylline in the blood (Chien *et al.*, 2010).

Table 2.1: Marketed major formulations containing *Andrographis paniculata* extracts

S. No.	Name of formulation	Indication(s)	Manufacturer
1.	Not-So-Well I Capsule	Immune system & Body defenses	Get Well Natural LLC, Las Vegas, NV 89109, USA URL: http://www.getwellnatural.com/index.aspx
2.	Kalmcold® Capsule	Assist in the relief of Cold and Flu symptoms	Natural Remedies Pvt. Ltd., Plot No. 5B, Veerasandra Industrial Area, 19th K.M. Stone, Hosur Road, Electronic City (Post), Bangalore -560 100, Karnataka, India. URL:http://www.kalmcold.com/
3.	Paractin Capsule	Natural anti-inflammatory (COX-2 inhibitors)	Marvelous Products, 105A Lew Dewitt Blvd Suite 128 Waynesboro, VA 22980 URL:http://www.marvelousproducts.com/aboutus.asp

4.	Kan Jang® Tablet	Respiratory tract infection, Common cold or Sinusitis	Swedish Herbal Institute Prinsgatan 12 Goteborg-SE-41305, Sweden URL:http://www.hellotrade.com/swedish-herbal-institute/profile.html
5.	Livfit Vegecaps Tablets and Syrup	Liver protection	Dabur India Limited, Corporate Office Kaushambi Ghaziabad - 201010 Uttar Pradesh, India URL:http://www.dabur.com/default.aspx
6.	Purim (Hemocare) Tablets	Acute and chronic dermatitis, Hyperpigmentation in chronic dermatitis, Cutaneous manifestations in worm infestations, Acne vulgaris and rosacea associated with acneiform pustulation	The Himalaya Drug Company, Makali Bangalore - 562 123 India. URL:http://www.himalayahealthcare.com/products/purim.htm

Table 2.2: Reported preclinical and clinical observations made with diverse types of *Andrographis paniculata* extracts

S. No.	Type of extract	Plant part used	Pharmacological activity	Dose, duration and route of administration	References
1.	Ethanolic extract	Whole plant	Inhibition of alpha-glucosidase and alpha-amylase enzymes	250, 500 and 1000 mg/kg, single dose, p.o. (*in vivo*)	(Subramanian *et al.*, 2008)
2.	Hydroethanolic extract	Whole plant	Chemoprotective effect	10 mg/animal, single dose, i.p. (*in vivo*)	(Sheeja and Kuttan, 2006)
3.	Hydroethanolic extract	Whole plant	Antiangiogenic activity	10 mg/animal, i.p. (*in vivo*) 10 µg/ml (*in vitro*)	(Sheeja *et al.*, 2007)
4.	Aqueous extract	Whole plant	Safety in pregnancy	200, 600 and 2000 mg/kg, for 19 days, p.o. (*in vivo*)	(Panossian *et al.*, 1999)
5.	Hydroethanolic extract	Aerial parts	Hepatoprotective activity	100 and 200 mg/kg, single dose, p.o. (*in vivo*)	(Nagalekshmi *et al.*, 2011)
6.	Hydroethanolic extract	Aerial parts	Antidiabetic activity	100, 200 and 400 mg/kg, twice daily for 14 days, p.o. (*in vivo*)	(Zhang and Tan, 2000)

7.	Hydromethanolic extract	Aerial parts	Anti-malarial activities	1.8 to 500 µg/ml, (*in vitro*) 7 mg/kg, i.p. (*in vivo*)	(Mishra *et al.*, 2009)
8.	Methanolic extract	Aerial parts	Hypnotic activity	100, 200 and 300 mg/kg, i.p. (*in vivo*)	(Mandal *et al.*, 2001)
9.	Methanolic extract	Leaves	Immunostimul-ant, cerebroprotec-tive and nootropic activities	100 mg/kg, seven days, p.o., (*in vivo*) 50 mg/kg, single dose, i.p. (*in vivo*)	(Radhika *et al.*, 2012a)
10.	Dichloromethane extract	Aerial parts	Cardiovascular activity	3 mg (*ex vivo*), perfused isolated rat heart	(Awang *et al.*, 2012)
11.	n-butanol extract	Aerial parts	Cardiovascular activity	5 mg/kg, single, i.p. (*in vivo*)	(Zhang and Tan, 1997)
12.	Hydroethanolic extract	Aerial parts	Antidiabetic and antihiperlipide-mic effect	434.6 and 1303.8 mg/kg, twice daily, 55 days, p.o. (*in vivo*)	(Nugroho et al,. 2012)

13.	Ethanolic extract	Aerial parts	Anti-inflammatory (Inhibition of TNF-α, IL-β)	0.1, 0.3, 1, 3, 10 and 30 µg/ml, (*in vitro*)	(Yan *et al.*, 2008)
			Beneficial in inflammatory bowel disease	500 mg/kg/day, for 7 days in mice and rats, p.o. (*in vivo*)	
14.	Hydromethanolic extract (KalmCold™)	Leaves	Anti-inflammatory (Inhibition of NO, IL-1β, IL-6, PGE_2, TXB_2 and LTB_4)	1.25 to 40 µg/ml, (*in vitro*)	(Chandrasekaran *et al.*, 2010)
15.	*Andrographis paniculata* extract (HMPL-004)	Aerial parts	Beneficial in ulcerative colitis	1200 and 1800 mg daily in human for 8-week (clinical study)	(Sandborn *et al.*, 2013)
16.	Hydromethanolic extract	Leaves	Anti-diabesity activity	50, 100 and 200 mg/kg/day, for 10 days in rats	(Thakur *et al.*, 2014b)
17.	Hydromethanolic extract	Leaves	Anti-depressant activity in diabetic rats	50, 100 and 200 mg/kg/day, for 10 days in rats	(Thakur *et al.*, 2014c)

Table 2.3: Some reported pharmacological activities of secondary plant metabolites isolated from *Andrographis paniculata*

S. No.	Chemical constituents	Pharmacological activities	Dose, duration and route of administration	References
1.	Andrographolide	Inhibition of α-glucosidase and α-amylase enzymes	10 mg/kg, single dose, p.o. (*in vivo*)	(Subramanian *et al.*, 2008)
		Antiangiogenic activity	500 µg/dose/animal, i.p. (*in vivo*); 0.25 µg/ml (*in vitro*)	(Sheeja *et al.*, 2007)
		Inhibitory effect on platelet aggregation	1 to 100 µM (*in vitro*)	(Thisoda *et al.*, 2006)
		Anti-inflammatory activity	3.56 to 57 µM (*in vitro*)	(Chandrasekaran *et al.*, 2011)
		Hypolipidemic activity	100 mg/kg, p.o. (*in vivo*)	(Yang *et al.*, 2013a)
		Gastroprotective activity	3 mg/kg, single dose for 30 days, p.o. (*in vivo*)	(Saranya *et al.*, 2011)
		Inhibition of NF-κB activation	10 µg/ml (*in vitro*)	(Xia *et al.*, 2004)

		Neuroprotective activity	0.01 to 1 mg/kg, single, i.p. (*in vivo*)	(Chan *et al.*, 2010)
		Antidiabetic and antihiperlipidem-ic activity	1.5 and 4.5 mg/kg, p.o. twice daily, 55 days, (*in vivo*)	(Nugroho *et al.*, 2012)
		Anti-inflammatory activity	0.01 to 10 µg/ml or 28.5 µM (*in vitro*)	(Parichatikan ond *et al.*, 2010)
2.	Neoandrographo-lide	Lack of inhibitory effect on platelet aggregation	1 to 100 µM (*in vitro*)	(Thisoda *et al.*, 2006)
		Hypolipidemic activity	100 mg/kg, p.o. (*in vivo*)	(Yang *et al.*, 2013a)
		Anti-inflammatory activity	100 and 150 mg/kg, (*in vivo*) 30 µM to 150 µM, (*in vitro*)	(Liu *et al.*, 2007)
		Anti-inflammatory activity	0.01 to 10 µg/ml or 20.8 µM (*in vitro*)	(Parichatikan ond *et al.*, 2010)
3.	Isoandrographol-ide	Anti-inflammatory activity	3.56 to 57 µM (*in vitro*)	(Chandrasek aran *et al.*, 2011)
4.	7-O-methylwogonin	Anti-inflammatory activity	33.5 to 67 µM (*in vitro*)	(Chandrasek aran *et al.*, 2011)

5.	14-deoxy-11,12-didehydroandrographolide	Inhibitory effect on platelet aggregation	1 to 100 µM (*in vitro*)	(Thisoda *et al.*, 2006)
6.	Dehydroandrogr-apholide	Anti-inflammatory activity	0.01 to 10 µg/ml (*in vitro*)	(Parichatikanond *et al.*, 2010)

2.2. Diabetes Mellitus

Diabetes mellitus (DM) refers to a group of metabolic disorder that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycemia include reduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. Type-2 diabetes mellitus has become a significant health problem in both developed and developing countries. 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 million by 2035 (International Diabetes Federation, 2013).

2.2.1. Etiologic classification of diabetes mellitus

I. Type-1 diabetes: *ß*-cell destruction, leading to absolute insulin deficiency (American Diabetes Association, 2014).

II. Type-2 diabetes: Ranges from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretion defect with insulin resistance (American Diabetes Association, 2014).

III. Gestational diabetes mellitus (GDM): A pregnant woman diagnosed with GDM may continue to be hyperglycemic after delivery and may be determined to have, in fact, type-2 diabetes (American Diabetes Association, 2014). Insulin resistance during pregnancy stems from a variety of factors, including alterations in growth hormone and cortisol secretion (insulin antagonists), human placental lactogen secretion which is produced by the placenta and affects fatty acids and glucose metabolism, promotes lipolysis, and decreases glucose uptake, and insulinase secretion which is produced by the placenta and facilitates metabolism of insulin (Gilmartin *et al.*, 2008).

2.3. Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in metres). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight. Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year because of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischaemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity (World Health Organisation, 2013). Obesity is also defined medically as a state of increased body weight, more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences (Spiegelman and Flier, 2001). Obesity results from increased energy intake, decreased energy expenditure, or a combination of the two. One-third of the American population is now considered obese and the prevalence of obesity in children is escalating dramatically. In India, prevalence of obesity was highest for urban population (male = 5.5%, female = 12.6%) followed by urban slum (male = 1.9%, female = 7.2%) and rural populations (male = 1.6%, female = 3.8%) (Yadav and Krishnan, 2008). The most widely used method to measure obesity is the BMI, which is equal to weight/height^2 (in kg/m^2) (Kopelman, 2000; Husain, 2011).

Obesity is frequently accompanied by hyperlipidemia. The increase in adipocyte mass and accompanying decreased insulin sensitivity associated with obesity has multiple effects on lipid metabolism. More free fatty acids are delivered from the expanded adipose tissue to the liver, where they are re-esterified in hepatocytes to form triglycerides, which are packaged into VLDLs for secretion into the circulation. The increased insulin level promotes fatty acid synthesis in the liver. Increased dietary intake of simple carbohydrates also drives hepatic production of VLDLs, resulting in elevations in VLDL and/or LDL in some obese

subjects. Plasma levels of HDL-C tend to be low in obesity, due in part to reduced lipolysis (Coenen *et al.*, 2007; Charlton *et al.*, 2009).

2.4. Co-morbid Brain Disorders Associated with Type-2 Diabetes

Type-2 diabetes has been associated with various comorbidities including CNS disorders such as depression, anxiety, cognitive dysfunctions, pain and inflammation.

2.4.1. Diabetes and depression

Diabetes and depression are two major chronic diseases with bidirectional relationship, and both of them are spreading like epidemics in almost all countries around the globe (Lin *et al.*, 2010). Co-occurrence of these two pathologies in same patients has strong negative impacts on their quality of life and shortens their life span (Dirmaier *et al.*, 2010; Roy *et al.*, 2012). Depression has been found also to be associated with alterations in diverse other diabetes related psychological and physiological processes (de Groot *et al.*, 2001; Lustman *et al.*, 2000), and it has been reported that prevalence of depression in diabetics is higher than prevalence of depression in normal population (Anderson *et al.*, 2001). Numerous structural, behavioural and biochemical alterations of the central nervous system are observed in diabetic patients and diverse such alterations are observed also in rodent models of diabetes where exaggerated symptoms of depression, anxiety and cognitive deficits are also observed (Hilakivi-Clarke *et al.*, 1990; Husain *et al.*, 2011a; Rowland and Bellush, 1989; Thakur *et al.*, 2013a; Thakur *et al.*, 2013b; Thakur *et al.*, 2014d). Although complex interactions of physical, psychological and genetic factors that contribute to such associations remain to be properly defined, available evidence strongly suggest that depression could as well a consequence of persistent metabolic abnormalities (MacKenzie and Trulson, 1978; Trulson and Himmel, 1985). However, it has been reported also that depression actually doubles the risk of type-2 diabetes, and that depression could as well be an independent risk factor for type-2 diabetes (Eaton *et al.*, 1996; Kawakami *et al.*, 1999; Nouwen *et al.*, 2010). It has been observed that the prevalence of depression was significantly higher in patients with type-2 diabetes compared with those

without (17.6 vs. 9.8%). These prevalence of depression was higher in females with diabetes (23.8%) compared with male (12.8%) diabetics (Ali *et al.*, 2006). Patients with diabetes and depression have been shown to have greater number of risk factors e.g. poor compliance with personal diabetic care (adherence to diet, checking blood sugar level), increased risk of retinopathy and macrovascular complications and have a decreased quality of life and increased disability burden (Aina and Susman, 2006).

All the three major neurotransmitters *viz.* serotonin, adrenaline and noradrenaline have been identified to be involved in pathophysiology of depression. This hypothesis is based on reserpine induced depression which is caused due to depletion of these three neurotransmitters and use of antidepressant drugs like tricyclic antidepressants (TCAs), monoamine oxidase (MAO) inhibitors and selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs) increase the amount of adrenaline, serotonin and noradrenaline in nerve synapses and alleviate depression. The decreased serotonin activity is essential for many antidepressant drugs have elicited feature of depression (Mann *et al.*, 1996) and enhancing effects on serotonergic activity and chronic antidepressant treatment facilitates serotonergic transmission (Blier *et al.*, 1987). 5-HT_{1A} receptors play an important role in depression. Pre-synaptic 5-HT_{1A} receptors are the primary target of several types of antidepressant drugs and long-term use of antidepressant drugs results in an increase in the 5-HT_{1A} receptors mediated hippocampal transmission (Marek *et al.*, 1989). The neuronal adaptative mechanisms of these receptors, both presynaptic and postsynaptic, account for the delay and limited response to the antidepressant drug (Celada *et al.*, 2004). Stressful conditions activating the hypothalamic–pituitary–adrenal (HPA) system and increasing the level of glucocorticoids have been also implicated in the etiology of depression (Pitchot *et al.*, 2001).

The currently available medication for treating depression, mainly restore the imbalance of monoaminergic transmission either by inhibiting the metabolism of monoamines or by inhibiting their reuptake at synapse (Owens *et al.*, 1997). The

antidepressant drugs may be classified into reversible inhibitors of monoamine oxidase (moclobemide, clorgyline), tricyclic antidepressant (imipramine, desipramine), selective serotonin reuptake inhibitors (fluoxetine), serotonin norepinephrine disinhibitor (mirtazepine), serotonin and noradrenaline reuptake inhibitor (venlafexin), and atypical antidepressants such as trazodone (α receptor antagonist), dopamine noradrenaline inhibitor (bupropion) (Stahl, 2009). In Ayurveda, Rasayana drugs constitute the group of drugs, which have over all beneficial effect on physical and mental faculty of health, can be used in variety of mental disorders such as depression and other mental disorders. Plant sources such as *Withania somnifera*, *Bacopa monniera* and *Fumaria indica,* which have been mentioned in *Ayurveda,* are frequently used herbal antidepressant. *Centella asiatica, Curcuma longa, Hypericum perforatum, Gingko biloba* and *Ocimum sanctum* are also well known for their antidepressant activity (Sharma, 2001). Treatment of depression by modern medicine has several shortcomings. Most medications for major depression have strong side effects (Hamilton and Opler, 1992; Johnston *et al.*, 1991; Glassman *et al.*, 1993; Fisher *et al.*, 2002). Chances of reoccurrence are also high with treatment of depression by modern medicine (Viguera *et al.*, 1998). Thus, search for effective and safer medications for comorbid depression is necessary and plant sources can prove to be a better option for that.

2.4.2. Diabetes and anxiety

Anxiety is related with emotional aspect of human being, which is universal in nature. Generally, anxiety stimulates and prepare individual to face potential threats and challenges, but its persistent and overwhelming occurrence precipitate pathological conditions known as 'anxiety disorders'.

Anxiety has also been associated with poor glycemic control seen in diabetes. According to a survey based study it was found that compared to rates seen in the general population, the rates of Generalised Anxiety Disorders and subsyndromal anxiety are higher (40 % of diabetic patients have elevated levels of anxiety symptoms) in diabetes (Grigsby, 2002). In addition, recent clinical studies have shown the prevalence of anxiety in diabetic patients is much higher

than the normal population, supporting the notion that there exists a strong relationship between diabetes and anxiety disorder (Clavijo *et al.*, 2006; Lin and Korff, 2008; Herzer and Hood, 2010). Anxiety in patients with diabetes is associated with less frequent blood glucose monitoring and suboptimal glycemic control (Shaban *et al.*, 2006), which further worse the diabetic condition. Previous reports have shown that diabetes results in anxiety-like behaviour in various preclinical testing paradigms. Diabetic rats demonstrated increased time spent in closed arms in elevated plus maze and reduced time spent in central arena in open field test (Aksu *et al.*, 2012; Thakur *et al.*, 2013b). Diabetes has also evidenced anxiety-like behaviours in rodents subjected to social interaction and zero maze tests (Ramanathan *et al.*, 1998). Moreover, decreased exploratory behaviour as less number and duration of head dips has been found in diabetic mice during hole-board test (Kamei *et al.*, 2001). These suggest that, diabetes significantly develops anxiety-like behaviour and is an effective tool for evaluating neuro-behavioural consequences of diabetes in rodents as well as in screening potential effects of various drug molecules in anxiety-like behaviour associated with diabetes.

Benzodiazepines (BZDs) have been used as the drug of choice for the treatment of anxiety disorders. However, long-term uses of BZDs do have various side effects such as development of tolerance, emergence of withdrawal symptoms and cognitive impairment (Papp *et al.*, 2010). Due to these disadvantages associated with use of BZDs, now antidepressants such as SSRIs and SNRIs are being frequently prescribed as first line anxiolytics. The other advantage of using SSRIs as anxiolytic is that they improve both psychic and somatic symptoms of anxiety disorders (Davidson *et al.*, 2005). An increased adrenergic activity in anxiety has been postulated as main factor behind poor glycemic control. On the other hand hyperglycemia associated with diabetes has also been found to exert anxiogenic effect (Lustman *et al.*, 1983). Some drugs used to treat anxiety such as Alprazolam have been found to have beneficial effects in glycemic control in patients suffering with comorbid diabetes and anxiety (Lustman *et al.*, 1995).

The currently available clinical treatment of anxiety includes use of BZD (diazepam, oxazepam), azapirones (buspirone, gepirone), sedative antihistaminic (hydroxyzine) and β blocker (propranolol) (Baldessarini, 2005). Now-a-days SSRIs and SNRIs are also prescribed as first line anxiolytic drugs (Davidson *et al.*, 2005). However, the side effects associated with these treatment modalities have pressed the need to search for alternate treatment system with lesser side effects (Papp *et al.*, 2010). Plants such as *Withania somnifera* (Bhattacharya *et al.*, 2000a), *Hypericum perforatum* (Kumar *et al.*, 2000a; Kumar, 2000), *Kava kava* (Smith *et al.*, 2001), *Marsilea minuta* (Bhattamisra *et al.*, 2007), *Bacopa monniera* (Bhattacharya and Ghoshal, 1998), and *Fumaria indica* (Singh, 2012) are some well known herbal anxiolytic drugs which have been claimed to be devoid of severe side effects as seen with conventional anxiolytic drugs such as BZD. There are many alternatives, which are being tried for effective treatment of anxiety. The 5-HT_2 receptor antagonist, dermaciclane is in phase 2 of clinical trial for anxiety treatment. Agomelatine, which has 5-HT_{2C} antagonist and melatonin agonist property, is in clinical trial phase also. Novel GABA modulator- pregabalin is showing promising antianxiety activity, is being tried. Anti-stress agents, which inhibit corticotropin releasing factor, have also shown positive results in preclinical studies for antianxiety effect. Recently a substance P antagonist (MK 869) has also shown antianxiety effect. Agents modulating glutamate functions (antagonist such as NMDA antagonist and inhibiting glutamate release such as MGLUr1 agonist) have been found effective in animal models of anxiety (Nutt and Ballenger, 2003). Various brain stimulation techniques such as electroconvulsive therapy, transcranial magnetic stimulation, vagus nerve stimulation and deep brain stimulation of the ventral caudate nucleus improved anxiety, depressive and compulsive symptoms (Eitan and Lerer, 2006).

2.4.3. Diabetes and memory impairments

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). Acute ingestion of high glycemic index carbohydrate foods

in diabetic patients has been found to aggravate memory impairment (Greenwood *et al.*, 2003). In contrast, better glycemic control has been found to improve cognitive performance (Strachan *et al.*, 1997; Biessels *et al.*, 2007). Brain imaging study in diabetic patients have revealed that changes observed in various brain region follow a similar pattern to that of ageing person's brain features suffering with cognitive decline (Biessels, 2010). A multifaceted role of insulin has been implicated in cognitive performance in normal as well as diabetic individual. Impairment of insulin activity in Alzheimer's disease having dementia as hallmark is well documented in various studies. In most of the animal research on diabetes and cognitive performance, it has been concluded that insulin deficiency may result in impairments in synaptic plasticity and cognitive processes while human studies suggest that insulin insensitivity may affect cognitive processing. Thus, overwhelming evidences from animal and clinical studies suggest functional link between diabetes and cognitive impairment underlying diverse mechanism of action (Park, 2001). Streptozotocin (STZ) is widely used to induce diabetes in rats and mice. STZ induce diabetes by destroying pancreatic β cells and creating shortage of insulin. STZ-induced diabetic rats have been found to display deficits in cognitive tasks, such as performance on the Morris water maze (Kamal *et al.*, 2000). STZ-induced diabetes in rats results in altered function of NMDA (Di Luca *et al.*, 1999) and AMPA (Chabot *et al.*, 1997) type glutamate receptors, which are involved in learning and memory processes. In most of the animal research on diabetes and cognitive performance, it has been concluded that insulin deficiency may result in impairments in synaptic plasticity and cognitive processes while human studies suggest that insulin insensitivity may affect cognitive processing (Park, 2001). There are ample evidences regarding prominent role played by neurotransmitter acetylcholine in memory function of animals. In patients of Alzheimer's disease, where dementia is hallmark of progression of disease there is 50–90% reduction in the content of ChAT, the enzyme that synthesizes acetylcholine in hippocampus, cortex and hypothamus regions of brain (Ojha *et al.*, 2010). At the same time, there is significant and selective degeneration of neurons having cholinergic intervention in hippocampus, cortex and

hypothamus of Alzheimer's disease patients. The core strategy to treat dementia of Alzheimer's disease has been to augment the level of acetylcholine by inhibiting the enzyme AChE. Currently the drug available to improve cognition in Alzheimer's disease such as galanthamine, rivastigmine and donepezil are AChE inhibitors (Figueiro *et al.*, 2010) but their limited efficacy and peripheral side effects impose a drawback for long time use (Birks and Flicker 2006).

The drugs used as cognition enhancers mainly constitute cholinergic activators (tacrine, rivastigmin and donepezil), glutamate antagonist (memantine) and miscelanious cerebroactive drugs such as piracetam, piribedil, pyrintol and dihydroerotoxin. Plants such as *Evolvulus alsinoid* (Nahata *et al.*, 2010), *Centella asiatica* (Howes and Houghton, 2003), *Bacopa monniera* (Russo and Borrelli, 2005), *Hypericum perforatum* (Kumar, 2000; Kumar et al., 2000b; Kumar *et al.*, 2002), *Terminalia chebula* (Manyam, 1999), *Curcuma longa* (Howes and Houghton, 2003), *Fumaria indica* (Singh, 2012) and edible vegetable like *Brassica juncea* (Thakur *et al.*, 2013a) are well known for their beneficial effect on cognitive functions. Researchers are now exploring the possible connection among Alzheimer's disease, vascular dementia, diabetes mellitus and cardiovascular diseases and cardiovascular therapies are speculated to prove useful in preventing Alzheimer's disease and dementia (Singh *et al.*, 2013; Thakur *et al.*, 2013a).

2.5. Inflammation and Pain Associated with Type-2 Diabetes

Diabetes mellitus has become the most common cause of peripheral neuropathy and many diabetes patients suffer from chronic pain. Central sensitization plays a pivotal role in the pathogenesis of pain hypersensitivity (von Hehn *et al.*, 2012) and its development results from augmented spontaneous and burst discharges in primary sensory neurons in neuropathic pain (Amir *et al.*, 2002).

The diabetic rat model has been studied, but the literature data are conflicting, and analysis of the animal's behaviour in response to pain has often been incomplete. With the hot-plate test, Forman *et al.* (1986) observed hyperalgesia in diabetic rats (age of diabetes: 8 weeks) as did Lee *et al.* (1990) after 3 days of diabetes with the tail immersion test at 50°C but other findings disagree with

these results. Thus Raz *et al.* (1988) reported that diabetic rats did not develop hyperalgesia in the hot-plate test, even after 16 weeks of diabetes. Levine *et al.* (1982) reported the same finding for mice, while Akunne and Soliman (1987) found loss of pain sensitivity in rats subjected to the hot-plate test. With the paw-pressure test, Wuarin-Bierman *et al.* (1987) reported hyperalgesia with focus on hyperactivity of nociceptive C-fibers in diabetic rats (duration of diabetes: 30 weeks). These discrepancy in findings can be avoid by correct study deign, preference in selection of rats over mice; use of pain tests with localised stimulus, use of noxious and non-noxious stimuli, longer test period after diabetes induction and selection of responder animals (Courteix *et al.*, 1993).

Similar to traumatic neuropathic pain, microglia activation is involved in the development and maintenance of central sensitization in Diabetic Neuropathic Pain (DNP). Since activity in primary afferents is decreased in diabetes, the development of hypersensitivity of spinal nociceptive neurons in DNP must involve some mechanisms that are distinct from traumatic neuropathic pain. Glucose toxicity on local site of spinal cord can contribute to the development of spinally mediated hyperalgesia and targeting on spinal sensory processing (spinal glutamatergic pathways) may assist development of novel therapeutic strategies for preventing and alleviating painful diabetic neuropathy (Calcutt, 2002; Calcutt and Chaplan, 1997). Hyperglycemia is an important factor in pain hypersensitivity associated with diabetes and results in altered pain sensitivity. Appropriate blood glucose control can help relieve pain in long-term diabetes through indirect mechanisms (Courteix *et al.*, 1996). The high concentration of glucose results in pain hypersensitivity characterized by allodynia and hyperalgesia (Pabreja *et al.*, 2011; Barriere *et al.*, 2012) probably by disrupting the functions of cell mitochondria and subsequent generation of reactive oxygen species (Stevens *et al.*, 2000) and oxidative stress (Feldman, 2003). Other possible target, activation of spinal microglia has been demonstrated in streptozotocin-treated animals, the most commonly used model of diabetes (Courteix *et al.*, 1993), and this activation can last 6 months (Cheng *et al.*, 2014). Andrographolide having some inhibitory activity in microglial activation in mesencephalic neuron-glia cultures (Wang *et al.*, 2004). Therefore, targeting

microglia and these receptors by antagonist or agonist might be considered as a novel approach to relieve neuropathy (Wang *et al.*, 2014).

2.6. Cytokines and Central Nervous System

Modern hectic lifestyle coupled with environmental changes and alarming level of pollution has posed human being to more risk of various types of stressors. Stress has been identified as causative and worsening factors of various diseases such as depression, anxiety, cognitive dysfunctions, sleep disorder, gastric ulcer, sexual dysfunctions and immunosuppression (Bhattacharya and Muruganandam, 2003; Charmandari *et al.*, 2005; Singh *et al.*, 2012). Stress and of stress-related illnesses are major contributing factors to public health problem that impairs job performance, increased risk, and generates large health care expenditures (Kanitz *et al.*, 2011).

Over activity of Hypothalamus-Pituitary-Adrenal axis (HPA axis) is significant aspect of stress. This axis is constituted by interlinked hormones corticotropin releasing hormone, adrenocorticotropin hormone and corticosterone, acting in a cascade manner to control release of next one by earlier with negative feedback mechanism exerted by corticosterone on hypothalamus (Calogero *et al.*, 1992). During stress these hormones are released in excess amount and they exert their catabolic, anti-reproductive and immunosuppressive effect (Charmandari *et al.*, 2005).

Cytokines constitute a large family of more than 100 small proteins that function as short-range mediators and involved in essentially all biological processes (Feldman, 2008). Cytokines have been identified to modulate various cell signaling processes and inhibition of cytokines and their receptors have yielded beneficial effects in various immune mediated disorders (Feldman, 2008). Cytokines are essentially protein or glycoprotein, ranging between 8 and 30 kDa size, and are produced ubiquitously within body (Hopkins, 2003). Cytokines are involved in diverse physiological and pathological processes of the living organism and are categorized into different subfamilies such as Interleukins, chemokines, tumor necrosis factors, interferon, colony stimulating factors, neuropoietins and growth factors each with their specific set of functionality.

However, cytokine(s) may show pleiotrophy/redundancy in activity (Hopkins, 2003). Cytokines have been identified to exert autocrine, paracrine and endocrine effects. Due to their diverse effects on physiological activities especially immune functions, cytokines are now synthesized and used clinically to treat various infectious and autoimmune disorders (Ikram *et al.*, 2004). Based on their function related to inflammation, cytokines are classified in two categories *viz.* pro-inflammatory and anti-inflammatory. Cytokines promoting inflammation such as IL-1, interleukin (IL)-1β and tumor necrosis factor (TNF)-α are pro-inflammatory cytokines, where as cytokines such as IL-4, IL-10 and IL-13, which suppresses the activity of pro-inflammatory cytokines are called anti-inflammatory cytokines (Dinarello, 2000). Owing to their capability to modulate neurological processes such as neurohumoral transmission, interaction with monoamine receptors, influence on neurodegenaration and neuronal plasticity etc., cytokines have been identified to interplay almost every aspects of neuropsychological disorders. The macrophage theory of depression proposed by Smith (1991), states that excessive secretion of macrophage cytokines such as IL-1, TNF-α and interferon (IFN)-α are the cause of some cases of major depression (Connor and Leonard, 1998). Pro-inflammatory cytokines have been identified to contribute directly to the development of depression (Anisman *et al.*, 2002). Activation of the inflammatory response system (IRS) which leads to increased production of pro-inflammatory cytokines such as interleukin IL-1β, IL-2, IL-6, IFN-γ and TNF-α results in precipitation of major depression (Licinio and Wong, 1999). Increased serum levels of TNF-α and other pro-inflammatory cytokines have been found in patients with major depression and several other psychiatric conditions (Simen *et al.*, 2006). The involvement of pro-inflammatory cytokines in etiology of depression is further corroborated by the fact that exogenous treatment of IL-2, TNF-α and IFN-α to previously psychiatrically healthy individuals, lead to develop depressive like symptoms such as depressed mood, increased somatic concern and stress reactions, cognitive impairment and difficulties with motivation and flexible thinking (Connor and Leonard, 1998).

Recent studies have indicated wide role of cytokines in mediating the stress response in conjunction with other stress mediating system such as HPA-axis

and monoaminergic system (Singh *et al.*, 2012; Singh, 2012; Bhattacharya and Muruganandam, 2003; Connor and Leonard, 1998). Acute stress has been found to induce pro-inflammatory activities in certain tissues through neural activation of the peripheral corticotropin-releasing hormone–mast cell–histamine axis (Elenkov and Chrousos, 1999). It has been observed that central and systemic administration of IL-2, IL-6 and TNF-α may stimulate the HPA-axis, which is essential feature of stress (Connor and Leonard, 1998). In a random study with school students, it was found that psychological stress significantly increased the stimulated production of TNF-α, IL-6, IL-10 and IFN-γ (Maes *et al.*, 1998). Furthermore, pro-inflammatory cytokines TNF- α and IL-1 β have been thought to exaggerate the oxidative stress (Floyd *et al.*, 1999).

Besides overactive HPA axis, cytokines also constitute important domain of pathophysiological aspects of stress. The role of cytokines has been implicated in both physiological and psychological maladies precipitated by stress such as ulcer, depression, anxiety, cognitive impairment etc. Gastric ulcer, in experimental induced model using rats shows higher level of IL-1β and TNF-α (Jainu and Devi, 2006). Pro-inflammatory cytokines Such as IL-1β and TNF-α have been identified to contribute directly to the development of depressive symptoms and they induce stress-reactive neuroendocrine and central neurotransmitter changes similar to depression (Dowlati *et al.*, 2010; Anisman *et al.*, 2002).

2.7. Toll-like Receptors and Central Nervous System

Toll-like receptors (TLRs) are a family of pattern recognition receptors that enable the recognition of conserved structural motifs in a wide array of pathogens (Kielian, 2006). They are homologous of Toll, a receptor found in insects, involved both in establishing dorsoventral polarity during embryogenesis and in immune response against fungal infections (Hashimoto *et al.*, 1988; Trotta *et al.*, 2014). The TLR family can be divided into extracellular and intracellular members. TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 are localized on the cell surface to recognize PAMPs. Conversely, TLR3, TLR7, TLR8 and TLR9 are intracellularly expressed in endosomal or lysosomal

compartments and the endoplasmic reticulum (Trotta *et al.*, 2014). TLRs are described not only in the immune cells, such as macrophages, dendritic cells, B and T cells, but also in non-immune cells, including fibroblasts and epithelial cells (Olson and Miller, 2004).

Neuroinflammation is known as a key player in a variety of neurodegenerative and/or neurological diseases. Brain TLRs are leading elements in the initiation and progression of neuroinflammation and the development of different neuronal diseases. Furthermore, TLR activation is one of the most important elements in the induction of insulin resistance in different organs such as the central nervous system (Hemmati *et al.*, 2014). The recent emergence of studies examining TLRs in the central nervous system (CNS) indicates that these receptors not only play a role in innate immunity in response to infectious diseases but may also participate in CNS autoimmunity, neurode-generation and tissue injury (Kielian, 2006).

TLRs are described in the brain where, until recently, their expression was believed to be limited to microglia (Olson and Miller, 2004), astrocytes (Bowman *et al.*, 2003), and oligodendrocytes (Bsibsi *et al.*, 2002). In addition, the expression of certain TLRs has recently been documented in mammalian neurons (Prehaud *et al.*, 2005; Wadachi and Hargreaves, 2006) and appears to be implicated in several non-immune processes, such as bone metabolism (Bar-Shavit, 2008), neurogenesis (Rolls *et al.*, 2007) and brain development (Ma *et al.*, 2007).

TLR-3 recognizes double-stranded RNA (dsRNA) released during viral infections, triggering the production of type-1 interferon and inflammatory cytokines/chemokines via the Toll/IL-1R domain-containing adaptor molecule-1 (TRIF) (Stridh *et al.*, 2013). TLR-3 activation exacerbates chronic neurodegeneration (Field *et al.*, 2010) and triggers nigrostriatal dopaminergic degeneration (Deleidi *et al.*, 2010). In addition, activation of the viral innate immune receptor TLR-3 sensitizes the neonatal brain to subsequent hypoxic–ischemic damage (Stridh *et al.*, 2010). However, contradictory reports suggest

that stimulation of the TRIF pathway may be neuroprotective by reprogramming the cerebral response to stroke (Marsh *et al.*, 2009).

TLR7 and TLR8 are highly homologous to each other and are important for immune responses elicited by GU-rich ssRNA as well as synthetic chemicals, including the imidazoquinoline compounds (i.e., imiquimod and resiquimod) and guanosine analogs (Hemmi *et al.*, 2002; Diebold *et al.*, 2004; Heil *et al.*, 2004). The latter compounds (guanosine analogs) were initially described for their ability to activate TLR7 and TLR8, and are potent immune response modifiers leading to the production of cytokines (i.e., IFNs) that exert important antiviral and antitumor activities (Hemmi *et al.*, 2002). Both imiquimod and resiquimod are used clinically for the localized treatment of herpesvirus infections of the skin, so systemic engagement of TLR7/8 would not be expected to occur. The structural similarities of these compounds to nucleic acids led to the identification of ssRNA as a natural agonist for TLR7/8 (Heil *et al.*, 2004; Diebold *et al.*, 2004). Interestingly, mammalian RNA also contains GU-rich sequences, suggesting that it may serve as an autoimmune trigger, which is supported by the finding that patients with systemic lupus erythrematosus (SLE) have autoantibodies against RNA (Lau *et al.*, 2005). Although there is evidence that TLR7 and TLR8 are expressed in microglia (Bsibsi *et al.*, 2002; Olson *et al.*, 2004) and astrocytes (Carpentier *et al.*, 2005), there are no available studies investigating either the consequences of TLR7/8 agonist treatment or the responses in TLR7 or TLR8-deficient glia. A recent report has identified a critical role for TLR8 in regulating the activity of CD4+ regulatory T cells, which may play an important role in controlling immune responses to cancer and autoimmune diseases (Peng *et al.*, 2005). Therefore, drugs targeting these TLRs might be a potential candidate for management of neuro-inflammatory disorders associated with neuro-immune functions.