CHAPTER – 2

LITERATURE REVIEW

LITERATURE REVIEW

2.1. *Withania somnifera*

Withania somnifera (L.) Dunal, often referred to as 'Indian ginseng' and commonly known as 'Ashwagandha' in Sanskrit and other modern Indian languages, is a plant of the genus Withania belonging to Solanaceae family. It is one of the most extensively used medicinal plant mentioned in the classical Ayurvedic texts composed in the Vedic period of Indian history (c. 1500 - c. 500 BCE) [S.K. Bhattacharya and A.V. Muruganandam, 2003]. In Ayurvedic system of medicine, the roots of *Withania somnifera* are used as a tonic, or for promoting longevity, revitalizing and immunity [S.K. Kulkarni and A. Dhir, 2008; S.K. Bhattacharya and A.V. Muruganandam, 2003; H.S. Puri, 2003]. *Withania somnifera* is also one of the chief active ingredients in numerous currently commercialized Ayurvedic formulations. Such *Withania somnifera* containing Ayurvedic formulations are now commonly prescribed for treatments of arthritis, rheumatism, hypertension, impotency, erectile dysfunction and in various gynecological disorders [L.C. Mishra and B.B. Singh, 2000; H.S. Puri, 2003]. It is also preclinically and clinically most extensively studied and wildly growing Indian medicinal plant, diverse pharmacological activities of different types of extracts obtained from different parts of the plants and some of their already known bioactive constituents have been reported during more recent decades [K. Bone, 1996; S.K. Kulkarni and A. Dhir, 2008; L.C. Mishra and B.B. Singh, 2000; P.U. Devi et al., 1992].

2.1.1. Botanical description

Withania somnifera is a green woody shrub of 2-8 m height, found all over the dried parts of South East Asia *viz*. India, Pakistan, Bangladesh, Sri-Lanka, Nepal and different other parts of America, Australia and Africa [F.N. Hepper, 1991; I. Ilayperuma et al., 2002; M.H. Mirjalili et al., 2009; D.J. Mabberley, 2008]. In India, it is broadly distributed in different

states like Uttar Pradesh, Madhya Pradesh, Punjab, Gujarat, Rajasthan, Westbengal and in northeastern states [S.K. Kulkarni and A. Dhir, 2008]. The flowers of *Withania somnifera* are small and green and the ripe fruit is orange-red in color. Its tomentose hairs extend radially from a central stem and the whole plant is covered with these short, silver-grey, branched hairs. Its leaves are alternate (opposite on flowering shoots), simple, margins are slightly wavy, largely ovate, obovate or oblong, 30–80 mm long and 20–50 mm broad, narrowed into the 5–20 mm long petioles, hairless and green, densely hairy below. The 1–7 inconspicuous bisexual flowers of the plant appear at the leaf knots on 2–5 mm long stems. Its 5-lobed calyx is ca. 5 mm long; which in its fruits are ca. 20 mm long, membranous, spherical shaped and 5–10-ribbed. Its corolla is 5-lobed, narrowly campanulate, 5–8 mm long and light yellow to yellow-green in color. The stamens are extended and yellow-orange in color and the fruit is a hairless spherical berry, orange-red to red when ripe and enclosed by the enlarged calyx. The plant produces numerous seeds, which are very pale brown in color, 2.5 mm across, kidney-shaped and compressed with a rough, netted surface [H.S. Puri, 2003; F.N. Hepper, 1991].

2.1.2. Taxonomy

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: *Withania*

Species: *somnifera*

Figure 2.1: *Withania somnifera* (L.) Dunal. A) Whole plant, B) aerial part and C) roots of the plant (adapted from: National Medicinal Plants Board, Ministry of Ayush, Government of India and www.ethnodyne.com).

2.1.3. Vernacular names

English : Winter cherry

Arabic : Kaknaj-e-Hindi

Sanskrit : Ashvagandha, Ashvakandika, Gandhapatri, Palashaparni

Hindi : Asgandh, Punir

Urdu : Asgand, Asgand Nagori

Bengali : Ashvaganda, Asvagandha

Gujarati : Asan, Asana, Asoda, Asundha, Ghodaasoda

Telugu : Asvagandhi, Penneru, Pennerugadda, Dommadolu

Tamil : Amukkira, Asubam, Asuvagandi

Malayalam : Amukkiram, Pevetti

Odiya : Asugandha

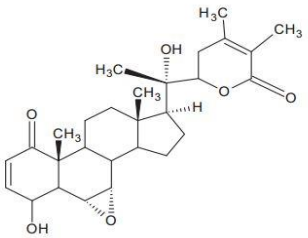
Marathi : Askandha, Kanchuki, Tilli

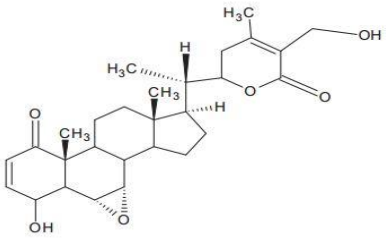
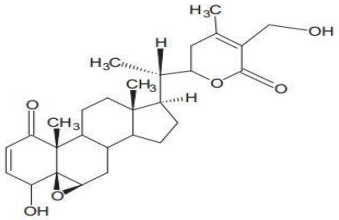
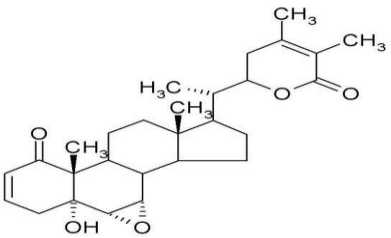
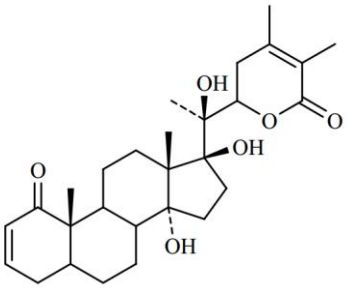
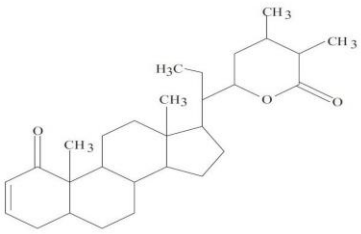
[Anonymous, 1982; R.N., Chopra et al., 1980; K.R. Kirtikar and B.D. Basu, 1980].

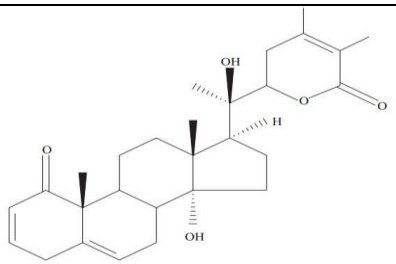
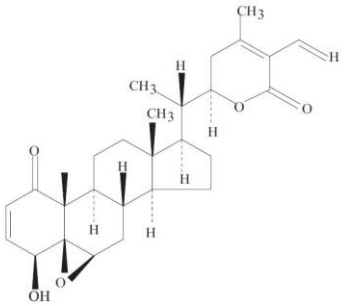
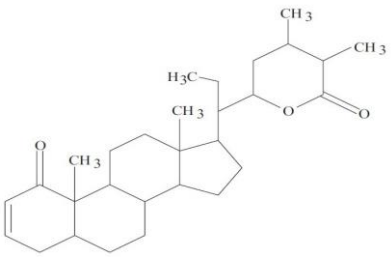
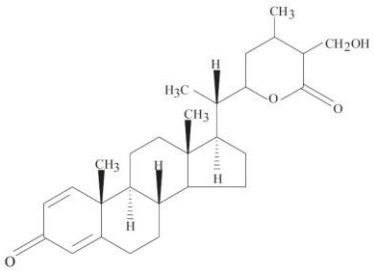
2.1.4. Medicinal phytochemistry of *Withania somnifera*

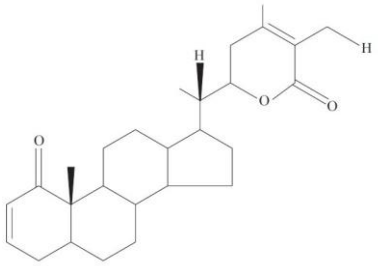
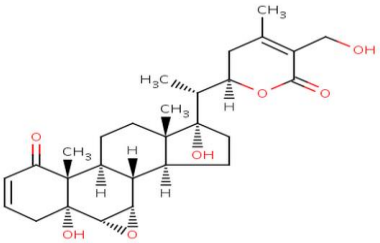
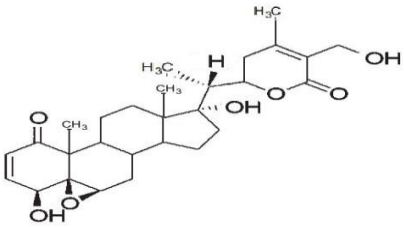
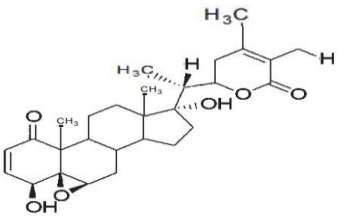
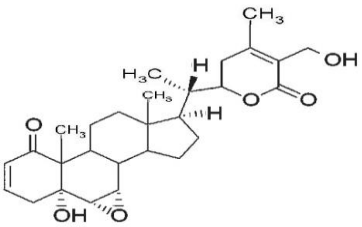
The major secondary metabolites of *Withania somnifera* often used as chemotaxononic markers of the plant are structurally diverse steroidal lactones (a class of substances commonly known as withanolides) and alkaloids [S.K. Kulkarni and A. Dhir, 2008; M. Elsakka et al., 1990]. Withanolides are some of the quantitively major secondary metabolites of *Withania somnifera* involved in its broad spectrum of therapeutically interesting bioactivity profile, and often used also for analytically standardizing its extracts for medicinal and commercial purpose [S.K. Kulkarni and A. Dhir, 2008; M.I. Choudhary et al., 2013; M. Elsakka et al., 1990]. Structurally, the withanolides are C_{28} steroidal lactones with ergostane skeleton which are often highly oxygenated at different positions of the skeleton [M.I. Choudhary et al., 2013; S.K. Kulkarni and A. Dhir, 2008]. Structural diversity of molecules often referred to withanolides [P.T. White et al., 2016; M.I. Choudhary et al., 2013; L.X. Chen et al., 2011] is apparent from the data summarized in the **Table 2.1**.

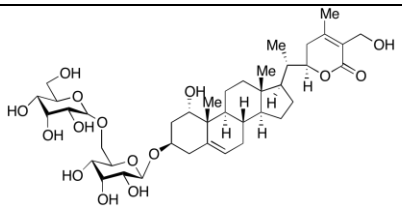
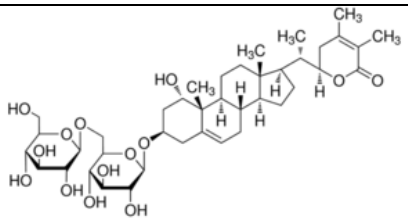
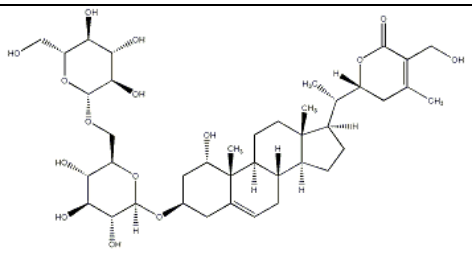
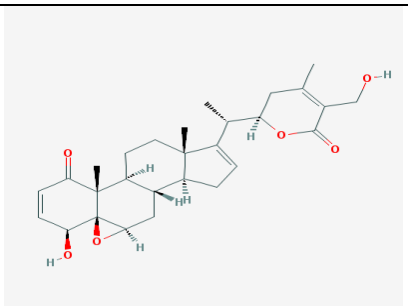
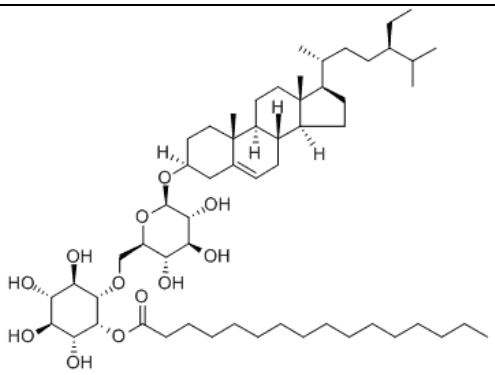
Table 2.1: Major steroidal bioactive phyto-constituents of *Withania somnifera* extracts.

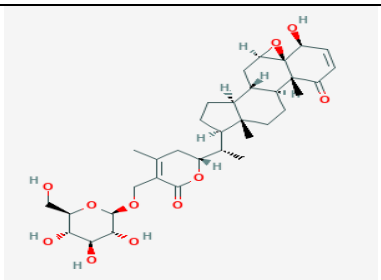
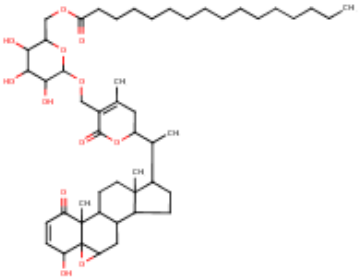
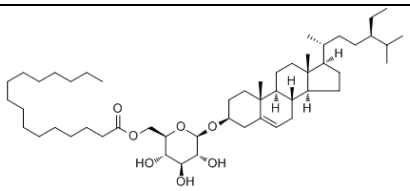
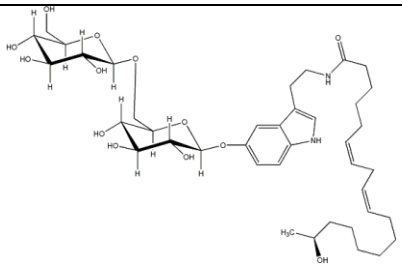
Sl. No.	**Chemical constituent**	**Plants part**	**Chemical Structure**	**Reference**
1.	Withanolide A	Root, Leaves, Shoot.		T. Dhanani et al., 2013; M. Sharada et al., 2007; N. Praveen and H.N. Murthy,

				2010
2.	12-deoxywithastramonolide	Root, Leaves, Shoot.		T. Dhanani et al., 2013
3.	Withaferin A	Root, Leaves, Shoot.		T. Dhanani et al., 2013; M. Sharada et al., 2007; A. Singh et al., 2010
4.	Withanolide B	Leaves, Shoot, Root.		M. Sharada et al., 2007
5.	Withanolide E	Leaves, Shoot, Root.		M. Sharada et al., 2007
6.	Withanone	Leaves, Shoot, Fruits, Root.		M. Sharada et al., 2007; N. Widodo et al., 2009;

				A. Singh et al., 2010
7.	Withanolide G	Leaves, fruit, root		A. Singh et al., 2010
8.	27-deoxy withaferin A	Leaves, root		A. Singh et al., 2010; N.D. Chaurasiya et al., 2008
9.	Withanolide D	stems, roots, leaves		A. Singh et al., 2010; D. Lavie et al., 1975; N.D. Chaurasiya et al., 2008
10.	Withasomidienone	Roots, leaves		A. Singh et al., 2010; A. Rahman and M.I. Choudhary, 1994

11.	Jaborosalactone A	Roots		A. Singh et al., 2010; V.V. Vande and D. Lavid, 1981
12.	27-hydroxy withanone	Roots and leaves		N.D. Chaurasiya et al., 2008
13.	17-hydroxy withaferin A	Roots and leaves		N.D. Chaurasiya et al., 2008
14.	17-hydroxy-27-deoxy withaferin A	Roots and leaves		N.D. Chaurasiya et al., 2008
15.	27-hydroxy withanolide B	Roots and leaves		N.D. Chaurasiya et al., 2008

16.	Withanoside IV	Roots		T. Kuboyama et al. 2006
17.	Withanoside V	Roots and leaves		S. Chatterjee et al., 2010
18.	Withanoside VI	Roots and leaves		S. Chatterjee et al. 2010
19.	Witharistatin	Roots		D. Benjumea et al., 2009
20.	Sitoindoside IV	Roots		S.K. Bhattacharya et al., 1987

21.	Sitoindosides IX	Roots		S. Ghosal et al., 1989
22.	Sitoindosides X	Roots		S. Ghosal et al., 1989
23.	Sitoindosides VIII	Roots		S.K. Bhattacharya et al., 1987
24.	Withanamide A	Fruits		A. Bhatia et al., 2013; O.P. Sidhu et al., 2011
25.	3ß-hydroxy-2,3-dihydro withanolide	Roots and leaves		A. Singh et al., 2010
26.	4β, 20-dihydroxy-i-oxo-5β,6β,-epoxy-witha-2,24-dienolide	Roots and leaves		A. Singh et al. 2010
27.	Withaferinil	Roots and leaves		L. Pabji et al., 1969; A. Singh et al., 2010

28.	Withanolide-5, 20α,(R)-dihydroxy-6α,7α-epoxy-1-oxo-5α-witha-2,24-dienolide	Roots and leaves		A. Singh et al., 2010; V. Bahr and R. Hansel, 1982
29.	Witharistatin	Roots		D. Benjumea et al., 2009
30.	Withasomidienone	Roots and leaves		A. Singh et al., 2010; A. Rahman and M.I. Choudhary, 1994;
31.	Withanolide sulfoxide	Roots		A. Singh, 2011

The first withanolide isolated from *Withania somnifera* was Withaferin-A in 1965 by Lavie and coworkers, and the name withanolide was given to such molecules because they were isolated from the plant genus *Withania* [D. Lavie et al., 1965; M.I. Choudhary et al., 2013]. Since then, a large number of withanolides have been isolated and characterized from other plants belongs to other families (Solanaceae, Taccaceae, Leguminosae, Labiatae, and Myrtaceae), and also from some oceanic organisms [M.I. Choudhary et al., 2013; E. Glotter, 1991; I. Kirson and E. Glotter, 1981; L.X. Chen et al., 2011; M.B. Ksebati and F.J. Schmitz, 1988; C. Srivastava et al., 1992]. Among all the plant families, the Solanaceae is the richest source of withanolides, to which both *Withania somnifera* and *Withania coagulans* belongs to [I. Kirson and E. Glotter, 1981; R. Jain et al., 2012]. Withanolides have received significant attention due to their wide range of biological activities observed. These include their

anticancer, immunomodulatory, anti-stress, anti-anxiety, anti-microbial, anti-oxidant and anti-neurodegenerative activities observed in diverse *in vitro* and/or *in vivo* experimental models [P.T. White et al., 2016; K. Narinderpal et al., 2013; V. Kumar et al., 2015a; S.K. Kulkarni and A. Dhir, 2008]. At present, most of Ashwaganda's pharmacological activities are attributed the two quantitatively major withanolides (withaferin A and withanolide D) biosynthesized and stored by the plant [K. Narinderpal et al., 2013]. More recently, a synthetic analogues of withaferin-A have also been identified as a therapeutically interesting cytotoxic agent [E.M. Wijeratne et al., 2014]. However, despite considerable efforts to obtain therapeutic leads from naturally occurring withanolides, many questions concerning their structure activity relationships or their pharmacological targets still remain open or speculative only.

Since structure activity studies with plant alkaloids have often lead to novel drugs and drug leads considerable efforts have also been made to identify alkaloids from *Withania somnifera*. Some of the alkaloids encountered in *Withania somnifera* are withanine, somniferine, somnine, somniferinine, withananine, psuedo-withanine, tropine, psuedotropine, 3-α-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine and anahydrine [S.K. Kulkarni and A. Dhir, 2008; A. K. Datta et al., 2010; G.L. Gupta and A.C. Rana, 2007]. Reportedly, the total alkaloid content isolated from the roots of *Withania somnifera* vary from 0.13 to 0.31%, and sometimes as high as 4.3% [A. K. Datta et al., 2010]. Such wide variations in the yield of alkaloids may be due to the genotype variability in the species and genotype/environmental interactions or may be due to the isolation method used [A. K. Datta et al., 2010; S.K. Srivastava et al., 1960]. Presences of some other alkaloids called ashwagandhine, ashwaganidhine, and somniferine in *Withania somnifera* have been reported also [A. K. Datta et al., 2010].

Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII, and two glycowithanoloids viz. sitoindoside IX or sitoindoside X possessing antistress activity have also been isolated from *Withania somnifera roots* [S.K. Kulkarni and A. Dhir, 2008]. Although such scattered reports on the antistress and other bioactivities of numerous phytochemicals encountered in *Withania somnifera* have continued to appear during more recent years, as yet no very definitive statements on their roles in traditionally known medicinal uses of the plant can be made. However, it is now well recognized that the contents of all bioactive secondary metabolites of the plant encountered in diverse parts and cultivars *Withania somnifera* can vary (both qualitatively and quantitatively) enormously [S. Chatterjee et al., 2010; A. Bhatia et al., 2013; N.A. Gajbhiye et al., 2015].

Apart from alkaloids and withanolides, numerous other bioactive plant metabolites of *Withania somnifera* are also encountered in numerous food plants commonly consumed with everyday meals [S. Chatterjee et al., 2010; A. Bhatia et al., 2013; S.K. Bharti et al., 2011; N.J. Dar et al., 2015]. Although their reported quantities in different part of the plant (0.08 to 0.10%) are much lower than those of withanolides or alkaloids, currently available information on quantitative systems pharmacology of many of them strongly suggest that they could also contribute to bioactivity profile of the extracts of the plant after their repeated daily oral doses. One such well studied food phytochemical encountered in Withania somnifera extracts is fumaric acid [S. Chatterjee et al., 2010], an intermediate of mitochondrial citric acid cycle involved in ATP synthesis and has diverse physiological functions in almost all terrestrial plants and all other cellular organisms [W.L. Araújo et al., 2011; A. Shakya et al., 2015a]. It is also biosynthesized gut microbiota bacteria by carbon capture mechanisms [C.A. Roa Engel et al., 2008].

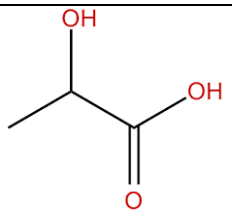
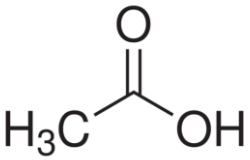
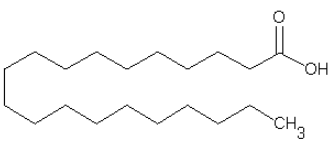
Fumaric acid was first isolated from a plant of the Fumaria species (*Fumaria officinalis*) often used in traditionally know systems of medicine for prevention and cure of inflammatory

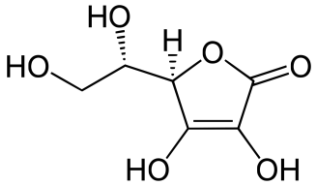
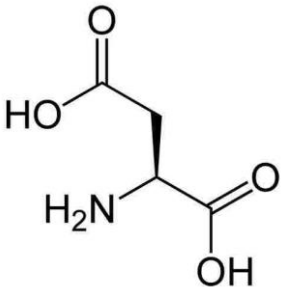
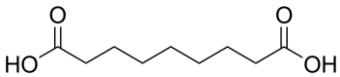
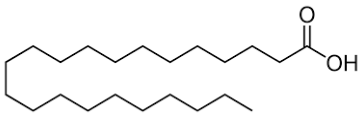
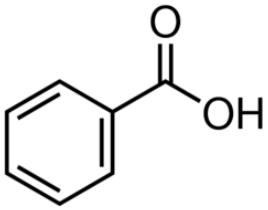
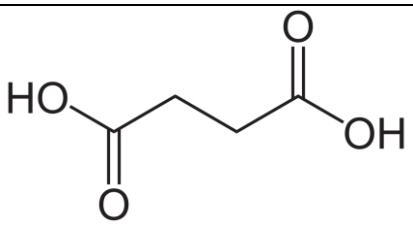
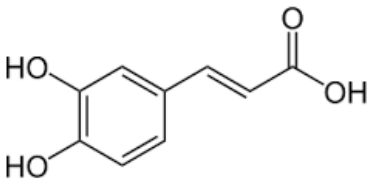
disorders of the skin. During more recent years fumaric acid and its easily hydrolysable conjugates have often been reported to be some of the bioactive constituents of diverse other traditionally known medicinal plants. Some of their bioactivities observed in animal models includes their central nervous system depressant [G.K. Singh and V. Kumar, 2010], anti-stress and adaptogenic [G.K. Singh et al., 2012a], anti-aggressive [G.K. Singh et al., 2012b], anti-anxiety and immune-modulatory [G.K. Singh et al., 2013a], anti-amnestic [G.K. Singh et al., 2013b], and analgesic and anti-inflammatory [A. Shakya et al., 2014] activities. Numerous other reports have consistently revealed and reaffirmed fumaric acid and its methyl esters (viz. mono- and di-methyl fumarate) are effective against inflammatory disorders such as psoriasis, multiple sclerosis, as well as Huntington's disease, and that this is due to their inhibitory effect on inflammatory cytokines release [R. de Jong et al. 1996; R.A. Linker et al. 2011; G. Ellrichmann et al. 2011; R. Gold et al. 2012; A. Shakya et al. 2016]. Fairly high daily oral doses of pure fumarates are now recommended by health authorities of USA and other countries for treatments of multiple sclerosis, and most preclinical reports on their diverse bioactivities deal mainly with their high doses or with their effects in cellular and other in vitro models [A. Shakya et al. 2016]. More recent observations in our laboratories have revealed that like therapeutically used methyl esters of the acid, daily oral doses of pure fumaric acid also possess anti-inflammatory, analgesic, anti-stress or adaptogenic activities in rodent models, and that in this respect fumaric acid is as effective as its esters with ED_{50} values $\leq$ 2 to 5 mg/kg/day [A. Shakya et al., 2015a; G.K. Singh et al, 2012a; 2012b]. Although contents of fumaric acid, fumarates and many other food phytochemicals with bactericidal activities encountered in *Withania somnifera* have not yet been quantified, observations in our laboratories have consistently revealed anti-stress, adaptogenic, and diverse other therapeutically interesting bioactivities of several of them after

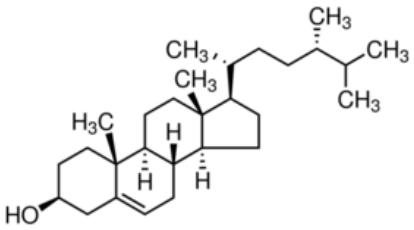
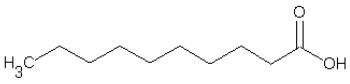
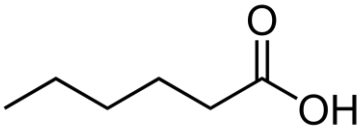
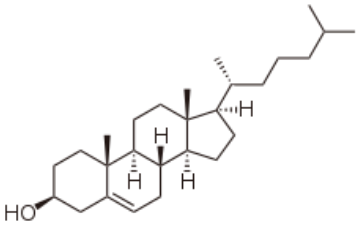
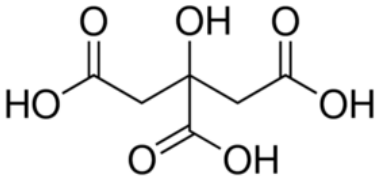
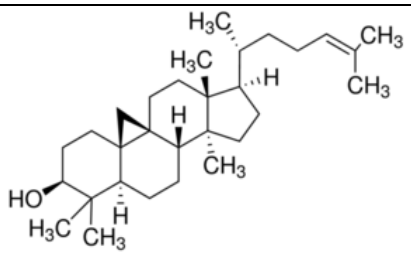
their fairly low daily oral doses [S.A. Khan et al., 2016; A.J. Langstieh et al., 2014; V. Yadav et al., 2015; N. Shrivastava et al., 2015].

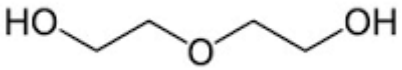
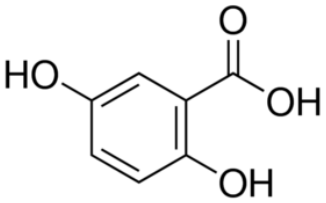
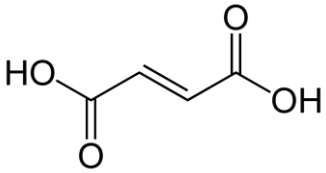
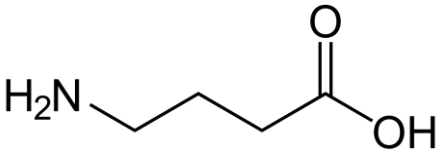
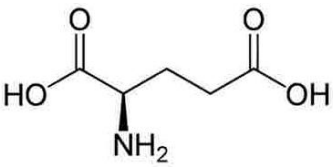
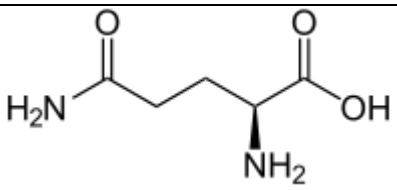
Structures of such phytochemicals, organic acids, and several other so-called bioactive "non-nutritive" substances already reported to be present in diverse types of *Withania somnifera* extracts are shown in **Table 2.2**. The ones for which low dose stress response modulating and other therapeutically interesting or metabolic function modulating activities are known, or are apparent, are given in bold letters and underlined in this table.

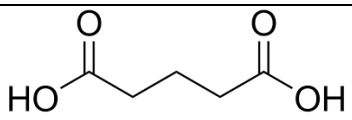
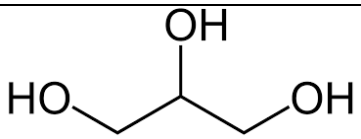
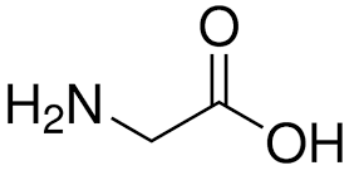
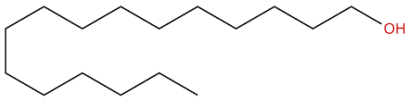
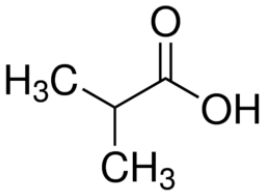
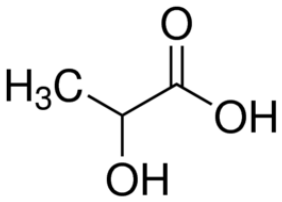
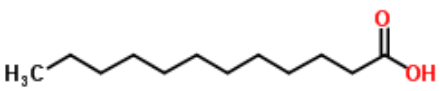
Table 2.2: So called "non-nutritive" constituents of *Withania somnifera* extracts reported to date.

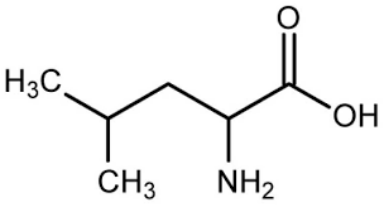
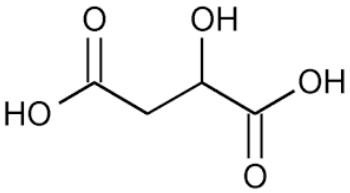
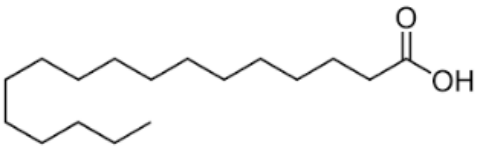
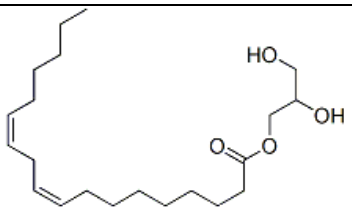
Sl. No.	Chemical constituents	Plant part(s)	Chemical Structure	References
1.	2-Hydroxy propanoic acid	Leaves		S. Chatterjee et al., 2010
2.	**<u>Acetic acid</u>**	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; S.K. Bharti et al., 2011
3.	Arachidic acid	Fruits		A. Bhatia et al., 2013

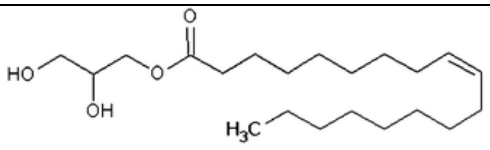
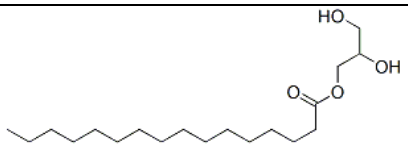
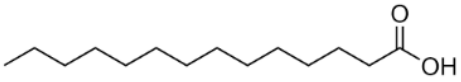
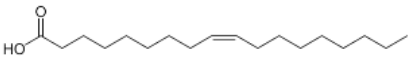
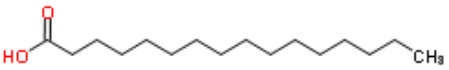
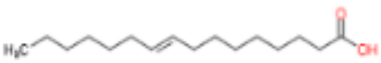
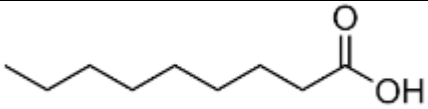
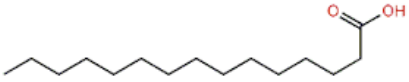
4.	**<u>Ascorbic acid</u>**	Leaves, fruits and roots		N. Alam et al., 2012; A.Q. Ansari et al., 2013
5.	Aspartic acid	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; O.P. Sidhu et al. 2011
6.	Azelaic acid	Fruits		A. Bhatia et al., 2013
7.	Behenic acid	Fruits		A. Bhatia et al., 2013
8.	**<u>Benzoic acid</u>**	Leaves and roots		S. Chatterjee et al., 2010
9.	Butandioic acid	Leaves and roots		S. Chatterjee et al., 2010
10.	Caffeic acid	Fruits		A. Bhatia et al., 2013; O.P. Sidhu et

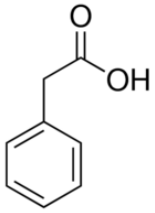
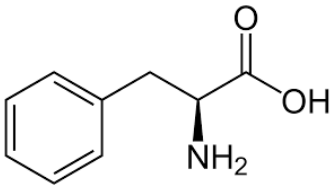
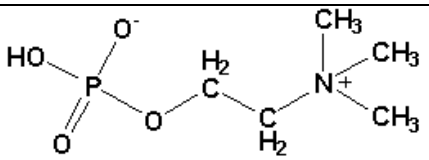
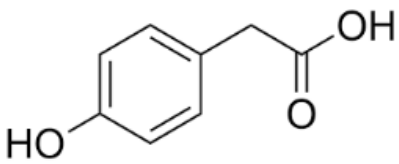
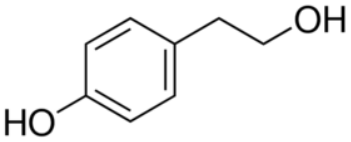
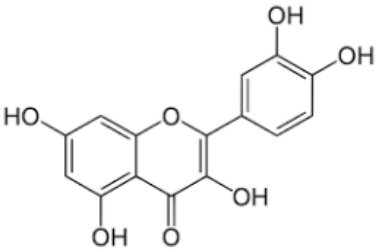
				al., 2011
11.	Campesterol	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
12.	Capric acid	Fruits		A. Bhatia et al., 2013
13.	Caproic acid	Fruits		A. Bhatia et al., 2013
14.	Cholesterol	Fruits		A. Bhatia et al., 2013
15.	**<u>Citric acid</u>**	Leaves and roots		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; O.P. Sidhu et al., 2011
16.	Cycloartenol	Fruits		A. Bhatia et al., 2013

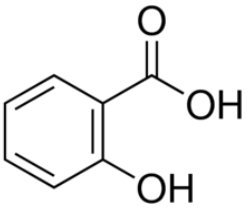
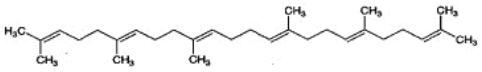
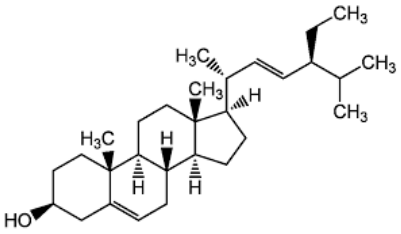
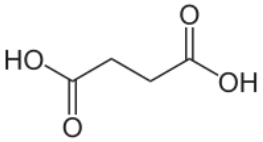
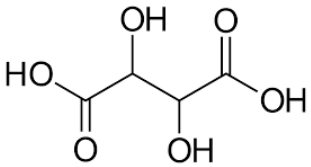
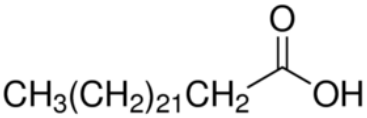
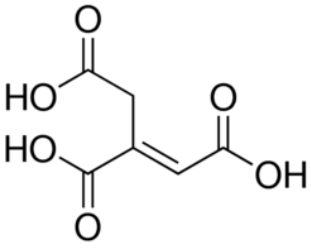
17.	Diethylene glycol	Fruits		A. Bhatia et al., 2013
18.	**Dihydroxy benzoic acid**	Leaves		S.B. Mekbib et al. 2009
19.	**Fumaric acid**	Leaves and roots		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; S.K. Bharti et al., 2011
20.	Gamma amino butyric acid (GABA)	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; O.P. Sidhu et al. 2011
21.	Glutamic acid	Fruits and leaves		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
22.	Glutamine	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et

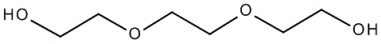
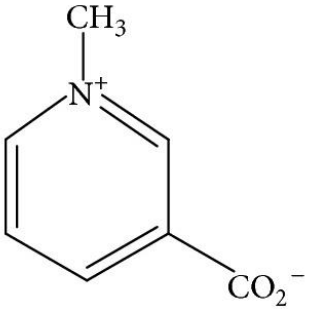
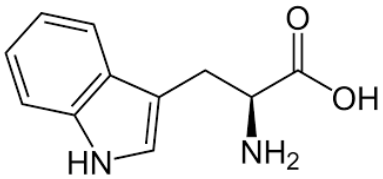
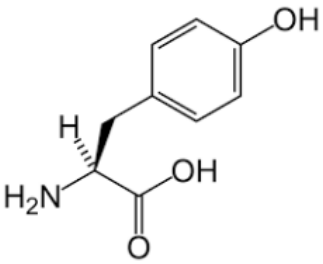
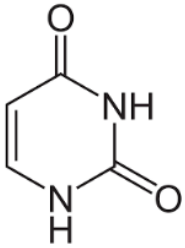
				al., 2013
23.	Glutaric acid	Fruits		A. Bhatia et al., 2013
24.	Glycerol	Fruits		A. Bhatia et al., 2013
25.	Glycine	Fruits and leaves		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
26.	Hexadecanol	Fruits		A. Bhatia et al., 2013
27.	Isobutyric acid	Fruits		A. Bhatia et al., 2013
28.	**<u>Lactic acid</u>**	Leaves and roots		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; S.K. Bharti et al., 2011
29.	Lauric acid	Fruits		A. Bhatia et al., 2013

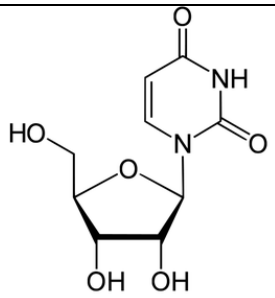
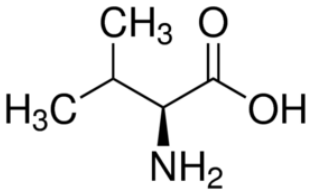
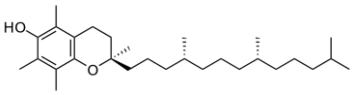
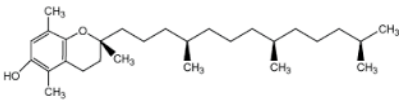
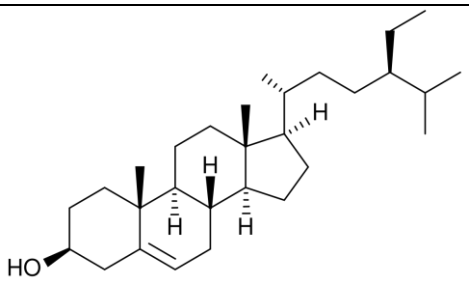
30.	Leucine	Fruits and leaves		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
31.	Linoleic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
32.	Linolenic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
33.	Malic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
34.	Margaric acid	Fruits		A. Bhatia et al., 2013
35.	Monolinolein	Fruits		A. Bhatia et al., 2013

36.	Monoolein	Fruits		A. Bhatia et al., 2013
37.	Monopalmitin	Fruits		A. Bhatia et al., 2013
38.	Monostearin	Fruits		A. Bhatia et al., 2013
39.	Myristic acid	Fruits		A. Bhatia et al., 2013
40.	Oleic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
41.	Palmitic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
42.	Palmitelaidic acid	Fruits		A. Bhatia et al., 2013
43.	Pelargonic acid	Fruits		A. Bhatia et al., 2013
44.	Pentadecanoic acid	Fruits		A. Bhatia et al., 2013

45.	Phenylacetic acid	Leaves and roots		S. Chatterjee et al., 2010
46.	Phenylalanine	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013; O.P. Sidhu et al. 2011
47.	Phosphocholine	Fruits		A. Bhatia et al., 2013
48.	P-hydroxy phenyl acetic acid	Leaves and roots		S. Chatterjee et al., 2010
49.	p-Hydroxy phenyl ethanol	Leaves and roots		S. Chatterjee et al., 2010
50.	**<u>Quercetin</u>**	Leaves		H.S. Bashir et al., 2013; S. Sivamani et al., 2014

51.	**<u>Salicylic acid</u>**	Leaves		S.B. Mekbib et al. 2009
52.	Squalene	Fruits		A. Bhatia et al., 2013
53.	Stigmasterol	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
54.	Succinic acid	Leaves, roots and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
55.	Tartaric acid	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
56.	Tetracosanoic acid	Fruits		A. Bhatia et al., 2013
57.	Trans-aconitic acid	Fruits		A. Bhatia et al., 2013

58.	Triacylglycerol	Leaves and roots		S. Chatterjee et al., 2010; O.P. Sidhu et al., 2011
59.	**Triethylene glycol**	Leaves		R. Wadhwa et al., 2013
60.	Trigonelline	Fruits		A. Bhatia et al., 2013
61.	Tryptophan	Fruits		A. Bhatia et al., 2013
62.	Tyrosine	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
63.	Uracil	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013

64.	Uridine	Fruits		A. Bhatia et al., 2013
65.	Valine	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013
66.	α-Tocopherol	Fruits		A. Bhatia et al., 2013
67.	β-Tocopherol	Fruits		A. Bhatia et al., 2013
68.	β -Sitosterol	Leaves and fruits		S. Chatterjee et al., 2010; A. Bhatia et al., 2013

Amongst all such plant metabolites known to date, salicylic acid (a circulating metabolite of aspirin) is pharmacologically and clinically the most extensively studied one. Salicylic acid is a plant hormone also involved in ecological survival processes of numerous plants [A.C. Vlot et al., 2009]. Together with its positional isomers, i.e. para- and meta-hydroxybenzoic acids, it is often considered to be a bioactive constituent of several medicinal and/or food plants now often pharmacologically classified as adaptogenic herbs [A.J. Langstieh et al., 2014, D. Zhou et al., 1993]. Amongst these three mono-hydroxy benzoic acids, the 3-hydroxybenzoic

acid is pharmacologically the least explored one [S. Khadem and R.J. Marles, 2010; F.A. TomásBarberán and M.N. Clifford, 2000]. It has recently been reported though, that like lactic acid, fumarates, and numerous other hydroxylated and other organic acids, 3-hydroxybenzoic acid is also an unspecific agonist of hydroxy carboxylic acid receptors, (HCA-receptors), and that modulations of such receptors in the digestive tract could also be involved in their metabolic and observed effects in animal models [H. Chen et al., 2014; M. Rahman et al., 2014; S. Offermanns and M. Schwaninger, 2015; B.H. Juurlink et al., 2014; S. Offermanns, 2017; C. Liu et al., 2009]. Since numerous phytochemicals and other ubiquitous substances encountered in *Withania somnifera* can also be metabolized to, or can modulate the production of diverse such acids inside the gastrointestinal tract, it is not impossible that HCA- and other fatty acid receptors are also involved in the traditionally known uses of *Withania somnifera* derived products as tonics, growth regulators, and longevity enhancers. Since gut microbiota regulated the metabolic and other functions of the enteric nervous system, often referred to as "the second brain" [A.K. Thakur et al., 2014b; S.S. Chatterjee, 2015], such bactericidal food phytochemicals encountered in *Withania somnifera* could as well be due to their modulating effects on gut microbial ecology.

Apart from such food phytochemicals, *Withania somnifera* extracts also contain numerous vitamins and polymeric nutritive and/or non-nutritive substances like lignans, nonnutritive proteins, and carbohydrates etc., which can also contribute to their pharmacological activity profiles by modulation of the functions of gut microbiota and enteric nervous systems. Presence of appropriate mixtures of such nutritive and non-nutritive substance in *Withania somnifera* and numerous other medicinal or food plants now often commercialized as food supplements or nutraceuticals have been pointed out by modern herbal researchers and scholars [P. Govind, 2011; S. Madhuri and G. Pandey, 2009].

2.1.4.1. Experimental pharmacology of withanolides

Withanolides have drawn considerable attention of experimental pharmacologists due to their uniquely complex structures, accessibility from plants, and diverse therapeutically interesting bioactivities of extracts of plants biosynthesizing and storing them in considerable amounts. Such bioactivities include their stress response modulating, anxiolytic, stimulant, cytotoxic, immunosuppressive, antimicrobial and anti-inflammatory activities [L.X. Chen et al., 2011]. Although Ashwagandha contains numerous other steroidal compounds, most of Ashwaganda's pharmacological activities have often been attributed to a few withanolides only, viz. Withaferin A, Withanolide D, Withanolide E, glycowithanolides and sitostinosides [R.D. Budhiraja and S. Sudhir, 1987]. There is now some evidence from animal studies that cognitive function enhancing and memory improving effects of withanolides are due to their effects on cholinergic receptors in the brain and that cholinomimetic like steroids encountered in *Withania* extracts have high affinity for cholinergic receptors [R. Schliebs et al., 1997]. It has been reported also that hypotensive effects of a *Withania* somnifera extract is due to its modulating effects on central cholinergic pathway which prolongs the effects of circulating adrenaline and minimizes the effect of acetylcholine [F. Ahumada et al., 1991].

Some other reports have indicated that sitostinosides, withanolides A, withaferin A isolated from Ashwagandha have no direct effects on the main inhibitory receptors in the central nervous system, i.e. the ionotropic $GABA_A$ receptor in the rat brain [R. Schliebs et al., 1997; M. Candelario et al., 2015]. However, they have been shown to have modulatory effect on $GABA_A$ receptor responses, and suggest that steroidal constituents present in the roots of *Withania somnifera* responsible for the development of pharmacological treatments for neurological disorders associated with GABAergic signaling dysfunction such as general anxiety disorders, sleep disturbances, muscle spasms, and seizures [M. Candelario et al., 2015; A.K. Mehta et al. 1991; S.K. Kulkarni et al., 2008].

Since steroidal withanolides also possess anti-inflammatory activities, they are often considered to be therapeutic leads for prevention and cure of chronic inflammatory diseases like arthritis, anti-tumor, cancer, and neuro-degeneration. Their anti-inflammatory activities have been attributed to their modulatory effects on several cellular inflammatory pathways like NF-κB, JAK/STAT, AP-1, PPARγ, Hsp90 Nrf2, HIF-1, etc. These naturally occurring compounds possess fairly broad spectrum of biologic activities across such complex disease processes, and have minimal adverse effect potentials [P.T. White et al., 2016]. Most notably, the antitumor and associated anti-inflammatory activities of withanolides results from targeting multiple signaling pathways simultaneously, particularly the nuclear factor kappa B (NF-κB), signal transducer and activator of transcription (STAT), and ubiquitin proteasome pathways. The antitumor and anti-inflammatory properties of Withaferin A and tubocapsenolide A have been attributed to the presence of key structural features such as an α,β-unsaturated ketone in ring A, a 5β,6β-epoxide in ring B, and a lactone side chain [E.M. Wijeratne et al., 2014; M. Ozawa et al., 2013; A.G. Damu et al., 2007; H.C. Wang et al., 2012; M. Ishiguro et al., 1974; B. Jayaprakasam and M.G. Nair, 2003a; B. Jayaprakasam et al., 2003b; H. Zhang et al., 2012; L.X. Chen et al., 2011].Cysteine residues in proteins are often implicated to react with these key electrophilic sites on the withanolide molecule [E.M. Wijeratne et al., 2014; M. Ozawa et al., 2013; Y. Yu et al., 2010; L. Ji et al., 2012]. While other withanolides also possess α,β-unsaturated ketone and/or epoxide (e.g., paraminabeolides, capsisteroids, and chantriolides) and are bioactive, in general they are often less potent than those withanolides possessing all three crucial functional groups.

Several withanolides have been demonstrated to possess significant immunomodulatory activities. Such effects have also been reported for *Withania somnifera* extracts (primarily aqueous extract), withanolide A, physalins and coagulins. However, both immunostimulatory and inhibitory actions have been attributed to different withanolides. *Withania somnifera*

extract (primarily withanolide A and 2,3 dihydro-3-sulphonile withanone components) is able to shift the immune response toward Th-1 polarization, activate cytotoxic natural killer cells [J. Mikolai et al., 2009; L. Davis and G. Kuttan, 2002], and recover depleted T-cells and increase expression of Th-1 cytokines IL-2 and IFN-γ in models of stress [F. Malik et al., 2007; K. Kour et al., 2009; B. Khan et al., 2006; S. Bani et al., 2006]. Conversely, coagulins isolated from *Withania coagulans*, primarily coagulin-H, have been demonstrated to posses immunosuppressive effects similar to prednisolone, via inhibiting stimulated T and B-cell lymphocyte proliferation, and Th-1 cytokine production (possibly through IL-2 receptor binding) [C.F. Huang et al., 2009; M.A. Mesaik et al., 2006].

There is now strong preclinical and clinical evidences reaffirming that inflammation initially started by immune cell mediators may persist chronically, resulting in ongoing stimulation of inflammatory mediators and regulatory pathways that contribute to the pathogenesis of chronic diseases including cardiovascular, neurologic, and pulmonary diseases, as well as cancer, diabetes, and obesity [B.B. Aggarwal et al., 2009; G. Costa et al., 2012; P. Libby, 2007]. As with acute inflammation, chronic inflammation is mediated through various signaling factors, which include proinflammatory cytokines such as TNFα, IL-1, IL-6, IL-8, IL-12, NO, adhesion molecules, and chemokines [G. Costa et al., 2012; P. Libby, 2007]. Additionally, transcription factors that regulate the expression of inflammatory mediators such as NF-κB, activator protein 1 (AP-1), peroxisome proliferator-activated receptor (PPAR)-γ, STAT3, hypoxia inducible factor-1 (HIF-1), β-catenin/Wnt, hedgehog, and nuclear factor erythroid 2-related factor 2 (Nrf2) have been linked to chronic diseases. The DNA-binding capacity of these transcription factors is modified by several signaling cascades such as JAK/STAT, MAPKs, PI3K/Akt/mechanistic target of rapamycin (mTOR), and ubiquitin proteasome system [L.A. O'Neill, 2006]. These signaling pathways have a wide range of functions and show complex crosstalk which depend on the cell type and the chronic

disease involved. Withanolides have now emerged as potential therapeutics leads against chronic diseases due to their unique ability to modulate these multiple intracellular signaling pathways.

Alternatively, it has also been demonstrated that withanolides decreases glucocorticoid receptor activity in the brain, thus down regulating corticosterone level and reversing hypoxia mediated neurodegeneration [I. Baitharu et al., 2014]. It has been reported indeed, that inhibition of corticosterone synthesis or blockade of glucocorticoid receptor activity during oxygen scarcity decreased neurodegeneration and enhanced memory weakness [I. Baitharu et al., 2012; I. Baitharu et al., 2013a; I. Baitharu et al., 2014]. Thus withanolides can provide protection against neurodegenerative disorders caused by hypoxia and diverse pathological condition leading to hypoxic damages. It has been reported indeed that Withaferin-A possesses potent antiproliferative activity against pancreatic malignancy (Panc-1, MiaPaCa2 and BxPc3) cells by blocking heat shock protein (Hsp)-90 chaperone action through collaboration with the C-end of Hsp90, and thereby actuating degradation of Hsp90 proteins, for example, Akt, Cdk4 and glucocorticoid receptor [Y. Yu et al., 2010].

It has also been demonstrated that the withanolide tubocapsenolide A induce cycle arrest and apoptosis in human breast cancer cells, which was associated with the inhibition of heat shock protein 90 (Hsp90). The 2,3-unsaturated double bond-containing withanolides inhibited Hsp90 function, as evidenced by selective depletion of Hsp90 client proteins and induction of Hsp70. Importantly, Hsp90 inhibition by the withanolides was correlated with their ability to induce cancer cell death. In addition, the withanolides reduced constitutive NF-κB activation by depleting IκB kinase complex (IKK) through inhibition of Hsp90. In estrogen receptor positive breast cancer (MCF-7) cells, the withanolides also reduced the expression of estrogen receptor, and this may be partly due to Hsp90 inhibition [H.C. Wang et al., 2012]. The withanolides exhibits potent inhibition of estrogen receptor expression as

well, an effect potentially lined to suppression of ER-dependent proliferation of breast cancer cells [G. Castoria et al., 2010]. Additionally, it has been demonstrated that Withaferin A has the ability to alter numerous cancer-associated growth factor receptors, kinases, and transcription factors [M. Kaileh et al., 2007]. Through various *in vitro* cell line studies, it has been suggested that steroidal withanolides induced apoptosis in the breast cancer cell lines was associated with down regulation or inhibition of the expression of estrogen receptors. Thus withanolides could become an important therapeutic option for the treatment of breast cancer [H.C. Wang et al., 2012; E.R. Hahm and S.V. Singh, 2013; X. Zhang et al., 2011; E.R. Hahm et al., 2011].Diverse reports on therapeutically interesting bioactivities of withanolides and their doses and treatment regimen used in those studies are summarized in **Table 2.3**. However, as yet no very systematic efforts have been made to assess their therapeutically interesting doses and dosing regimen necessary for obtaining therapeutic benefits from them, or for comparing the therapeutic potentials of structurally diverse withanolides encountered in *Withania somnifera.*

Table 2.3: Pharmacological activities of withanolides observed in experimental animal and *in vitro*.

Sl. No.	Pharmacological activity	Dose, duration and route of administration	References
1	Antioxidant activity on rat brain	Sitoindosides VII-X and withaferin A (glycowithanolides) (10 and 20 mg/kg; intraperitoneally) for 21 consecutive days	S.K. Bhattacharya et al., 1997b
2	Antihyperglycemic activity	Withanolide (50 and 100 mg/kg; orally) for 10 consecutive days	R. Maurya et al., 2008

3	Diuretic activity	Single dose of withaferin A and witharistatin, and a mixture of them (5 and 10 mg/kg; orally) for 6 hrs.	D. Benjumea et al., 2009
4	Active against Alzheimer treatment	Withanolide A (10 µmol/kg/day; oral) for 13 consecutive days. Withanoside IV from methanolic root extract of *Withania somnifera* (10 µmol/kg/day; orally) for 11 consecutive days. Withanolide A (active against multiple targets associated with Abeta pathways (BACE1, ADAM10, IDE, and NEP)	T. Kuboyama et al., 2005; T. Kuboyama et al., 2006; S.P. Patil et al., 2010
5	Anti-stress activity	Glycosides (sitoindosides VII and VIII, 50 to 100 mg/kg; orally as a single dose)	S.K. Bhattacharya et al., 1987
6	Nootropic activity	Equimolar mixture of sitoindosides VII-X and withaferin-A (40 mg/kg; intraperitoneally) for 7 consecutive days	R. Schliebs et al., 1997
7	Antioxidant and Immunomodulatory Activity	Glycowithanolides and a mixture of sitoindosides IX and X (50-200 mg/kg; orally).	S. Ghosal et al., 1989
8	Antimicrobial Activity	*In-vitro* activity of withanolide (2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/ml Conc.) *In-vitro* activity of withaferin A (6–100µg/ml Conc. against Grampositive microorganisms)	P. Kharela et al., 2011; P.D. Sethi et al., 1974
9	Central nervous system depressant activity	3β -hydroxy-2,3-dihydrowithanolide-F (625 mg/kg; intraperitoneally)	R.D. Budhiraja and S. Sudhir, 1987

10	Cardiovascular effects (Antihypertensive activity)	Withanolide (5 mg/kg; orally)	R.D. Budhiraja et al., 1983
11	Hepatoprotective activity	3β-hydroxy-2-3 dihydro withanolide – F (10 and 30 mg/kg; intraperitoneally) for 4 consecutive days.	R.D. Budhiraja et al., 1986
12	Immunosuppressive activity	*In-vitro* withacoagulins A – F (IC_{50}<20 µm on the inhibition of both ConA-induced T cell proliferation and LPS-induced B-cell proliferation)	C.F. Huang et al., 2009
13	Anti-inflammatory activity	3β-hydroxy-2-3 dihydro withanolide – F (10 and 30 mg/kg; intraperitoneally) for 14 consecutive days	R.D. Budhiraja et al., 1984
14	Antifungal activity	Withanolides (MIC 300 µg/ml)	M.I. Choudhary et al., 1995
15	Immunoactivating and immunosuppressive activity	Withaferin A (10 mg/kg) for 6 consecutive days. *In-vitro* 5,20a(R)-dihydroxy-6a,7a-epoxy-1-oxo-(5a)-witha-2,24-dienolide (1µg/ml; on spleen cell culture).	R. Maurya, 2010; B. Shohat and H. Joshua, 1971; V. Bahr and R. Hansel, 1982
16	Anti-cancer activity	*In-vitro* Withaferin A (40µg/ml; on Sarcoma-180 ascites tumour cells cell culture). *In-vitro* (Withanolides inhibited TNF-α induced nuclear factor-kappa B (NF-κB) activation with IC_{50} values in the range of 1.60−12.4µM.)	K. Chowdhury and R.K. Neogy, 1975 I.U. Haq et al., 2013
17	Anti-cholinesterase Activity	*In-vitro* withanolides inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities with IC_{50} values ranging between 29.0	M.I. Choudhary et al., 2005

		and 85.2 mM for AChE and BChE, respectively.	
18	Cytotoxic activity against liver and breast cancer cell lines	*In vitro* 5,6-De-epoxy-5-en-7-one-17-hydroxy withaferin A, of *Withania somnifera* (IC_{50} of 1.0 μg/ml)	A.A. Siddique et al., 2014

2.1.5. Phytopharmacology of *Withania somnifera*

The term "phytopharmacology" was first coined in 1930s by the American pharmacologist David Macht for the field of study of the effects of drugs on plants [D.I. Macht and R.E. Gardner, 1936]. The term has since changed its meaning to become an established field of drug research, where the active substances come from plants, or are isolated and/or derived from their secondary metabolites. For such purposes, experimental pharmacologists often start from plant extracts obtained from different parts of plants using arbitrarily chosen processing and extraction procedures. However, several such researchers still continue to use powdered plant materials for initial screening. Currently available reports revealing broad spectrums of pharmacological activity profiles of *Withania somnifera* extracts and powdered roots of the plant are summarized in **Table 2.4.,** and their more often reported therapeutically interesting bioactivities are summarized in the following.

Table 2.4: Pharmacological activities of *Withania somnifera* extracts and root powder.

Sl. No.	**Pharmacological activity**	**Dose, duration and route of administration**	**References**
1	Anticonvulsant activity	*Withania somnifera* root extract (100 – 200 mg/kg, orally)	S.K. Kulkarni et al., 2008

2	Nephroprotective activity	*Withania somnifera* root extract (500 mg/kg/day; orally) for 22 consecutive days.	S.C. Shimmi et al., 2011
3	Neuroprotective activity	*Withania somnifera* root extract (100-400 mg/kg/day; orally) for 28 consecutive days. *Withania somnifera* root hydroalcoholic extract (20 mg/kg; orally) for 28 consecutive days.	M.J. Manjunath and Muralidhara, 2013; S. Jain, et al., 2001
4	Antioxidant activity and improves fertility in Man	Infertile men were prescribed *Withania somnifera* root powder (5 g/day) orally for 3 months with milk.	M.K. Ahmad et al., 2010
5	Hypocholesteremic and antioxidant activity	*Withania somnifera* root powder (0.75 - 1.5 gm/animal/day; orally) for 28 consecutive days.	N.P. Visavadiya and A.V. Narasimhacharya, 2007
6	Hypoglycaemic and hypolipidaemic activity	*Withania somnifera* leaf and root extracts (100-200 mg/kg/day; orally) for 56 consecutive days.	R. Udayakumar, et al., 2009
7	Immunomodulatory Activity	*Withania somnifera* (200 mg/kg/day; orally) for 42 consecutive days.	P. Amaravathi, et al., 2012
8	Anxiolytic and antidepressant activity	Bioactive glycowithanolides isolated from *Withania somnifera* root extract (20 – 50 mg/kg/day; orally) for 5 consecutive days.	S.K. Bhattacharya, et al., 2000;

		Clinical trial *Withania somnifera* extract (250 mg/tab; orally) for 6 weeks.	C. Andrade et al., 2000
9	Adaptogenic activity	*Withania somnifera* root extract (25 and 50 mg/kg/day; orally) for 42 consecutive days.	S.K. Bhattacharya and A.V. Muruganandam, 2003
10	Antiulcer and antioxidant activity	*Withania somnifera* root extract (100 mg/kg/day; orally) for 15 consecutive days.	M. Bhatnagar, et al., 2005
11	Antiinflammatory activity	*Withania somnifera* root extract (1000 mg/kg; orally); *Withania somnifera* root extract (600 and 800 mg/kg/day; orally) for 25 consecutive days. *Withania somnifera* root powder (1000 mg/kg; orally); for 3 consecutive days	Y.P. Sahnia and D.N. Srivastavaa, 1993; A. Gupta and S. Singh, 2014. V.H. Begum and J. Sadique, 1987
12	Anticancer Activity	*Withania somnifera* leaf extract (100-250 mg/kg/day; orally) for 12 times on alternate day.	R. Wadhwa, et al., 2013
13	Hepatoprotective Activity	*Withania somnifera* powder (500 and 1000 mg/kg; orally)	E.P. Sabina, et al., 2013

14	Anti-stress activity	*Withania somnifera* aqueous root suspension (360 mg/kg; orally) as a single dose; *Withania somnifera* alcohol seed extract (100 mg/Kg; intraperitoneally) as a single dose; *Withania somnifera* aqueous root suspension (100 mg/kg; orally) for 7 consecutive days *Withania somnifera* extract (100 mg/kg; orally) for 14 consecutive days.	P. Kaur et al., 2001; N. Singh et al., 1982; R. Archana and A. Namasivayan, 1999; A. Bhattacharya et al., 2002
15	Cardioprotective activity in Myocardial Infarction	*Withania somnifera* alcoholic root extract (25, 50 and 100 mg/kg/day; orally) for 28 consecutive days.	I. Mohanty et al., 2004
16	Hypothyroid activity	*Withania somnifera* aqueous root extract (1.4 g/kg/day; orally) for 20 consecutive days.	S. Panda and A. Kar, 1998; S. Panda and A. Kar, 1999
17	Antiarthitic activity	*Withania somnifera* aqueous root extract (500 and 1000 mg/kg/day; orally) for 3 to 8 consecutive days. *Withania somnifera* root powder (1000 mg/kg/day; orally) for 15 consecutive days.	M. Rasool and P. Varalakshmi, 2006; M, Rasool et al., 2000; V.H. Begum and J. Sadique, 1988.

18	Anti-malarial activity	*Withania somnifera* root extract (600 mg/kg/day; orally) for 4 consecutive days.	D. Dikasso et al., 2006
19	Spermicidal and Antifertility activity in male	*Withania somnifera* hydroalcoholic fruit extract (50 mg/kg/day; orally) for 60 consecutive days.	P.C. Mali et al., 2008
20	Active against Skin Carcinogenesis	125 g (2.5%, w/w) and 250 g (5%, w/w) of the powdered root were mixed uniformly with 5 kg of standard laboratory diet each and made into pellets and were given for a period of 2 weeks as dietary feeding.	B. Padmavathi et al., 2005
21	Activity against Alzheimer's disease	*Withania somnifera* Chloroform-methanol root extract (1000 mg/kg/day; orally) for 60 consecutive days	N. Sehgala et al., 2012
22	Anti-Osteoarthritic Activity	*Withania somnifera* root powder (450 mg/day; orally) for 3 months.	R.R. Kulkarni et al., 1991
23	Antitumor Activity	*Withania somnifera* ethanolic extract (200 mg/kg/day; orally) for 7 months. *Withania somnifera* ethanolic root extract (400 mg/kg/day; orally) for 15 consecutive days.	N. Singh et al., 1986; P.U. Devi et al., 1992
24	Aphrodisiac Activity.	*Withania somnifera* ethanolic root extract (225 mg/capsule thrice daily; orally) for 90 consecutive days.	V.R. Ambiye et al., 2013

25	Antioxidant activity	*Withania somnifera* root extract (100 mg/kg; orally) for single day.	J.N. Dhuley, 1998
26	Hemopoetic activity (Anti-leucopenia activity)	*Withania somnifera* 70% methanolic root extract (20 mg/dose/animal, intraperitoneally) 10 doses in 30 days experiment.	L. Davis and G. Kuttan, 1998
27	Rejuvenating activity (Clinical Study on healthy children, age 8-12 years)	*Withania somnifera* powder (2 g/day fortified in 100 cc of milk) for 60 consecutive days.	S. Venkataraghavan et al., 1980
28	Memory-enhancing activity	*Withania somnifera* methanolic root extract (40 mg/kg; intraperitoneally) for 7 consecutive days. *Withania somnifera* hydro-methanolic root extract (50, 100, 150, 200 and 250 mg/kg; orally)	R. Schliebs et al., 1997; I. Baitharu et al., 2013b
29	Active against excitotoxic neuronal injury (Anti-excitotoxic activity)	*Withania somnifera* ethanolic root extract (20 mg/kg; mixed in diet) for 3 weeks.	M.S. Parihar and T. Hemnani, 2003
30	Antiradical and DNA cleavage protective activity	*In-vitro Withania somnifera* methanolic root extract [3, 6, 12, 25 µg/ml and 50, 100, 200, 400 µg/ml showing free radical-scavenging activity; and 800 µg/ml concentration, on the protection of supercoiled DNA	A. Russo et al., 2001

		against -OH generated by the photolysis of H_2O_2 (2.5mM)] [At conc. 12–25 µg/ml active against H_2O_2-induced toxicity on human fibroblasts (MTT test) and H_2O_2-induced DNA damage]	
31	Antiparkinson's activity	*Withania somnifera* extract (100, 200 and 300 mg/kg; orally) for 3 weeks. *Withania somnifera* root extract (100 mg/kg; orally) for 28 consecutive days.	M. Ahmad et al., 2005; S.R. Sankar et al., 2007
32	Antiepileptic/anticonvulsant activity	*Withania somnifera* extract (50 and 100 mg/kg; orally) for single day. *Withania somnifera* root extract (100 mg/kg; orally) for 9 weeks. *Withania somnifera* ethanolic root extract (30, 100 and 200 mg/kg; orally)	A. Kumar and S.K. Kulkarni, 2006; S.K. Kulkarni and B. George, 1996; S.K. Kulkarni et al., 1993
33	Active against Tardive dyskinesia	*Withania somnifera* root extract (50 and 100 mg/kg; orally) for 4 weeks.	P.S. Naidu et al., 2006
34	Nootropic activity	*Withania somnifera* root extract (50, 100 and 200 mg/kg., orally)	J.N. Dhuley, 2001

		for 6 consecutive days.	
35	Activity against Cerebral ischemia (Anti-stroke activity)	*Withania somnifera* hydroalcoholic extract (1 g/kg/day, orally) for 15 to 30 consecutive days.	G. Chaudhary et al., 2003
36	Anti-addiction activity	*Withania somnifera* root extract (100 mg/kg/day, orally) for 9 consecutive days.	S.K. Kulkarni and I. Ninan, 1997
37	Antibacterial activity	*In-vitro Withania somnifera* crude root and leaves extract (MIC 0.8 mg/ml for *S. aureus*; 0.25 mg/ml for *S. typhimurium;* 0.5 mg/ml for *E. coli*) *In-vivo Withania somnifera* crude root and leaves extract (100 mg/kg/day; orally) for 7 consecutive days in *S. typhimurium* infected female rats.	M Owais et al., 2005
38	Activity against Orofacial Dyskinesia	*Withania somnifera* crude root extract (100, 200 and 300 mg/kg/day; orally) for 21 consecutive days	P.S. Naidu et al., 2003.
39	Antifungal Activity	*In-vitro Withania somnifera* crude leaves extract (0.1ml vol. against *Aspergillus niger, A. flavus, Fusarium oxysporium, F. moniliformis,*)	S.P. Singh et al., 2010

40	Antiviral activity	*In-vitro Withania somnifera* hydroalcoholic root extract (25µg/ml conc. shows 99.9% inhibition on Bursal Disease Virus Replication)	M. Pant et al., 2012
41	Anti-leishmanial activity	*In-vitro Withania somnifera* methanolic root extract (IC_{50} value 78±5 µg/ml against promastigote, and 63±6 µg/ml against amastigote forms of *L. donovani*)	U. Sharma et al., 2009.
42	Nephroprotective and immunomodulatory activity in *Leishmania donovani*-infected BALB/c mice	*Withania somnifera* extract (350 mg/kg/day; orally) for 15 consecutive days	H. Sachdeva et al., 2013
43	Blocking protein kinase C signaling pathway Within Melanocytes.	*In-vitro Withania somnifera* extract (10 µg/ml treated human melanoma cells inhibit PKC activity)	H. Nakajima et al., 2011
44	Anti-carcinogenic activity (on peritoneal macrophage functions)	*Withania somnifera* alcoholic root extract (100 mg/kg/day; orally) for 17 weeks	J.N. Dhuley, 1997

2.1.5.1. Anticancer activity: *Withania somnifera* has been reported for its anti-cancer activity against various type of cancer such as leukemia, lung, prostate, breast, colon, renal, pancreatic, head and neck cancer cells of humans [R. Nema et al., 2013; K. Patel et al., 2013; N. Singh et al., 2011a; 2011b; B. Yadav et al., 2010; V. Kumar et al., 2015a] and skin cancer and fore stomach cells in rodents [B. Padmavathi et al., 2005]. Recently the research have focused in the evaluation of the anticancer potential of *Withania somnifera* and underlying molecular mechanism of anticancer activity by using various *in vitro* and *in vivo* techniques. *Withania somnifera* as well as its bioactive principle withanolides have shown potent anticancer activity against variety of cancer cell lines by various mechanisms such as cancer cell toxicity, COX-2 inhibition and induction of phase II detoxifying enzymes such as quinine reductase. Withanolides are the main bioactive principle of *Withania somnifera* and might be responsible for its antitumor activity [V. Mulabagal et al., 2009; K. Patel et al., 2013]. Angiogenesis is an obligatory step in cancer progression, inhibiting angiogenesis might be one treatment strategy for cancer. In this context researcher have evaluated the anti-angiogenic activity of Withaferin-A against cancer cells. Withaferin-A have shown potent anti-angiogenic activity against cancer cells [K. Patel et al., 2013]. *Withania somnifera* also has been reported to induce apoptosis in cancer cells via different mechanisms such as, activation of tumor suppressor proteins such as p53 and pRB [R. Wadhwa et al., 2013], inhibiting the activation of nuclear factor kappa-B (NF-κB) [J.H. Oh and T.K. Kwon, 2009], increased free radical (reactive oxygen species) generation and activation of p38 MAP kinase [K. Patel et al., 2013].

Withaferin-A have shown anticancer activity via activating prostate apoptosis response-4 (Par-4), inhibiting chymotrypsin like activity of proteasome and by modifying the intermediate filament protein vimentin and inducing actin microfilament aggregation in human cancer cell lines [Z. Yang et al., 2013; Y. Yu et al., 2010].

Withaferin-A also depicts apoptotic activity in colon and breast cancer cells through inhibition of pro-survival pathways, such as Akt/NF-κB/Bcl-2, generating oxidative stress [S. Koduru et al., 2010] and inhibition of cell migration/invasion through down regulation of STAT3 activity [K. Patel et al., 2013]. Other reported possible mechanisms are suppression of X-linked inhibitor of apoptosis protein (XIAP) and cIAP-2 protein [E.R. Hahm and S.V. Singh, 2013].

2.1.5.2. Neuroprotective activity: *Withania somnifera* have shown marked neuroprotective activity against various neurological such as cognitive disorders, senile dementia and Parkinsonism and Alzeimer's disorder [M. Ahmad et al., 2005]. The neuroprotective activity of *Withania somnifera* is attributed to its significant antioxidant activity. Active principles of *Withania somnifera* such as glycowithanolides, withanolides and sitoindosides have shown substantial antioxidant activity via increased level of antioxidant enzymes in rat frontal cortex and striatum. *Withania somnifera* was also found to affect the cortical acetylcholine pathway in lateral septum and frontal cortex and have shown potential of reverse treating the cognitive impairment in patients [R. Schliebs et al., 1997].

Withania somnifera also improved the athletic performance and endurance performance in healthy individuals via increasing oxygen carrying capacity of red blood cells (RBCs) [S. Shenoy et al., 2012; K. Arman and J.S. Sandhu, 2007]. *Withania somnifera* which is rich in amino acids (aspartic acid, tyrosine, glycine, alanine, tryptophan, glutamic acid, cysteine, etc.), proteins, starch, reducing sugars, steroidal lactones and alkaloids increases its nutritional value and acts as a tonic, stimulant and energy rejuvenator [V. Kumar et al., 2015a].

Withania somnifera root extract have shown neuroprotective activity via down regulating nitric oxide pathway in the brain [M. Bhatnagar et al., 2009] and increased levels of brain

antioxidant enzymes such as SOD, CAT, GSH and GST [S. Sharma et al., 2011]. It is also reported that, the potential of withanolide-A and withanoside IV in neurite and synapses regeneration in damaged neurons [T. Kuboyama et al., 2005; T. Kuboyama et al., 2006]. Various studies with mamalian neuronal cell lines have shown the protective effect of *Withania somnifera* against β-amyloid (1-42) and HIV-1_{Ba-L} (clade B) toxicity [K.R.V. Kurapati et al., 2013]. The root extract of *Withania somnifera* have shown protective effect against Alzheimer's disease in a transgenic mice model via increased β-amyloid clearance, decreased behavioral and memory deficits.

In an animal model of Parkinson's disease root extract of *Withania somnifera* has been reported to normalized the brain dopamine, catecholamine level, dopaminergic D_2 receptor binding affinity and tyrosine hydroxylase expression and reduced the overall oxidative stress via reducing lipid peroxidation, and increasing the antioxidant enzymes level [S. RajaSankar et al., 2009]. Clinical trial studies have also shown promising results of *Withania somnifera* root powder in treating Parkinson's disease [M.F. Saleem, 2010].

2.1.5.3. Anti-epileptic activity: Since ancient times *Withania somnifera* has been used in the treatment of seizures and epilepsy. Now various *in vitro* and *in vivo* preclinical studies have supported the use of *Withania somnifera* against various types of epilepsy. *Withania somnifera* extracts as well as withanolides have shown anti-epileptic activity against various animal model of epilepsy. It modulates the $GABA_A$ receptor activity in the brain, and increases the seizure threshold [S.K. Kulkarni et al., 2008]. Oxidative stress effects membrane constitution resulting in decreased NMDA receptor density leading to impaired spatial memory. However, *Withania somnifera* root extract and withanolide-A have ameliorated spatial memory deficits by enhancing antioxidant system and restoring altered NMDA receptor density [S. Soman et al., 2012]. In another study *Withania somnifera* root

extract and withanolide-A have decreased the expression of AMPA receptor and glutamate levels [S. Soman et al., 2013]. These findings revealed the potential of *Withania somnifera* and its bioactive withanolides in the treatment of epilepsy.

2.1.5.4. Anxiolytic and antidepressant activity: The root extract of *Withania somnifera* has been used extensively from ancient Indian practice for the treatment of anxiety and depression. Various scientific reports have supported the traditional use of *Withania somnifera* as antianxiety and antidepressant [S.K. Bhattacharya et al., 2000; M.K. Jayanthi et al., 2012]. In clinical trials the aqueous extracts of *Withania somnifera* have shown improvement in the psychomotor symptoms of anxiety and depression [U. Pingali et al., 2014] via antioxidant and decreased production of nitric oxide in the brain tissues [Z.A. Khan and A.R. Ghosh, 2011; T. Maity et al., 2011].

2.1.5.5. Anti-inflammatory and anti-arthritic activity: *Withania somnifera* possess potent anti-arthritic and anti-inflammatory activities might be due to its steroidal nucleus of its bioactive principles. Recent experimental studies have reported the anti-inflammatory and antiarthritic activity of *Withania somnifera* through inhibition of inflammatory markers and improving the movements of the joints [A. Gupta and S. Singh, 2014]. *Withania somnifera* and withanolides are also effective in other inflammation associated disorders such as cystic fibrosis and irritable bowel syndrome. Various experimental studies have proposed several mechanisms such as inhibition of NF-κB activation, COX-2 expression, prostaglandins, histamine, endothelial cell protein C receptor activity, interleukins, and cytokines release [S.K. Ku et al., 2014; V. Mulabagal et al., 2009; J.H. Oh and T.K. Kwon, 2009; A. Gupta and S. Singh, 2014; J. Paval et al., 2009].

2.1.5.6. Spermatogenic activity: *Withania somnifera* has been used in the treatment of male infertility where sperm count remains very low. *Withania somnifera* has been reported to improved testicular development and spermatogenesis by directly affecting the seminiferous tubules in experimental animals. It also improved the pro-sexual behavior of sexually inactive animals, and increased the daily sperm production and testosterone level. *Withania somnifera*, by its potent antioxidant activity counteract the reactive oxygen species and thus prevent oxidative damage to the sperm and abnormal sperm parameters [V.R. Ambiye et al., 2013]. In recent years, clinical trials also supported the spermatogenic activity of *Withania somnifera*, it improves the semen quality and reproductive hormone levels in infertile male patients [M.K. Ahmad et al., 2010; K.K. Shukla et al., 2011]. The seminal plasma concentration of histidine, lactate, alanine, glycerylphosphorylcholine, citrate and phenylalanine is altered in infertile individuals while *Withania somnifera* significantly normalized the concentration of all the contents of seminal plasma and it recovers the quality of semen [V.R. Ambiye et al., 2013; A. Gupta et al., 2013].

2.1.5.7. Hepatoprotective activity: *Withania somnifera* exhibits hepatoprotective activity against various hepatotoxic animal models where it significantly reduces the hepatic damage biomarkers such as aspartate transaminase and alanine transaminase. It normalized the antioxidant enzymes and reduces the lipid peroxidation of liver tissue [T. Malik et al., 2013; E.P. Sabina et al., 2013]. In another rodent model of gamma radiation induced toxicity, *Withania somnifera* significantly decreases the level of elevated biomarker enzymes, malondialdehyde (MDA), total nitrate/nitrite NO(x) and heme oxygenase activity in the liver. In addition *Withania somnifera* normalized the antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and glutathione respectively [M.H. Hosny and H.H. Farouk, 2012].

2.1.5.8. Antimicrobial activity: *Withania somnifera* has been reported to show antibacterial activities against both Gram-positive and Gram-negative pathogenic bacteria [P. Singariya et al., 2012]. The leaves and roots extract of *Withania somnifera* have been reported to possess antimicrobial activity against various Gram-negative pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Citrobacter freundii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [N. Alam et al., 2012]. Furthermore the isolated alkaloids and flavonoids from *Withania somnifera* also exhibit antimicrobial activity against various pathogen such as *Enterobacter aerogens, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Raoultella planticola* and *Agrobacterium tumefaciens* [G. Singh and P. Kumar, 2011; 2012].

The crude leaves extract of *Withania somnifera* is also having antimicrobial activity against pathogens like *Staphylococcus aureus, Proteus mirabilis, Streptococcus sobrinus, Streptococcus mutans* and *Salmonella paratyphi B* [V. Kumar et al., 2015a; N. Al-Ani et al., 2013; S. Pandit et al., 2013]. On the other hand, *Withania somnifera* have shown moderate activity against *Microsporum gypseum, Candida albicans* and *Cryptococcus neoformans* [P.G. Mwitari et al., 2013]. The bioactive principle of *Withania somnifera* such as withanolide D, E and F also possess inhibitory activity against *Mycobacterium tuberculosis* PknG, which is a protein kinase that regulates key metabolic processes within the bacterial cell [N. Santhi and S. Aishwarya, 2011].

2.1.5.9. Hypoglycaemic and hypolipidemic activity: In the traditional and Ayurvedic medicine system *Withania somnifera* is commonly used to cure diabetes and obesity. Furthermore preclinical and clinical studies support the traditional claims for the use of *Withania somnifera* against diabetes and obesity. *Withania somnifera* significantly reduces the blood glucose and lipid levels [U. Rajangam et al., 2009] via stimulating insulin secretion

from pancreatic β-cells. Other possible reported mechanisms are increased transportation of glucose into the cells, increased insulin release and increased GLUT transporters activity [T. Anwer et al., 2008; M. Khalili, 2009; A. Sarangi et al., 2013; N.P. Visavadiya and A.V. Narasimhacharya, 2007]. In another experimental study *Withania somnifera have significantly* increased plasma HDL-cholesterol level and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity and bile acid content of liver [N.P. Visavadiya and A.V. Narasimhacharya, 2007].

2.1.6. Toxicology of *Withania somnifera*

Numerous studies and the statistics obtained from various clinical and toxicological research on *Withania somnifera* was established that the plant is non-toxic in a wide range of applied doses and it can be assumed that the doses in which its formulations are specified both in humans and in animals are likely to be very safe [G. Kumar et al., 2015; S.B. Patel et al., 2016]. As of today, no herb-herb or herb–drug interactions have been observed with *Withania somnifera* [S.K. Kulkarni and A. Dhir, 2008; P.C. Prabu et al., 2013; A.C. Sharada et al., 1993; J. Savai et al., 2013]. The common side effects and the long-term safety of *Withania somnifera* are still under consideration. However, very large doses of *Withania somnifera* might cause stomach discomfort, diarrhoea, nausea and vomiting. On the basis of limited clinical research it was reported that *Withania somnifera* might cause calmness, low blood pressure, respiratory depression and might cause abnormal heart rhythms [Z. Gardner and M. McGuffin, 2013; H.S. Puri, 2003].

Information available on the pharmacology of diverse types of *Withania somnifera* extracts and formulations obtained from different parts of the plant have strongly suggested that appropriate combinations with other plants could be used for prevention of diverse side effects those are commonly observed with *Withania somnifera* [V. Kumar et al., 2015a].

2.2. Adaptogens

The term adaptogen was first coined in the year 1947 by the Russian scientist N.V. Lazarev, after his discovery of the potential role of Dibazol (2-Benzyl-benzimidazol) as an adaptogenic agent in experiments designed to stimulate non-specific resistance in humans [N.V. Lazarev, 1947; H. Wagner et al., 1994]. In the year 1958, the term adaptogen was introduced into scientific literature to indicate substances that increase the state of nonspecific resistance during stress situations, basis the Hans Selye's theory of stress and the general adaptation syndrome [A.G. Panossian, 2013]. Adaptogens are the biologically active substances obtained from plant sources, which increases the state of non-specific resistance in stress conditions and improve physical endurance.

Stress has been postulated to be involved in the development of variety of diseases, including psychiatric disorders such as depression and anxiety etc., and endocrine disorders (including diabetes mellitus, immunosuppression, cognitive dysfunctions, hypertension, peptic ulcer, male sexual dysfunction and ulcerative colitis) [S.K. Bhattacharya and A.V. Muruganandam, 2003; G.R. Elliott and C. Eisdorfer, 1982].

Numerous studies have shown that chronic unpredictable stress can induce oxidative stress, glucose intolerance, increase in plasma corticosterone levels and significantly disrupts monoamine levels in different brain areas [S.K. Bhattacharya, et al., 2000; S.K. Bhattacharya and A.V. Muruganandam, 2003; A.V. Muruganandam et al., 2002; A. Bhattacharya et al., 2002; A. Bhattacharya et al., 2001]. Although benzodiazepine therapy has significant anti-stress or anxiolytic effect on acute stress conditions [G.R. Elliott and C. Eisdorfer, 1982], issues related to the development of long term drug dependencies and tolerance or failure of therapy against chronic stress induced adverse effect on psychiatric disorders are still remain unanswered. Additionally, benzodiazepines have adverse effects during pregnancy and on the newborn during lactation [A.J. Trevor and W.L. Way, 2001]. Giving importance to the

stressful situations, especially when the individual can't adapt to the metabolic and/or environmental stressor (often leading to increased incidences of stress-related physical and mental health problems), it seems reasonable that practitioners of traditionally known systems of medicine have often prescribed herbal adaptogens with stress response regulating activities. Earlier in the 1950–60, herbal medicinal plants were also used to increase stamina and survival in harmful environment conditions [G. Ahmad et al., 1998]. Now, various herbal drugs have been studied for their adaptogenic activity during stressful events. Efforts are made to evaluate the phytochemistry, cellular and molecular mechanisms of stress protective activity. Efficacy and safety of adaptogens in stress related disorders are also studied in preclinical and clinical level. Various experimental studies have shown interesting findings with adaptogenic herbs, and suggested that, adaptogens can increases the nonspecific resistance against stress both *in vitro* and *in vivo* studies [F.A. Wiegant et al., 2009; F.A.C. Wiegant et al., 2008; M. Jafari et al., 2007; S.E. Schriner et al., 2009].

In light of recent research, adaptogens are redefined as "metabolic regulators of natural origin which increase the capacity of a living organism to adjust to ecological stressors and counteract harm to the living being by such stressors" [A. Panossian et al., 1999]. Botanists refer to adaptogens as rejuvenating herbs, tonics, rasayanas or restoratives because they believed to help the body return to a balanced state [A.G. Panossian, 2013]. Adaptogenic agents increase nonspecific resistance or develop tolerance towards stress and reduce exhaustion through effects on mediators of stress response, homeostasis, energy metabolism and the neuroendocrineimmune system [A.G. Panossian, 2013]. Adaptogenic herbs have ability to make homeostasis in endocrine hormones and immune system. The endocrine system hypothalamic-pituitary-adrenal axis has been demonstrated a key role in the pathophysiology of stress. Adaptogens possesses beneficial stress-protective activity by regulating hypothalamic-pituitary-adrenal axis involving stress hormone cortcosterone,

neuropeptide Y [A. Panossian et al., 2012] and key mediators of the stress response such as nitric oxide, membrane bound G-protein receptors, molecular chaperons; Heat shock proteins Hsp70 and Hsp72 [A. Panossian et al., 2012], which are directly involved in stress-induced cytoprotection [A.G. Panossian, 2013; A. Panossian et al., 2009; P.A. Prodius et al., 1997]. On the cellular level, adaptogens modulate gene expression (transcriptional control of metabolic regulation) of key mediators of intracellular communications involved in stress-induced signal transduction pathways, including G-protein signaling cyclic adenosine monophosphate (cAMP)-mediated pathway [A. Panossian et al., 2013], G-protein signaling phosphatidylinositol, phospholipase C pathways [A. Panossian et al., 2013], stress activated protein kinase JNK (MAPK-9) [A. Panossian et al., 2009], heat shock factor 1 (HSF-1) [A. Panossian et al., 2012], cortisol and glucocorticoid receptors [A. Panossian et al., 2007], beta-endorphin [IuB. Lishmanov et al., 1997], nitric oxide [A. Panossian et al., 2007] and biosynthesis of energy source such as ATP [A. Panossian et al., 2009]. Adaptogens have apparent stimulating and tonic activity due to their stress-protective activity. The CNS stimulating and tonic effects of adaptogens have been reported by various research teams [A. Panossian and H. Wagner, 2005]. However, adaptogens do not possess tolerance and addiction or substance abuse potentials and they do not impair normal mental activity in long term use as compared to conventional CNS stimulants such as ephedrine, fenfluramine and phentermine [A. Panossian and G. Wikman 2010]. In addition to therapeutic effects in stress-induced disorders, adaptogens have shown positive results as an adjuvent therapy on the quality of life of patients, receiving standard therapy of many chronic diseases and pathological conditions such as post-surgery recovery, asthenia, and congestive heart failure. Adaptogens may be also used in age related disorders, such as neurodegenerative diseases, and cardiovascular diseases [A. Panossian and G. Wikman 2010]. Although numerous researches have established the importance of adaptogens in combating stress disorders, the

adaptogen has not yet been accepted in medicine. This is probably due to the difficulties in discriminating adaptogenic drugs from immunostimulators, anabolic drugs, nootropic drugs, and tonics. There can be no question, in any case, that, at any rate in animal experiments, there are plant derived drugs equipped for balancing particular periods of the adaptation syndrome as defined by Hans Seyle. These drugs reduce stress reactions in the alarm phase or retard / prevent the exhaustion phase and thus provide a certain degree of protection against long-term stress.

2.3. Stress

Daily stress and recurrent nerve-racking events over the life course will take a big physiological toll on the body [B.S. McEwen and E. Stellar, 1993]. The stress response in itself doesn't cause adverse effects on health; it really protects the body from harmful consequences. On the other hand, whenever the strain response is activated, physiological adjustments should be created and over time these adjustments lead to accumulated wear and tear [S. Read and E. Grundy, 2012]. Stress is a common experience in our everyday lives that is blamed for causing many health problems [G.P. Chrousos and P.W. Gold, 1992] and yet we have very little idea how stressful experiences may lead to disease. According to B.S. McEwen concepts, stress is very subjective and doesn't take into consideration the huge individual variations that exist in managing the environment. There are various aspects of everyday life that may not qualify as stress but yet may have adverse effects on the body; however, it is difficult to quantify the physiological responses of these daily troubles except those accumulated and result in a physiological change or a disease. Additionally, we do not fully aware the setup of the body systems that promote adaptation through their ability to respond towards stressful environments, because these systems can also cause harm under extreme conditions [B.S. McEwen, 1998]. The body systems, such as autonomic nervous

system and hypothalamic–pituitary–adrenal axis or a number of other systems promote adaptation by producing hormonal and neurotransmitter mediators, a process called "allostasis" [P. Sterling and J. Eyer, 1988]. Sometimes these systems that promote stability, or homeostasis, can also cause problems for the body if they are overactive or underactive [B.S. McEwen and E. Stellar, 1993; B.L. Ganzel et al., 2010]. Thus, the method of physiological stability or allostasis typically incorporates a price; and that we have referred to as the worth of adaptation that promotes pathophysiology "allostatic load" [B.S. McEwen and E. Stellar, 1993; B.S. McEwen, 1998]. Allostatic load results once the allostatic systems are either overworked or fail to shut off when the stressful event is over or once these systems fail to respond adequately to the initial stress, leading alternative systems to overreact [B.S. McEwen, 1998]. During stressful conditions, the maintenance of life is critically dependent on keeping our internal milieu stable in the face of changing environmental conditions (homeostasis). Hens Selye (1956) used the term 'stress' to denote anything that extremely threatens the homeostasis. The actual cause of threat is called "stressor" and the response against this stressor is referred as 'stress response.' Humans and other animals invoke integrated coping responses against the perceived threat via central nervous system (CNS) [R.S. Lazarus and S. Folkman, 1984; S.M. Hilton, 1975]. In response to an internal or external stressor such as fight-or-flight situation, there are increased autonomic and hormonal responses which maximize the possibilities for muscular exertion [W.B. Cannon, 1929, W.R. Hess, 1957], leading to a harmonization of physiological responses to stress circumstances. The pattern of stress response to stressor may vary according to type of stressor and individuals. In some individuals the stress response is associated with active coping, while others tend to show aversive vigilance responses [A.L. Kasprowicz et al., 1990]. There are huge individual differences in how people react to potentially stressful situations, and these depends on mainly two factors, firstly how the individual perceives and interprets the

situation – if it is a threat, and secondly individual differences concern the physical condition of the body itself. Moreover, metabolic imbalances that leads to diabetes and obesity can increase the vulnerability of an individual to stress, and these may have a genetic component [B.S. McEwen, 1998]. Genetic inheritance also plays an important role in determining individual differences in stress response, and early life stress has been also reported to produce long-term effects in cognitive-emotional responses [S. Levine 1957; M.J. Meaney et al., 1993] and it was reported that, the effect of early life nutrition on serotonin level in rats, rats grown by fostering mothers have increased levels of central serotonin activity as compared to lack of fostering mothers.

2.4. Depression

In the past two decades, there has been increasing recognition of patients with depression which affects hundreds of millions of people all over the world. Depression is a psychopathological state, manifested by low or depressed mood, anhedonia or loss of interest, sleep and psychomotor disturbances, feelings of guilt, low self-esteem, suicidal tendencies, and autonomic and gastrointestinal disturbances. Depression is highly prevalent disorders with a lifetime prevalence of 20% in the general population worldwide and female are more prone to depression than male. The female to male prevalence ratio is of about 5:2 [B. Brigitta, 2002]. Typically, depressive symptoms are recurrent and most patients recover from major depressive episodes [M.M. Weissman et al., 1996]. Suicidal ideation and attempts are common in chronic case of depression which is a considerable risk for mortality in depressive patients. The rate of suicide is among young age group of 15 to 24 years [M.L. Wong and J. Licinio, 2001]. The coexistence of anxiety, especially panic disorder, makes depression more morbid and fatal [B. Brigitta, 2002]. The influence of stressful environment and adverse life events on the development of depression has been investigated by various preclinical studies

[E.S. Paykel 2001]. According to monoamine hypothesis of depression, a functional deficiency of the brain monoaminergic neurotransmitters such as norepinephrine (NE), 5-hydroxytryptamine (5-HT) and dopamine (DA), which are responsible for depressive symptoms [J.J. Schildkraut, 1965; A. Coppen 1967]. Depression is also highly prevalent comorbid disease with diabetes and cardiovascular disease. The low rate of diagnosis of depression is one of the biggest challenges in management of depressive disorders among those suffering from diabetes. In diabetic patients 45% of the cases of mental disorder remain undiagnosed [C. Li et al., 2010]. It has also been reported that occurrence of depression in diabetics is higher than that of normal population [R.J. Anderson et al., 2001]. Several neurochemical alterations are observed in the CNS of diabetic patients and diverse such behavioural and biochemical variations are also observed with diabetes rodent [L.A. Hilakivi-Clarke et al., 1990; N.E. Rowland and L.L. Bellush, 1989].

The availability and use of antidepressant drugs has expanded greatly, but there are several undesirable adverse effects which limit their application. The first-generation antidepressant drugs, tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs), are widely used antidepressants which increase the concentrations of 5-HT and NE. In addition, due to undesirable side effects of TCAs and MAOIs such as sedation, hypotension, blurred vision, dry mouth, and hypertension limit their use in depression [J.P. Feighner, 1999; A.H. Glassman and P.A. Shapiro, 1998].

However, introduction of newer antidepressants such as selective serotoninreuptake inhibitor (SSRI), selective NE-reuptake inhibitor and both 5-HT and NE reuptake inhibitor have shown good efficacy and safety in depressed patients [J.P. Feighner, 1999]. These newer antidepressants are safer as compare to TCA and the incidence of severe adverse effects were certainly abridged, but failure of these drugs in the treatment of resistant types of depression is again one limitation [B. Brigitta, 2002]. The development of new pharmacological

intervention having diverse pharmacological action and safety is needed for treatment of severe depressive symptoms as well comorbid depression associated with other disorders. However, the classic antidepressant having efficacy and good safety of margin, well tolerated and contribute to the overall health of the patient remains to be discovered.

2.5. Anxiety

Anxiety is manifested by various behavioural and physiological responses such as vigilance, avoidance and arousal, which advanced to protect the individual from threat [R.C. Kessler et al., 1994]. Despite their high prevalence and comorbidity anxiety has not received the same recognition as other major syndromes such as mood and psychotic disorders. Although, the primary care physicians are involved in the assessment and management of anxiety which may results in less visibility than schizophrenia, depression and bipolar disorder [B. Roy et al., 2005; M. B. Stein et al., 2004]. Advances in psychological disorders research including anxiety over the previous decade are likely to be reflected in modifications of diagnostic measures.

Neural circuitry of HPA axis, sympathetic sympathetic nervous system and hippocampus are involves in the pathophysiology of anxiety [R.B. Jeffrey and J. Schulkin 1998]. In addition, chronic anxiety can lead to overactivation of the limbic system, which may increase future anxiety [F. Gregory, 2011]. Genetics and family history may also included in etiology of anxiety, recent study reported the role of genetic differences in the variance of psychological disorders [O.J. Bienvenu et al., 2011]. The genetic difference may influence neurotransmitters such as serotonin and norepinephrine and stress hormones (cortisol), which play an important role in anxiety [A.D. Moser et al., 2015]. Anxiety is also frequently caused other health problems such as asthma or chronic obstructive pulmonary disease (COPD),

heart diseases, nurodegenarative diseases like Parkinson's disease, multiple sclerosis, diabetes and stroke [American Psychiatric Association, 2008].

Anxiety has also been associated with metabolic disorders such as diabetes mellitus. According to a survey report it was found that as compared to general population, the rates of anxiety behavour are higher (approximately 40 %) in diabetic patients [A.B. Grigsby, 2002]. In addition, clinical reports have clearly point out that, occurrence of anxiety disorders in diabetic individual is higher than the normal population, evidences have clearly revealed that there is a strong relationship between diabetes and anxiety disorder [M. Clavijo et al., 2006].

Pharmacological interventions for anxiety disorders are safer and more tolerable as compared to the past 30 years. But on the other hand, treatment efficacy and duration is not improved which emerges the need of novel pharmacological interventions. Benzodiazepines (BZDs) are the first choice of drug for the treatment of anxiety disorders. However, long-term side effects such as development of tolerance, substance dependence, withdrawal symptoms and cognitive impairment are always associated with BZDs, which leads to poor therapeutic outcomes [L. A. Papp et al., 2010]. Currently, anxiety disorders are managed by antidepressant such as tricyclic antidepressant (TCA), selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) and MAO inhibitors (MAOIs), alpha–delta calcium channel anticonvulsants, beta blockers and azapirones [F. J. Farach et al., 2012].