Chapter 2
Phytopharmacology of Ashwagandha as an Anti-Diabetic Herb

Vikas Kumar, Amitabha Dey, and Shyam Sunder Chatterjee

Abstract Ashwagandha (*Withania somnifera*) extracts and several pharmaceutical formulations containing them are currently often used as tonics useful for prevention and cure of mental health problems, including sleep disturbances, accompanying or caused by diverse slowly progressing chronic diseases. The possibility that *W. somnifera* could be used for treatments of diabetes and associated metabolic disturbances were first suggested by the results of an exploratory clinical study conducted with its root powdered in diabetic patients and published in 2000. Since then, numerous preclinical and a randomized, double blind and placebo controlled clinical study with extracts of the plant have continued to add experimental evidences in favor of the convictions of the scholars and practitioners of Ayurvedic and other traditionally known systems of medicine that the plant could also be used for prevention and cure of diabetes and other metabolic disorders associated physical and mental health problems. Currently available information suggesting such possibilities are summarized and critically analyzed in this chapter. Potential uses of our current knowledge on phytopharmaclogy and medicinal phytochemistry of the plant and its bioactive constituents for obtaining more sustainable and reproducible health benefits from the plant in patients suffering from, or at risk to, metabolic disorders associated mental health problems, or for discovering novel therapeutic lead against such health problems of the twenty-first century are also discussed.

Keywords *Withania somnifera* • Hyperglycemia • Diabetic complications • Oxidative stress • Psychosomatic disorders • Multi-targeted therapy

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2.1 Introduction

Diabetes is a slowly and silently progressing metabolic disorder, which ultimately leads to structural and functional deteriorations of almost all bodily organs including those of circulating fluids and central and peripheral nervous systems. It is one of the major health problems now affecting almost all countries, irrespective of their socioeconomic, cultural, and genetic backgrounds (Shaw et al. 2010). According to recent estimates, more than 85% of diabetic patients live in India, China and others developing countries where herbal remedies and advices of the practitioners of traditionally known systems of medicine still continue to be the only health care and therapeutic possibilities for a vast majority of their population. Most probably, prevalence of diabetes in those countries is also continuing to increase during the next two decades (Guariguata et al. 2014).

Co-morbidities of anxiety, depression and other mental health problems are more often encountered in diabetic patients than in normal population (Mendenhall et al. 2014; Balhara and Sagar 2011; Snoek et al. 2015; Petrak et al. 2015; Bajor et al. 2015), and it is now well recognized that serious psychological distress has a significant negative impact on the initiation and progression of diabetes and associated comorbidities (Egede and Dismuke 2012; Egede et al. 2014). However, cardiovascular diseases are the leading causes of death and other health problems of patients suffering from, or at risk to, type 2 diabetes. While lowering blood glucose levels in such patients have major beneficial effects on microvasculature complications, as yet no very definitive statements on beneficial effects of available psychoactive drugs and other treatments on mental health problems and cardiovascular outcome are possible (Huqi 2015; McMurray et al. 2014).

The most ancient text of traditionally known system of medicine, i.e. Ayurveda, mention that metabolic abnormalities leading to sweet tasting urine are associated with abnormal body weight changes and sedentary behaviour (Sharma and Chandola 2011a, b; Puranik and Patwardhan 2012). The Ayurvedic concept that proper choices of food and eating habits in combination with physical and mental exercises and medicine is useful for prevention and cure of such disorders, now referred to as diabetes or diabesity, have now been well accepted by all other traditional known or modern systems of medicine (Ahmed 2002; Henschen 1969; White 2014; Zajac et al. 2010; Zhang et al. 2010). Specific combinations of diverse edible and psychoactive plants with anti-stress or adaptogenic properties are often used as therapeutics in Ayurvedic and other traditionally known systems of medicine for prevention and cure of physical and mental health problems accompanying almost all chronic diseases. Amongst them, *W. somnifera* (Ashwagandha, or Winter cherry) is one of the clinically and preclinically more extensively scrutinized ones now also attracting considerable attention of modern drug designers. It is one of the few plants of the *Withania* genus of flowering plants of the Solanacea family with 23 species that are native to many Afro-Asian countries (Mirjalili et al. 2009). The two other medicinally used ones in India and other Afro-asiatic countries are *W. coagulans*

and *W. obtusifolia* (Kumar et al. 2011; Alali et al. 2014), some scattered reports on their beneficial effects against diabetes have also appeared during more recent years.

A group of naturally occurring poly-oxygenated C-28 estrogen-type steroids commonly known as Withanolides (Misico et al. 2011; Singh et al. 2010; Choudhary and Yousuf 2013) are chemotaxonomic markers of the plants of the *Withania* genus (Alali et al. 2014; Chen et al. 2011; Zhang et al. 2014; Samadi 2015). Since anti-hyperglycemic and diverse other therapeutically interesting bioactivities of structurally diverse Withanolides have been reported (Gorelick et al. 2015; Khodaei et al. 2012), they are now often considered to be their quantitatively major bioactive secondary metabolites. It cannot be ignored though, that numerous other extractable secondary metabolites of *W. somnifera* with Withanolides like bioactivity profiles in preclinical models are now known also (Singh et al. 2001, 2003; Wadhwa et al. 2013). Therefore, it is apparent that like for all other medicinal plant extracts, bioactivity profiles or therapeutic benefits of *W. somnifera* extracts do not also depend entirely on their contents of Withanolides, or any other chemotaxonomic marker of the plant, only. Since our current knowledge on biological interactions between the structurally diverse extractable bioactive constituent of the plant still remain far from being satisfactory, as yet no very definitive statements on therapeutic potentials of diverse types of medicinally used *W. somnifera* extracts, or on their bioactive constituents involved in their such potentials, can yet be made.

Critical analysis of currently available preclinical and clinical information on such potentials of *W. somnifera* extracts and their quantitatively major bioactive constituents strongly suggest though, that appropriately processed and standardized extracts of the plant and several bioactive metabolite of the plant could be useful therapeutic leads against diabetes mellitus and associated co-morbidities. Therefore, efforts are now being made in several laboratories, including ours, to better define the medicinal phytochemistry and phytopharmaclogy of the plant. Currently available preclinical and clinical information on such extracts and their bioactive constituents potentially involved in their glucose and insulin homeostasis regulating effects are summarized and critically analyzed in this chapter.

2.2 Why Should *Withania somnifera* Be Considered as an Anti-Diabetic Plant?

Together with sedentary behaviour, malnutrition (both over and under-nutrition) is a major risk factor for diabetes mellitus (Zimmet et al. 2014; Schmidt and Duncan 2003), defined as a disease characterized by hyperglycemia and secretion and excretion of excessive amount of urine. Available therapeutic options for prevention and cure of diabetes (Colagiuri 2010) either do not meet the therapeutic demands of patients, or are not affordable or available to a vast majority of global population. Diabesity, *i.e.* obesity associated type-2 diabetes, is the spreading epidemic of the twenty-first century and is also major economic burden in many countries including

USA and other economically developed ones (Farag and Gaballa 2010). Amongst all currently available antidiabetic drugs, metformin is the only one highly recommended for prevention and cure of type-2 diabetes, which is the most prevalent type (ca. 90%) encountered amongst all diabetic patients (Liu et al. 2010; Anwer et al. 2008). The development of type-2 diabetes begins with an impairment of glucose tolerance and insulin resistance, i.e. a condition when the cells in the body are unable to use insulin secreted from the β-pancreatic and other cells, and which eventually leads to hyperglycemia and hyperinsulinemia (Anwer et al. 2008; Zimmet and Thomas 2003; Robertson and Harmon 2006). The symptoms of diabetes include high blood sugar, polydypsia, polyuria, unusual thirst, polyphagia, weakness and tiredness, intense hunger and sudden weight loss. In addition to high blood sugar, other factors including dyslipidemia or hyperlipidemia also play important roles in the development of micro and macro-vascular complications of Type-2 diabetes, which ultimately leads to cardiovascular disorders and other co-morbidities and death (Tiwari et al. 2014). Cognitive deficits and diverse spectrums of mental health problems, including anxiety and depression, are more often encountered in diabetic patients than in normal population, and it is now well documented that serious psychological suffering has a major negative impact on diabetes process and outcome (Thakur et al. 2015; Balhara and Sagar 2011; Sarkar and Balhara 2014).

Like for numerous other lifestyles associated medical conditions, sleep disturbances has now also been well recognized to be another major risk factor for diabetes development (Anothaisintawee et al. 2015; Kowall et al. 2016). Changes in diet, physical activity, body eight, and obesity (as judged by body mass index) do not seem to be effective in decreasing diabesity risk of sleep disturbances (Cespedes et al. 2016; Lee et al. 2016). Due to their diabetogenic and obesogenic effects, numerous currently available psychoactive and sleep inducing or tranquilizing drugs are often contraindicated in patients suffering from or at risk to diabetes (Flanagan 2008). Environmental and metabolic stress triggered anxiety and depression are the root causes of sleep disturbances (Spoormaker and van den Bout 2005; Leroith and Vinik 2008), and *W. somnifera* has since long been well recognized to be a sleep inducing plant without sedative or hypnotic effects (Archana et al. 2015; Pingali et al. 2013; Choudhary et al. 2015). It has recently been pointed out indeed, that while most herbal adaptogens possess stimulating effects, *W. somnifera* is a calming adaptogenic herb (Winston and Maimes 2007). Numerous preclinical and some clinical studies have consistently revealed and reconfirmed beneficial effects of diverse types of *W. somnifera* extracts against stress triggered psychopathologies leading to sleep disturbance (Chandrasekhar et al. 2012; Ashok and Shende 2015; Archana et al. 2015; Pratte et al. 2014; Dar et al. 2015; Wadhwa et al. 2015). Therefore, *W. somnifera* seems to be a particularly well-suited adaptogenic herb for prevention and cure of sleep disturbances triggered metabolic disorders like diabetes and diabesity.

2.3 Pathophysiology of Diabetes Comorbidities

It has since long been well recognized that diabetes is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia (Anderson and Auslander 1980). Insulin is the principle hormone that regulates the uptake of glucose from the blood into most other cells. Therefore, deficiency of insulin or insensitivity of its receptors plays a crucial role in all forms of diabetes. The body can obtains glucose either from food, digested and processed inside the gastrointestinal tract, or by breakdown of from stored glucose precursor glycogen or from gluconeogenesis i.e., the generation of glucose from non-carbohydrate substrates in the body. Glucose is used by about two thirds of the body's cells for maintaining their energy balance and for conversion of glucose to other needed molecules or for storage and other functions.

Lower glucose levels results in decreased insulin release from pancreatic cells and in breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in opposite manner to insulin (Gardner and Shoback 2011). When the glucose concentration in blood remains high for a long time, the kidney reach a threshold of glucose reabsorption, and glucose is excreted in the urine causing glucosuria. This increases the osmotic pressure of the urine and inhibits water reabsorption by kidney, resulting in increased urine production (polyuria) and increased fluid loss. Resulting loss of blood volume is then replaced osmotically from water held in the body cells and other body compartments, causing dehydration and increased thirst.

Apart from insulin and glucagon, numerous other hormones of the gastrointestinal tract are also involved in pathogenesis of obesity and type-2 diabetes (Vincent et al. 2008; Adamska et al. 2014; Thomas et al. 2003; Scheen and Paquot 2015). It is now becoming increasingly apparent that gut microbial ecology also plays a crucial role in regulating the homeostasis of gastrointestinal hormones, and that dysbiosis or dysbacteriosis inside the gastrointestinal tracts often leads to numerous chronic diseases, including diabetes and obesity (Nicholson et al. 2012; Miele et al. 2015; Durg et al. 2015; Choudhury et al. 2016). Diverse gastrointestinal hormones are encountered or even biosynthesized inside the brain, and that they are also involved in the pathogenesis and progression of diabesity associated co-morbid psychopathologies (Torres-Fuentes et al. 2015; Thakur et al. 2014). Since like structurally diverse plant derived molecules and other products, *W. somnifera* extracts and substances derived from them also possess bactericidal activities (Kapoor et al. 2015; Singh and Kumar 2011; Girish et al. 2006), it could as well be that modulation of gut microbial ecology are also involved in broad spectrums of beneficial effects of the plant observed in diabetic patients and animals. Efforts to verify this possibility could not only be useful for better understanding of therapeutic potential of the plant, but also for obtaining novel therapeutic leads against diabesity and associated co-morbidities.

2.4 Preclinical Pharmacology of *Withania somnifera*

The very first reports dealing with preclinical pharmacology of *W. somnifera* started appearing in English language journals during 1980s, and dealt mainly with adaptogenic or stress response regulating effects of the herb. Since several observations have revealed Ginseng like effects of the plant in diverse animal models, it is now often referred to as "Indian ginseng" (Kulkarni and Dhir 2008). It was not until the year 2000 when the very first reports on beneficial effects of the plant in diabetic patients had appeared (Andallu and Radhika 2000). This clinical trial was conducted to corroborate an earlier preclinical finding made with an Ayurvedic formulation of the plant (Trasina) in diabetic rats (Bhattacharya et al. 1997a). Currently available experimental evidences reaffirming that *W. somnifera* is indeed another anti-diabetic, or anti-hyperglycemic, or glucose homeostasis regulating plant are summarized in the following. For more detailed currently available preclinical and clinical information on neuro-psycho-pharmacology (Durg et al. 2015), and other therapeutically interesting bioactivities of the plant, more recent reviews mentioned before and other chapters of this book has to be consulted.

2.4.1 Anti-Hyperglycemic Activities in Rodent Models

Prevention of diabetes is the primary goal of almost all health care authorities around the globe (WHO 2006). The biomarker or diagnostic criteria now regularly used in all epidemiological or clinical studies for identifying patients prone to, or suffering from, diabetes are blood glucose responses to meals and to diverse versions of glucose-tolerance and insulin sensitivity tests in relation to body weights or body mass index of a person (Bentley-Lewis et al. 2008; Marrero et al. 2014; Hostalek et al. 2015). Several reports on beneficial effects on body weight changes, blood glucose and insulin levels, and glucose or insulin tolerance in stressed, or obese, or diabetic rodents have appeared during the past decade. Some of the most cited and more recent reports revealed such effects of diverse types of *W. somnifera* extracts are summarized in Table 2.1. One such report dealing with effects of *W. somnifera* extracts in alloxan-induced diabetic rats attributed the observed effects of the extract to the flavonoids encountered in roots of the plant, and suggested that this is due to their ability to stimulate insulin release from pancreatic cells (Udayakumar et al. 2009). Such and numerous other *W. somnifera* extracts like beneficial effects of pure flavonoids and their metabolites have often been reported (Dall'Asta et al. 2015). It cannot be ignored though, that numerous other phytochemicals and other substances capable of modulating glucose homeostasis and with antidiabetic activity in animal models are also encountered in such extracts, and that pancreatic cells are not the only insulin producing and secreting cells involved in etiology and pathogenesis diabetes (Kojima et al. 2006; Lehner et al. 2012).

Table 2.1 Some often cited and more recent reports on anti-diabetic (anti-hyperglycemic) potential of *Withania somnifera*

Part of plant	Type of extract/formulation	Dose, duration and route of administration	Mechanism of action	References
Leaves and roots	Ethanolic	100 and 200 mg/kg/day, for 56 days, p.o.	Increased insulin secretion from pancreatic β cells	Udayakumar et al. (2009)
Roots	Aqueous	200 and 400 mg/kg/day, for 35 days, p.o.	Increased insulin sensitivity and inhibited insulin resistance	Anwer et al. (2008)
Leaves and roots	Ethanolic	200 mg/kg/day, for 56 days, p.o.	Insulin mimetic and increased insulin secretion from pancreatic β cells	Sarangi et al. (2013)
Leaves	Ethanolic	1 ml/rat/day (30 and 200% concentration), for 10 days, i.p.	Increased hepatic metabolism of glucose and increased insulin secretion	Navinder et al. (2013)
Roots	Dried powder	3 g/day (500 mg/capsule) for 30 days, p.o.	Increased serum level of insulin, decreased serum level of lipids	Andallu and Radhika (2000)
Leaves and roots	Methanolic	100 μg/ml in cellular model of diabetes	Increased insulin secretion and increased insulin sensitivity	Gorelick et al. (2015)
Roots	Aqueous	200 and 400 mg/kg/day for 35 days, p.o.	Increased glucose metabolism, increased insulin sensitivity	Safhi and Anwer (2011)
Roots	Hydromethanolic	25, 50 and 100 mg/kg/day for 10 days, p.o.	Insulin mimetic and anti-oxidant action	Thakur et al. (2015)
Roots	Ethanolic	1.4 g/kg/day for 15 days, p.o.	Increased insulin secretion and insulin sensitivity	Jatwa and Kar (2009)
Root	Dried powder	5, 10, 20 mg/kg, p.o. for in vivo assay and 0.3, 0.6, 0.9, 1.2 and 1.5 mg/ml for in vitro assay	Increased transport f glucose, increased insulin sensitivity and inhibition of α- amylase enzyme	Nirupama et al. (2014)
Roots	Acetone	20 mg/kg/day for 1 month, p.o.	Antioxidant action and increased insulin sensitivity	Parihar et al. (2004)

(continued)

Table 2.1 (continued)

Part of plant	Type of extract/formulation	Dose, duration and route of administration	Mechanism of action	References
Leaves	Aqueous and methanolic	1 ml of extract used for in vitro assay	Inhibition of α- amylase enzyme	Prabhakar et al. (2013)
Whole plant	Methanolic	Withanolides (IC_{50}–38.20 µg/ml) isolated were used for in vitro assay	Inhibition of α-glucosidase enzyme	Khan et al. (2014)
Roots	Aqueous	0.58 mg/ml for in vitro assay	Inhibition of both α- amylase and α-glucosidase enzyme	Balaji et al. (2015)
Roots	Standardized extract	500 mg/kg/day for 15 days, p.o.	Antioxidant action and increased insulin secretion	Kyathanahalli et al. (2014)
Roots	Aqueous	200 and 400 mg/kg/day for 35 days, p.o.	Antioxidant action and protected pancreatic β cells from oxidative damage	Anwer et al. (2012)
Roots	Hydoalcoholic	25 and 50 mg/kg/day for 21 days, p.o.	Antioxidant and adaptogenic action	Bhattacharya and Muruganandam (2003)
Fruits of *W. coagulans*	Aqueous and choroform	1 g/kg/day for 14 days, p.o.	Hypoglycemic, antioxidant and immunomodulation activities	Hoda et al. (2010)
Roots	Powder	62.5 mg/g WS powder with standard pellet diet for 56 days	Increased insulin sensitivity and decreased insulin resistance	Samadi Noshahr et al. (2015)
Roots and leaves	Ethanolic	100 mg/kg/day for 56 days, p.o.	Antioxidant action, and rejuvenation of β-cells leading to increased insulin production and secretion	Udayakumar et al. (2010)
Roots	Ethanolic	1.4 g/kg/day for 15 days, p.o.	Reduced oxidative stress and increased glucose metabolism	Jatwa and Kar (2009)
Trasina (Ayurvedic formulation)	Powder containing *W. somnifera*	100 and 200 mg/kg/day for 28 days, p.o.	Anti-oxidant action and increased insulin sensitivity	Bhattacharya et al. (1997a)

Extra-pancreatic insulin production and secretion have been observed in streptozotocin-induced diabetic rats (Cunha et al. 2007), and antidiabetic activity of repeated daily treatments with *W. somnifera* extracts have also been reported in such animals as well (Sarangi et al. 2013; Safhi and Anwer, 2011). In one of the reports dealing with anti-diabetic activity of *W. somnifera* (Anwer et al. 2008), no effects of even 400 mg/kg daily oral doses of an extract on blood glucose and glycosylated haemoglobin (HbA1C) levels, or in glucose and insulin tolerance tests, or on hepatic insulin resistance, were observed, whereas 200 mg/kg daily doses of the same extract was effective in affording protections against all these altered parameters in diabetic rats. Others using other animal models for hyperglycemia and in non-diabetic animals have also reported analogous observations. Such observations strongly suggest that beneficial effects of *W. somnifera* in diabetic rodents is not necessarily due to its effects on insulin secretion, and that its observed bioactivity profile in normal animals is not very predictive for its potential therapeutic benefits observed in diabetic and other animals with disturbed glucose homeostasis.

The alternative possibility that stimulatory and enhancing effects of Withanolides on glucose transporters and cellular glucose transport are involved in anti-diabetic activity of the plant is suggested by the observations made in some cellular as well as in animal models (Gorelick et al. 2015; Kumar et al. 2015a, b; Safhi and Anwar 2011). Plant metabolites structurally analogous to Withanolides (Coagunolides) encountered in *W. coagulans* have also been reported to be effective in suppressing postprandial hyperglycemia and anti-hyperglycemic activity in streptozotocin diabetic rats (Maurya et al. 2008). Since diverse steroidal lactones are encountered in varying concentrations in roots and leaves of *W. somnifera*, and antidiabetic activities of diverse parts of this and other plants of *Withania* family has been reported, it seems logical that they are also involved in traditionally known medicinal uses in diabetic patients. However, since several other fairly common plant metabolites like fumaric, succinic, 4-hydroxybenzoic, nicotinic and several other organic acids and other bioactive molecules with antidiabetic and other glucose homeostasis influencing bio-activities are also found in *W. somnifera* (Chatterjee et al. 2010), they could also be involved in observed effects of its crude extracts and root powders in animal models and clinical trials. Moreover, since depending on cultivations and extraction procedure used, the relative contents of Withanolides and other bioactive phytochemical in different parts and types of *W. somnifera* extracts vary considerably (Bharti et al. 2011; Dhanani et al. 2017; Fernando et al. 2013), observations made with one type of extract in a study cannot be generalized for all types of extracts.

In any case, there is now more than sufficient preclinical evidences suggesting preventive and curative potentials of diverse types of extracts from different parts of *W. somnifera* against diabetes and other chronic diseases caused by or associated with disturbances of glucose homeostasis. Recent observations in our laboratories and elsewhere (Thakur et al. 2015; Nirupama et al. 2014; Bhattacharya and Muruganandam 2003) have revealed that fairly low daily oral dose (25 mg/kg/day or lower) of *W. somnifera* root extracts are highly effective in regulating body weights and glucose metabolism in diabetic as well as in stressed non-diabetic rodents. These observations, taken together with the fact that such low oral doses of

W. somnifera extracts also modulate brain functions, strongly suggest that their observed anti-diabetic activities are most probably due to their modulating effects against environmental and metabolic stress triggered alterations in brain functions and that their regulating effects on biological oxidative processes are involved in their anti-hyperglycemic activities observed after their repeated daily doses. This inference is well supported by currently available evidences on the neurobehavioral activity profiles of *W. somnifera* (Durg et al. 2015; Dar et al. 2015).

2.4.2 Mechanistic Studies

The initial report (Bhattacharya et al. 1997a) appearing in 1997, and leading to the very first exploratory clinical study with *Withania somnifera* root powder in diabetic patients (Andallu and Radhika 2000), had indicated that hypoglycemic effect of the *Withania somnifera* containing Ayurvedic formulation (Trasina) could be due to its protective effects against oxidative free radicals triggered pathologies in pancreatic β-cells. A report suggesting that Glycowithanolides (Sitoindosides VII to X) and Withaferin A are involved in the antioxidative and stress response modulating or adaptogenic activity of *Withania somnifera* extracts had also appeared during the same year (Bhattacharya et al. 1997b). Another study revealing protective effects of fairly low daily oral doses (25 mg/kg/day) of a *Withania somnifera* extract against chronic stress triggered alteration in insulin sensitivity and glucose homeostasis had appeared after a few years thereafter (Bhattacharya and Muruganandam 2003). Since then the numbers of reports reaffirming protective effects of diverse types of *Withania somnifera* derived products and their bioactive constituents against diverse oxidative stress triggered pathologies have continued to increase (Mirjalili et al. 2009; Rai et al. 2016). Although it is now almost certain that Glycowithanolides and their aglycones are some of the bioactive anti-diabetic and oxidative stress protective constituents of *W. somnifera* extracts, the fact that they also contain numerous other bioactive molecules with anti-oxidative and other activities similar to those of Withanolides in numerous *in vitro* and completely animal models cannot be neglected.

In patients suffering from diabetes mellitus, oxidative stress induced by dysregulation of glucose homeostasis accompanies chronic inflammation, which eventually leads to tissue damages, and the crucial role of oxidative stress in the development of diabetic endothelial dysfunctions is also underlined in numerous studies (Rochette et al. 2014; Domingueti et al. 2015; Fiorentino et al. 2013; Hasnain et al. 2016). Diverse cellular heat shock proteins are involved in regulation of stress triggered alterations in glucose homeostasis and insulin sensitivity (Hooper and Hooper 2005; Hooper et al. 2014). It has been suggested that manipulation these cellular chaperons in metabolically relevant tissues represent a therapeutic avenue for prevention and cure of obesity associated diabetes and other metabolic disorders (Hooper 2014; Henstridge et al. 2014; Molina et al. 2016). *W. somnifera* root extracts and Withanolides, especially Withaferin A and Withanone, modulate the functions of

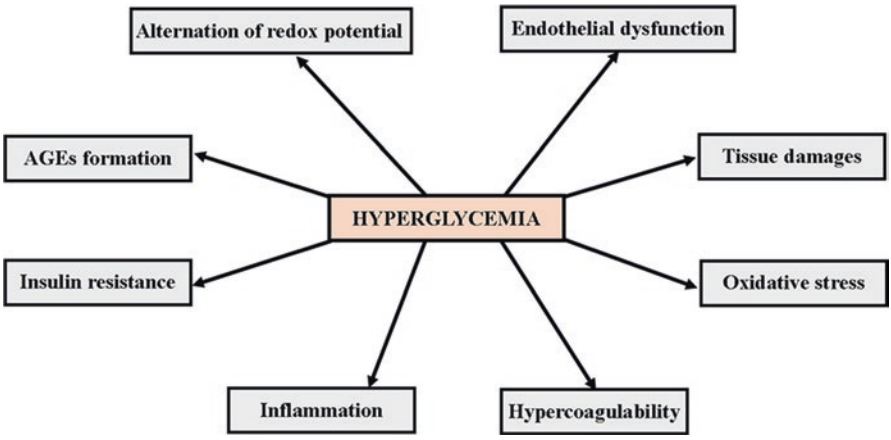

Fig. 2.1 The major pathological mechanisms involved in the development of vascular and other complications in diabetes mellitus (Adapted from: Domingueti et al. 2015)

such cellular chaperons and other nuclear processes regulating oxidative stress and cellular stress responses (Vanden Berghe et al. 2012; Gao et al. 2014; Misico et al. 2002; Mohan and Bargagna-Mohan 2016; Heyninck et al. 2014; Vaishnavi et al. 2012; Cui et al. 2014). Covalent or irreversible binding to sulfhydryl groups of proteins and other biological targets have been implicated in their broad spectrums of activities observed against diverse cellular metabolic processes (Antony et al. 2014). However, yet little concentrated efforts have been made to identify such mechanistic possibilities for other *W. somnifera* metabolites with anti-oxidative and anti-diabetic activities in animal and cellular models. Available information on such bioactivities of numerous phytochemicals encountered in *W. somnifera* (Chatterjee et al. 2010) strongly suggest though, that many of them can modulate the functions of diverse biological targets and processes involved in diverse hyperglycemia triggered pathological changes involved in diabesity associated vascular and other complications. Some such major mechanisms and processes involved in hyperglycemia-triggered pathologies are summarized in Fig. 2.1.

Apart from intracellular, several extracellular sites of actions for *W. somnifera* extracts have been suggested during more recent years (Fig. 2.2). Glucose transporters and other cellular processes regulating glucose uptake in skeletal muscle myotubes and adipocytes and some others involved in insulin secretion from basal pancreatic β cells are just a few of them (Jonathan et al. 2015; Kumar et al. 2015a; Safhi and Anwer 2011; Nirupama et al. 2014). Inhibition of enzymatic activities of α-amylase and α-glucosidase involved in starch degradation and digestive processes regulating glucose homeostasis has also been implicated in anti-hyperglycemic activities of *Withania somnifera* extracts and their bioactive constituents (Balaji et al. 2015; Prabhakar et al. 2013; Khan et al. 2014). In one of these reports (Khan et al. 2014), α-glucosidase inhibitory IC_{50} value of *W. somnifera* derived Withanolides in isolated cells was estimated to be 38.20 μg/ml. In another study (Khan et al.

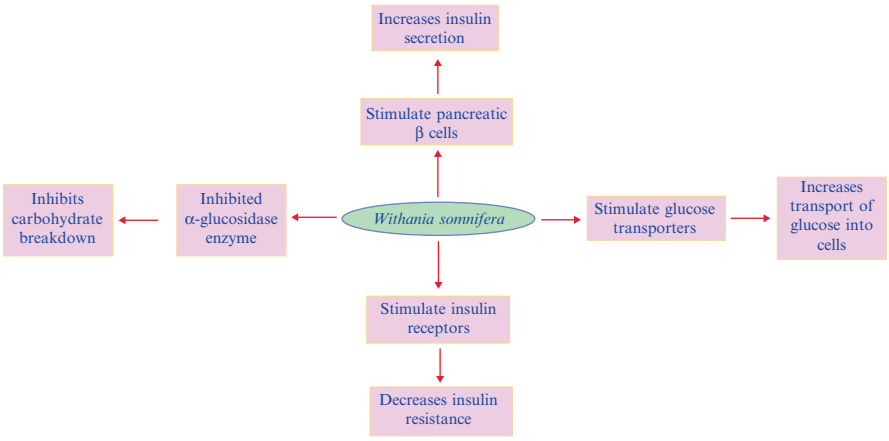

Fig. 2.2 Diverse proposed mechanisms that could be involved in glucose and insulin homeostasis influencing effects of *Withania somnifera* and its bioactive constituents. All such mechanisms could be influenced diverse *Withania somnifera* extract constituents by reversible as well as irreversible binding to diverse biological targets regulating glucose homeostasis

2014a) the same authors have reported that a roots extract of *W. somnifera* at 0.1 and 0.2 mg/ml dose levels possess anti-glycating activity (*in vitro*) via inhibition and reduction of cross-link breaker of glycated proteins or of extra cellular matrix proteins. Since after oral intake, the high concentrations can be expected only inside the gastrointestinal tract, and both α-amylase and α-glucosidase are involved in digestive process and other functions of the gastrointestinal tract, it could as well be that their anti-hyperglycemic and diverse other effects observed after their repeated daily oral doses in metabolically or otherwise stressed animals are also due to their modulating effects on the physiological functions of the digestive system inside this tract. Considering the facts that gut microbial ecology influences the functions of almost all innervated bodily organs (Fig. 2.3), and numerous *W. somnifera* constituents possess bactericidal activities as well as inhibitory effects on enzymes hydrolyzing carbohydrate polymers, this possibility is theoretically a very plausible one also.

Although many questions concerning bioactive constituents, modes and sites of actions still remain unanswered, it is now certain that Glycowithanolides and their aglycones are some of the quantitatively major bioactive constituents of *W. somnifera*, and that like numerous other secondary plant metabolites, they are also pluripotent molecules with therapeutically interesting effects on homeostatic processes regulating blood glucose levels, insulin sensitivity, and physiological functions of the peripheral as well as of the central nervous system. Since numerous therapeutically interesting effects of *W. somnifera* derived products become apparent, or more pronounced, in metabolically abnormal or stressed animals only, it seems reasonable to assume that their ability to facilitate adaptability of diverse bodily organs against noxious stress responses are also involved in their anti-diabetic or anti-hyperglycemic activities. In any case, they do not seem to be carbon copies of any

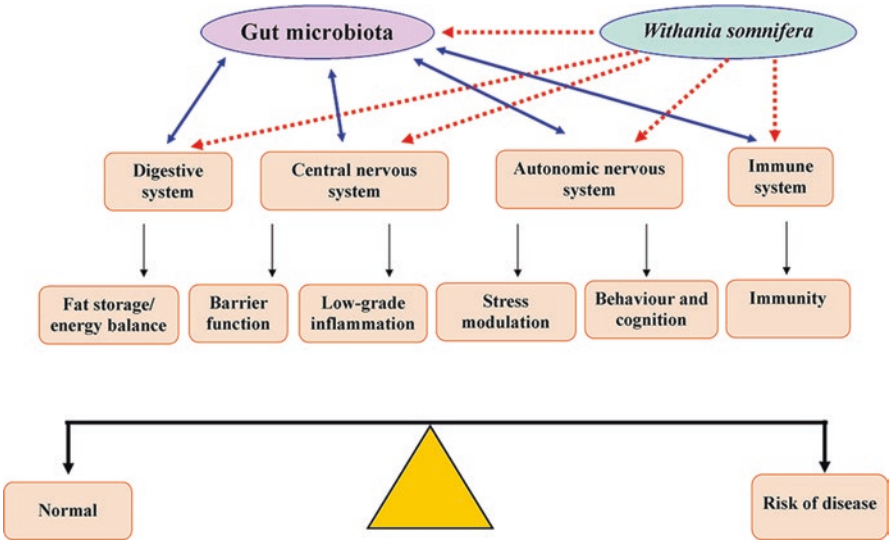

Fig. 2.3 Role of gut microbiota in regulating glucose homeostasis (Adapted from: Foster and Neufeld 2013). *Withania somnifera* extracts and many of their bioactive constituents posses bactericidal activity, and in addition can also modulate the functions of all organs functions regulating glucose homeostasis

currently known and therapeutically used anti-diabetic and other drugs. Since fairly high daily oral *W. somnifera* extract doses (400 mg/kg/day for several weeks) are well tolerated by diabetic or stressed rodents, and their estimated minimal daily stress response modulating and antidiabetic activities in rodent models are 25 mg/kg/day or lower (Thakur et al. 2015; Bhattacharya and Muruganandam 2003; Nirupama et al. 2014), it can also be said that safety range of products derived from the plant are very big. Thus, depending on the severity of diabetes in a given patient, daily oral doses of *W. somnifera* extracts could be fairly low or even very high.

2.5 *Withania somnifera* and Diabetic Complications

Hyperlipidemia is one of the major risk factor for cardiovascular disorders and premature deaths in patients suffering from diabetes mellitus, and it is now becoming increasingly apparent that dyslipidemia accompanying hyperglycemia can also eventually lead Alzheimer's disease and other dementia disorders (Saxena 2010; Tiwari et al. 2014; Aulston et al. 2013). Reports revealing anti-hyperlipidemic and other beneficial effects of *W. somnifera* and Withanolides against altered lipid metabolism in diabetic or hyperlipidemic animals are summarized in Table 2.2. In one of these reports (Visavadiya and Narasimhacharya 2007), enzymatic activity of HMG-CoA (the enzyme regulating cholesterol synthesis) in liver of cholesterol fed

Table 2.2 Some often cited and more recent reports on anti-hyperlipidemic potential of *Withania somnifera*

Part of plant	Type of extract/formulation	Dose, duration and route of administration	Mechanism of action	References
Leaves and roots	Ethanolic	100 and 200 mg/kg/day, for 56 days, p.o.	Increased high density lipoprotein-bound cholesterol and decreased very low density lipoprotein-bound cholesterol and low-density lipoprotein-bound cholesterol	Udayakumar et al. (2009)
Roots	Powder	Root powder combined with high fat diet and administered for 4 weeks	Increased hepatic antioxidant status and activity of hepatic HMG-CoA reductase enzyme	Visavadiya and Narasimhacharya (2011)
Roots	Powder	Root powder combined with diet at 0.75 and 1.5 gm/rat/day was administered for 4 weeks	Increased antioxidant activity and HMG-CoA reductase activity	Visavadiya and Narasimhacharya (2007)
Fruits of *Withaia coagulans*	Hydroalcoholic (60%)	1000 mg/kg/day for 4 weeks	Inhibited HMG-CoA reductase enzyme	Datta et al. (2013)
Leaves and roots	Ethanolic	200 mg/kg/day, for 56 days, p.o.	Increased high density lipoprotein-bound cholesterol and decreased very low density lipoprotein-bound cholesterol and low-density lipoprotein-bound cholesterol	Sarangi et al. (2013)
Roots	Dried powder	3 g/day (500 mg/capsule) for 30 days, p.o.	Decreased serum level of cholesterol, decreased serum level of lipids	Andallu and Radhika (2000)
Ambrex (a poly herbal formulation)	Powder containing *W. somnifera* (100 mg/g)	Ambrex (40 mg/kg/day) for 15 days, p.o.	Antioxidant action, increase HDL cholesterol and reduce LDL cholesterol	Devi and Rajkumar (2013)
Caps HT2 (herbal Ayurvedic formulation)	Capsule containing roots of *W. somnifera* (100 mg/g)	100–400 mg/kg/day for 30 days, p.o.	Antioxidant action, increased HDL cholesterol levels	Mary et al. (2003)

Trasina (Ayurvedic formulation)	Powder containing *W. somnifera*	100 and 200 mg/kg/day for 28 days, p.o.	Antioxidant and hypocholesterolemic activity	Bhattacharya et al. (1997)
Fruits of *Withania coagulans*	Aqueous	1 g/kg/day for 49 days, p.o.	Decresed elevated serum cholesterol, triglycerides and lipoprotein levels	Hemalatha et al. (2006)
Fruits of *Withania coagulans*	Chloroform	1 g/kg/day for 14 days, p.o.	Decrease in the blood triglyceride, total cholesterol, LDL and VLDL levels	Hoda et al. (2010)
Fruits of *Withania coagulans*	Coagulanolide isolated from aqueous decoctions	100 mg/kg for 10 days, p.o.	Lowered the level of plasma triglycerides, total cholesterol, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol	Maurya et al. (2008)
Leaves and roots	Methanolic and isolated Withanolides	100 µg/ml in cellular model of diabetes	Increased insulin secretion and increased insulin sensitivity	Gorelick et al. (2015)
Fruits of *Withania coagulans*	Aqueous	1000 mg/kg/day for 28 days, p.o.	Increased glucose metabolism and decreased elevated cholesterol levels	Saxena, (2010)
Roots	Ethanolic	1.4 g/kg/day for 15 days, p.o.	Increased antioxidant enzymes and decreased elevated cholesterol levels	Jatwa and Kar (2009)
Roots	Ethanolic (5%)	50 mg/kg/day for 56 days, p.o.	Antioxidant activity and decreased elevated cholesterol levels	Kumar et al. (2013)
Roots	Standardized	750 mg/day for 10 days followed by 1000 mg/day for next 10 days and 1250 mg/day for last 10 days, p.o.	Decreased lipid levels and increased fat metabolism	Raut et al. (2012)

rats was reduced by incorporating *W. somnifera* root powder in the hypercholesteremic diet. Increased bile acid synthesis and improvements in antioxidative status of the animals treated with *W. somnifera* were also observed in that study. Antihyperglycemic and anti-hyperlipidemic effects of some Withanolides isolated from *W. coagulans* (Coagulin: 50 mg/kg/day) have also been reported (Maurya et al. 2008). These observations suggest that appropriate structure activity studies and structural modification of Withanolides could eventually lead to structurally and functionally novel drug leads for prevention and cure of diabetes associated hyperlipidemia also. However, till now all such efforts have been made for obtaining potential anticancer drugs, or for obtaining structurally novel inducers of heat shock proteins only (Zhang et al. 2012; Wijeratne et al. 2014).

Functions and structures of all parts of the nervous system are eventually disrupted in diabetic patients causing encephalopathy and myelopathy in the central nervous system, and peripheral neuropathy in all divisions of peripheral nervous system. Autonomic neuropathy is most commonly associated with dysregulation of gastrointestinal (Drewes et al. 2016) and cardiovascular functions (Serhiyenko and Serhiyenko 2015), while peripheral sensory neuropathy manifests as pain, dysesthesia, and/or loss of sensation. Peripheral neuropathy ultimately affects more than 50% of all diabetic patients. It is most prevalent in poorly controlled diabetic patients and its complications start arising immediately after sudden improvement of glycaemic control (Lee-Kubli et al. 2014; Hosseini and Abdollahi 2013). Since currently available drugs are not effective in relieving pain in all patients, and all of them have their own drawbacks, efforts are now being made in several laboratories to identify novel therapeutic leads against diabetic neuropathy associated pain.

The very first report suggesting *Withania somnifera* roots could have anti-nociceptive activities had appeared also in 1997 (Kulkarni and Ninan 1997), and a very recent one has reconfirmed and further extended those observations (Orrù et al. 2014). However, efforts to identify the pharmacological receptors possibly involved in anti-nociceptive activity of diverse types of *W. somnifera* extracts and numerous of their known bioactive constituents using *in vitro* radio-ligand binding experiments were unsuccessful in pinpointing any specific neurotransmitter or opioid receptors for the extracts of for any of their bioactive constituents studied (Sonar et al. 2015). In any case, the observations summarized in this report strongly suggest that opioid, cannabinoid, GABAergic and glutamatergic receptors are not the primary pharmacological targets involved in anti-nociceptive and other brain function modulating activities of *W. somnifera* extracts and many of their bioactive constituents observed in animal models after their repeated daily fairly low oral doses.

Several other reports have continued to reconfirm anti-nociceptive effects of *W. somnifera* against the second phase of formalin-induced pain in diabetic rodents (Khalili 2009; Pradeep et al. 2010; Orrù et al. 2016; Roughani et al. 2007). Reported results of one of these studies (Pradeep et al. 2010) suggest that such effects of the tested dose of the extract (100 mg/kg/day for several weeks) is due to its antidepressant activities, and that protective effects of the extract against noxious stimuli triggered oxidative stress is involved in its such effects. They reveal also that antidepressant drugs like effects of the extract increases with increasing numbers of

treatment days, and that such effects of the extracts is qualitatively similar that to that of the flavonoid quercetin (10 mg/kg/day). In another study, similarities between the effects of *W. somnifera* and salicylic acid in formalin induced pain after their repeated daily doses were observed (Khalili 2009). Since both salicylic acid and quercetin are also encountered in *W. somnifera* extracts, they could also contribute to the observed effects of the extracts in pain models. Available preclinical and clinical information on therapeutic potentials salicylic acid (Rainsford 2013; Ugurlucan et al. 2012; Rumore and Kim 2010; Berk et al. 2013; Khan et al. 2016) and quercetin (Nabavi et al. 2015; Gormaz et al. 2015) are in agreement with this possibility.

Salicylates and the anti-hyperlipidemic drug simvastatin are currently often prescribed for prevention of diabetes and metabolic syndromes associated with or caused by environmental stress and psychiatric disorders. Although, their potential uses as antidepressants or for treatments of psychiatric disorders are now controversially discussed (Rahola 2012), it cannot be denied that psychiatric disorders often accompany or are caused by metabolic disorders, and that prevalence of morbidity and mortality are higher in diabetes with psychosomatic abnormalities (Balhara and Sagar 2011; Sarkar and Balhara 2014; Maia et al. 2012). Several reports during more recent years have consistently revealed antidepressive, anxiolytic and other beneficial effects of *W. somnifera* and its metabolites against diverse neurological disorders in diabetic and stressed or intoxicated rodents after their repeated daily oral doses (Parihar et al. 2004; Xu et al. 2013; Thakur et al. 2015; Kumar et al. 2015a; Roughani et al. 2006; Parihar et al. 2015; Wadhwa et al. 2015; Sehgal et al. 2012; Kuboyama et al. 2014). Several of these reports, have reconfirmed that such effects of the extracts of the plant are due to their protective effects against hyperglycemic stress triggered alterations in oxidative defense systems in brain and other bodily organs. However, yet no very definitive statement on possible involvements of bioactive metabolites of the plant, other than Withanolides, in such effects of *W. somnifera* derived crude products can be made.

Except for a very few reports dealing with stress resistance increase or adaptogenic and anxiolytic and antidepressant other bioactivities activities of the plant, most of them deal with arbitrarily chosen extraction procedures, doses and dosing regimen for the tested extracts. However, in one such more recent report (Bharathi et al. 2015) it was inferred that 30 mg/kg daily oral doses of a commercially available *W. somnifera* extract is its minimally effective anti-depressive ones in albino mice. Dose finding studies conducted with another commercially available extract of the roots of the plant (containing only 2.6% Withanolides and their glycosides) have revealed that 25 mg/kg/day or lower doses of the extract for 10 days is high enough not only for its statistically significant antidepressant and anxiolytics like effects in diabetic and non-diabetic rats, but also for its protective effects against chronic unpredictable mild stress triggered alterations in body weight and temperature changes in such rats (Thakur et al. 2015). Such were also the more recent observations made with root, leaf, and stem extracts of *W. somnifera* extracts containing different contents of Withanolides, and the minimally effective daily oral doses of a *W. somnifera* root extract devoid of Withanolides for analogous activities was only

a bit higher than that of the root extracts containing Withanolides (Dey et al. 2014, 2016a, b).

These observations reaffirm and strongly suggest that constituents of *Withania somnifera* extracts other than Withanolides also contribute to the bioactivity profiles of its extracts, and that such profiles of Withanolides present in *W. somnifera* roots and other parts of the plant are modulated by numerous other bioactive constituents of the plants, or those of other edible or medicinal plants. Salicylic and other hydroxylated aromatic acids, quercetin and other flavonoids, nicotinic, fumaric and ascorbic acids, an triethylene glycol are some such phytochemicals present in *W. somnifera* extracts, and their diverse combinations are encountered also in numerous other medicinal or edible plants often used in Ayurvedic and other traditionally systems of medicine for prevention and cure of diabetes and other metabolic disorders (Singh et al. 2014; Kumar et al. 2015a). Unfortunately, as yet only very little attention has been paid to the possibility that neurohormetic and stress response modulating properties of structurally and functionally diverse phytochemical (Son et al. 2008; Mattson and Cheng 2006; Calabrese et al. 2012) of *W. somnifera* could also be involved in antidiabetic and other therapeutically interesting bioactivities of diverse types of extracts of the plant. It is now becoming increasingly apparent though, that heat shock proteins and other cellular chaperons are also involved in their hormetic and stress response regulating activities (Dattilo et al. 2015). Diverse biomarkers of such physiological processes are useful tools for diagnosing and treatments of neurodegenerative and other comorbidities commonly encountered in chronic diseases, including those leading to or caused by insulin resistance and dysregulation of glucose homeostasis (Bhakta-Guha and Efferth 2015; Farooqui 2013). Appropriate uses of these biomarkers for better understanding of therapeutic potentials of *W. somnifera* and its metabolites could eventually lead to novel therapeutic leads and pharmacological strategies urgently needed for prevention and cure of diabetes and associated comorbidities.

2.6 Clinical Studies

The very first exploratory clinical observations suggesting that *W. somnifera* could be an antidiabetic plant with anti-hyperlipidemic activities (Andallu and Radhika 2000) was conducted with its root powder containing capsules administer for 30 consecutive days to mild non-insulin dependent mildly diabetic patients maintained on antidiabetic diabetic drug Daonil (glibenclamide), and in mildly hypercholesteremic patients not undergoing any drug treatments. Daily dose of the root powder used was 3 g/day, and the effects of *W. somnifera* treatments on hyperglycemia, hyperlipidemia and other parameters were compared with those observed in subjects not undergoing any other additional treatments. This study indicate that regular intake of *W. somnifera* root powder can induce potassium sparing diuretic effects in diabetic patients, and can also reduce serum cholesterol and triglycerides in hyperlipidemic patients. However, the magnitude of effects of root powder treatment on

hyperglycemia in diabetic patients maintained on glibenclamide (12% reduction in blood glucose level) was equal to that observed in only glibenclamide treated diabetic patients. No adverse effects of treatments were observed in this study.

Report of another exploratory clinical study dealing with effects of *W. somnifera* extract on blood glucose and lipid levels have appeared 12 years after that exploratory study (Raut et al. 2012). In this study published in 2012, increasing numbers of capsules of a *W. somnifera* aqueous extract (extraction procedure used and analytical characteristics of the extract used was not mentioned) was administer to young healthy volunteers during 30 days for evaluating its dose related safety, tolerability and effectiveness against diabetes associated vascular abnormalities. Increasing doses of the extract used were equivalent to 6, 8, and 10 g of crude pulverized of *W. somnifera* roots (not mentioned whether dried or not). The extract was well tolerated by 17 of the 18 volunteers included in the study. One of the volunteers showed increased appetite, libido and hallucinogenic effects with vertigo even after the lowest dose of the extract tested (750 mg/day for 10 days) and was excluded from the study. Improvement in sleep quality was observed in 6 subjects. Although significant reduction in mean total blood cholesterol and blood urea nitrogen values were observed, extract treatments for 30 days had no statistically significant effects on blood sugar, triglyceride, and HDL-, LDL-, and VLDL-cholesterol values.

However, significant and dose dependent lowering effects of an analytically well characterized *W. somnifera* root extract on total cholesterol, triglyceride, and LDL-cholesterol levels were observed in a more recently reported randomized, double blind, and placebo controlled study in Type-2 diabetic patients maintained on 1500–2500 mg/day metformin (Usharani et al. 2015). This study was designed to evaluate the effects of the extract on endothelial functions of type-2 diabetic patients and to verify whether antioxidative effects of *W. somnifera* extracts observed in animals could be confirmed in patients or not. Daily 250 and 500 mg doses of the extract were administered for 12 weeks in this study. Significant improvements of endothelial function, as well as in biomarkers of oxidative stress, systemic inflammation, and HbA1C levels were also observed in this study, and the extract was well tolerated by the patients. The authors conclude that the extract can be used as a therapeutic adjunctive in type-2 diabetic patients. Since diabetic patients included in the study were maintained in on metformin and yet a significant reduction in the HbA1aC level was observed in *W. somnifera* treated group, the observation of the study are also in agreement with several observations made in laboratory rodents suggesting preventive effects of oxidative processes as well as glycation reactions (Fig. 2.4) are involved in the modes of action(s) of the *W. somnifera* extract tested.

The same research group had also reported similar effects of an ayurvedic polyherbal formulation (CardiPro) containing *W. somnifera* as one of its several active ingredients (Fatima et al. 2012), and also were the authors of another report revealing analgesic activity a *W. somnifera* extract currently commercialized in the USA (Sensoril®) and containing ca 15.7% Withanolides glycosides, 40.2% oligosaccharides and 0.24% Withaferin A (Usharani et al. 2013; Kumar et al. 2015b). The same author (Pingali et al. 2014) has also reported mental function improving effects of the same extract in healthy persons. Although several other reports revealing

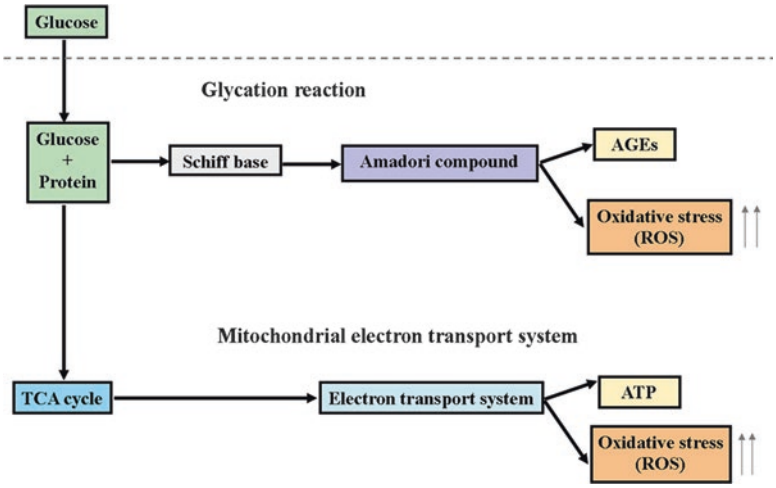

Fig. 2.4 Increase in oxidative stress in the diabetic state: Acceleration of glycation response and the intramitochondrial electron transfer system is observed in the diabetic (hyperglycemic) state, causing oxidative stress as the responses accelerated. [*Black arrows* indicate pathway; *grey arrows* indicate increase; *AGE* advanced glycosylation end products, *ROS* reactive oxygen species, *TCA cycle* tricarboxylic acid cycle, *ATP* adenosine tri-phosphate (Adapted from: Kawahito et al. 2009)]. *Withania somnifera* extracts can inhibit both glycation reactions leading to elevated HbA1c levels as well as oxidative damages

anti-stress, anxiolytic, and safety in volunteers and patients have appeared during more recent years (Chandrasekhar et al. 2012; Pratte et al. 2014), as yet only two reports (Andallu and Radhika 2000; Usharani et al. 2015) on potential health benefits of *W. somnifera* in diabetic patients have appeared in more recent English language journals. Both of them and several others made in volunteers and non-diabetic patients do reaffirm that fairly high daily *W. somnifera* extracts doses are well tolerated without any severe adverse effects and that they could be effective and safe herbal therapeutic alternatives against diabetes associated hyperlipidemia and other comorbidities. Identification of bioactive metabolites of the plant involved in their anti-hyperlipidemic and Hb1Ac level suppressing effects of its extracts could eventually lead to novel therapeutic leads and strategies urgently needed for prevention and cure of diabetes and other metabolic disorders, i.e. the most widely spreading epidemics of the twenty-first century affecting ca. 20–25% of global adult population (Alberti et al. 2006).

2.7 Conclusions and Future Perspective

Although it is now evident that *W. somnifera* is an anti-diabetic plant, many questions concerning its bioactive constituents and pharmacological interactions between them as well as on their pharmacological targets and their locations of

actions in different bodily organs remain unanswered. From the preclinical and clinical information summarized in this chapter it is evident though, that homeostatic processes and mechanisms regulating circulating glucose levels and its biological functions are involved in the modes of actions of diverse types of extracts obtainable from different parts of the plant. Hereupon, modulations of oxidative processes and mechanisms regulating glucose and/or insulin homeostasis by the extracts of the plant and their numerous bioactive constituents play important roles. A few, but not all, such effects of *W. somnifera* derived products could be due their antioxidative properties, and due to their irreversible and nonspecific binding to diverse biological targets involved in regulation of biological oxidative processes. Like for numerous other plant-derived products, the so-called synergy and polyvalence paradigms (Houghton 2009) can explain their therapeutically interesting bioactivity profiles.

Although glycosylated Withanolides and their aglycones are certainly some of the quantitatively major secondary metabolites with modulating effects on glucose and insulin homeostasis, they must not necessarily be the only ones involved in such effects of extracts of the plant. However, since they are structurally and functionally the unique constituents biosynthesized by *W. somnifera* and many other plants, better understanding of their sites and modes of action are certainly be useful not only for drug discovery purposes, but also better understanding of therapeutic potentials of numerous other plants synthesizing and storing them in different concentrations in their different anatomical parts. Since medicinal values of other plants synthesizing and storing structurally and functionally diverse Withanolides for prevention of metabolic and other disorders has been well recognized by practitioners of Ayurvedic and other traditionally known systems of medicine, better understanding of their sites and modes of actions could be useful for identifying novel therapeutic leads from several such plants as well.

Since *W. somnifera* is a fairly nontoxic psychoactive plant with traditionally known and reasonably validated sleep quality improving and anti-diabetic activities in experimental and some clinical models, it seems to be particularly well suited for prevention and cure of diabesity and other metabolic disordered triggered by, or associated with sleep disorders. Proper understanding of its bioactive constituents and pharmacological interactions between them, involved in such effects of the plant is an essential prerequisite for obtaining sustainable and reproducible therapeutic benefits from the plant. To date, only very little concentrated efforts have been made to identify a convenient and validated bioassay system necessary for such purposes. It is now well recognized though, that environmental and/or metabolic disturbances triggered stress leads to sleep disturbances (Fig. 2.5), which in turn leads to diabesity and further diabetic complicates. Therefore, quantification of the extracts and their bioactive constituents in stressed or metabolically disturbed rodent models for such purposes can be warranted.

More recent observations made in our laboratories and elsewhere using stressed rodents have revealed sleep quality improving effects of *W. somnifera* extracts (Kumar and Kalonia 2007, 2008) and their antihyperglycemic activities on stress diabetic and non-diabetic rats (Thakur et al. 2015). The most sensitive and easily

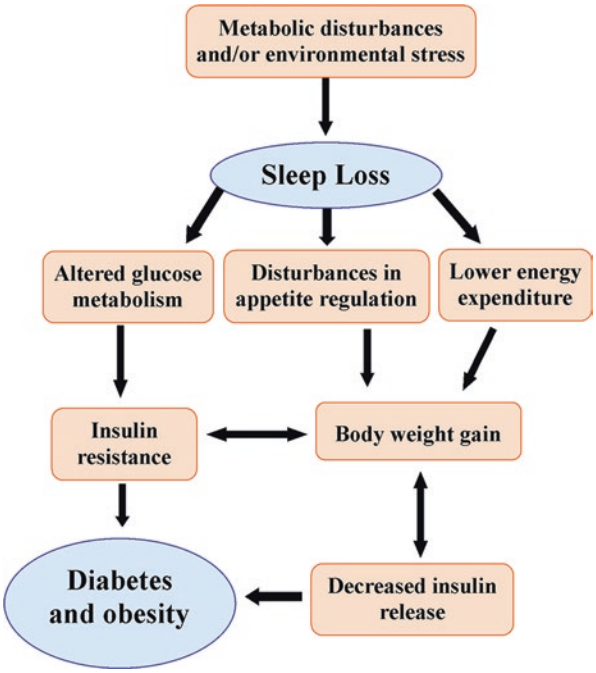

Fig. 2.5 Pathways leading to sleep loss to diabetes risk (Adapted from: Knutson et al. 2007)

quantifiable effects of such extracts identified to date in our laboratory are their protective effects against alterations in stress triggered body weight and temperature of laboratory rodents after their repeated daily fairly low oral doses. Using a bioassay procedure identified and validated during these efforts we have already led us identify several bioactive constituents of their extracts other than Withanolides with very high oral efficacies against both these biological markers of environmental or metabolic stress. Therefore, it seems reasonable to suggest that appropriate uses of this bioassay system and conventionally known activity guided fraction procedures could be used not only or better understanding of therapeutic potentials of *W. somnifera* derived products for prevention and cure of diabetes and associated comorbidities, but also for clarifying pharmacological interactions between Withanolides and other bioactive constituents of the plant often encountered in many other edible and medicinal plants.

China, India and South American countries are the most populous countries now affected the most from diabetes, and these are the also the ones spending the most on drug discovery efforts based on the uses of medicinal plants. However, the phytopharmacological and other strategies often used by preclinical and clinical researches are those based on reductive and specific target based ones evolving from efforts to better understand the biological processes and mechanisms involved in modes of actions of already known drugs. Unfortunately, even diverse combinations of currently available and more costly antidiabetic and other drugs evolving from such

strategies are not meeting the therapeutic needs and demands of the patients suffering from, or at risk to, diabetes and other metabolic disorders. The phytopharmacological lessons learned from *W. somnifera* strongly suggest that more realistic and holistic strategies are necessary for better understanding of therapeutic potentials, of this and other plants many of which also biosynthesize and store many stress response modulating secondary metabolites of *W. somnifera*.

A quotation cited in the introduction of recently published book on herbal adaptogens (Winston and Maimes 2007), states: "*All plants contain adaptogenic/tonic compounds, because plants have to contend with good deal of stress themselves*". Currently available information on the effects of metabolites of *W. somnifera* and other adaptogenic plants on brain functions (Kennedy 2014), have already added more than sufficient experimental evidences that numerous of them are also adaptogenic or stress resistance improving agents in all mammals including human beings. Numerous preclinical and clinical observations made with Withanolides and other metabolites synthesized and stored by *W. somnifera* have continued to add further evidences in favor of the conviction that loss of adaptability to metabolic stress and leading to diabetes can be compensated by their regular oral intake. What needs to be done now is to assemble their appropriate combination and doses in a capsule, or in foods and drinks, for obtaining reliable and reproducible therapeutic benefits from them. Judicious uses of the knowledge and knowhow evolving from the efforts of numerous phyto-pharmacologists and medicinal phytochemists during the past decade, and leading to our current understanding of therapeutic potentials of the plant, will certainly be useful for such purposes.

References

Adamska E, Ostrowska L, Górska M, Krętowski A (2014) The role of gastrointestinal hormones in the pathogenesis of obesity and type 2 diabetes. Prz Gastroenterol 9:69–76

Ahmed AM (2002) History of diabetes mellitus. Saudi Med J 23:373–378

Alali FQ, Amrine CSM, El-Elimat T, Alkofahi A, Tawaha K, Gharaibah M, Swanson SM, Falkinham JO III, Cabeza M, Sanchez A, Figueroa Oberlies NH (2014) Bioactive Withanolides from Withania obtusifolia. Phytochem Lett 9:96–101

Alberti G, Zimmet P, Shaw J (2006) The IDF consensus worldwide definition of the metabolic syndrome. In: Grundy SM (ed) International diabetes federation. Brussels, Belgium

Andallu B, Radhika B (2000) Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root. Indian J Exp Biol 38:607–609

Anderson BJ, Auslander WF (1980) Research on diabetes management and the family: a critique. Diabetes Care 3:696–702

Anothaisintawee T, Reutrakul S, Van Cauter E, Thakkinstian A (2015) Sleep disturbances compared to traditional risk factors for diabetes development: systematic review and meta-analysis. Sleep Med Rev 30:11–24

Antony ML, Lee J, Hahm ER, Kim SH, Marcus AI, Kumari V, Ji X, Yang Z, Vowell CL, Wipf P, Uechi GT, Yates NA, Romero G, Sarkar SN, Singh SV (2014) Growth arrest by the antitumor steroidal lactone withaferin A in human breast cancer cells is associated with down-regulation and covalent binding at cysteine 303 of β-tubulin. J Biol Chem 289:1852–1865

Anwer T, Sharma M, Pillai KK, Iqbal M (2008) Effect of Withania somnifera on insulin sensitivity in non-insulin-dependent diabetes mellitus rats. Basic Clin Pharmacol Toxicol 102:498–503

Anwer T, Sharma M, Pillai KK, Khan G (2012) Protective effect of Withania somnifera against oxidative stress and pancreatic beta-cell damage in type 2 diabetic rats. Acta Pol Pharm 69:1095–1101

Archana SS, Mishra HS, Yadav KN (2015) Ashwagandha: solution to stress and insomnia. Indian J Ancient Med Yoga 8:153–159

Ashok GA, Shende MB (2015) A clinical evaluation of antistress activity of ashwagandha (Withania somnifera Dunal) on employees experiencing mental stress at work place. Int J Ayur Pharma Res 3:37–45

Aulston BD, Odero GL, Aboud Z, Glazner GW (2013) Alzheimer's disease and diabetes. InTech Open, Hampshire

Bajor LA, Gunzler D, Einstadter D, Thomas C, McCormick R, Perzynski AT, Kanuch SW, Cassidy KA, Dawson NV, Sajatovic M (2015) Associations between comorbid anxiety, diabetes control, and overall medical burden in patients with serious mental illness and diabetes. Int J Psychiatry Med 49:309–320

Balaji RM, Jeyaram C, Sundaram KM, Ramasamy MS (2015) Studies on antidiabetic activity of Indian medicinal plants using α–amylase and α-glucosidase inhibitory activity: a pathway to antidiabetic drugs. World J Med Sci 12:207–212

Balhara YPS, Sagar R (2011) Correlates of anxiety and depression among patients with type 2 diabetes mellitus. Indian J Endocrinol Metabol 15:S50–S54

Bentley-Lewis R, Levkoff S, Stuebe A, Seely EW (2008) Gestational diabetes mellitus: postpartum opportunities for the diagnosis and prevention of type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab 4:552–558

Berk M, Dean O, Drexhage H, McNeil JJ, Moylan S, O'Neil A, Davey CG, Sanna L, Maes M (2013) Aspirin: a review of its neurobiological properties and therapeutic potential for mental illness. BMC Med 11:74

Bhakta-Guha D, Efferth T (2015) Hormesis: decoding two sides of the same coin. Pharmaceuticals 8:865–883

Bharathi P, Seshayamma V, Jagannadharao GH, Sivakumar N (2015) Evaluation of antidepressant activity of aqueous extract of Withania somnifera [Aswagandha] roots in albino mice. IOSR J Pharma Biol Sci 10:27–29

Bharti SK, Bhatia A, Tewari SK, Sidhu OP, Roy R (2011) Application of HR-MAS NMR spectroscopy for studying chemotype variations of Withania somnifera (L.) Dunal. Magn Reson Chem 49:659–667

Bhattacharya SK, Muruganandam AV (2003) Adaptogenic activity of Withania somnifera: an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav 75:547–555

Bhattacharya SK, Satyam SK, Chakrabarti A (1997a) Effect of Trasina an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. Indian J Exp Biol 35:297–299

Bhattacharya SK, Satyam KS, Ghosal S (1997b) Antioxidant activity of Glycowithanolides from Withania somnifera. Indian J Exp Biol 35:236–239

Calabrese V, Cornelius C, Dinkova-Kostova AT (2012) Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. Biochim Biophys Acta 1822:753–783

Cespedes EM, Bhupathiraju SN, Li Y, Rosner B, Redline S, Hu FB (2016) Long-term changes in sleep duration, energy balance and risk of type 2 diabetes. Diabetologia 59:101–109

Chandrasekhar K, Kapoor J, Anishetty S (2012) A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. Indian J Psychol Med 34:255–262

Chatterjee S, Srivastava S, Khalid A, Singh N, Sangwan RS, Sidhu OP, Roy R, Khetrapal CL, Tuli R (2010) Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry 71:1085–1094

Chen LX, He H, Qiu F (2011) Natural Withanolides: an overview. Nat Prod Rep 28:705–740

Choudhary MI, Yousuf S (2013) Withanolides: chemistry and antitumor activity. In: Ramawat KG, Me'rillon JM (eds) Natural products. Springer, Berlin/Heidelberg, pp 3465–3495
Choudhary B, Shetty A, Langade DG (2015) Efficacy of Ashwagandha (Withania somnifera [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. Ayu 36:63–68
Choudhury SM, Tan TM, Bloom SR (2016) Gastrointestinal hormones and their role in obesity. Curr Opin Endocrinol Diabetes Obes 23:18–22
Colagiuri S (2010) Diabesity: therapeutic options. Diabetes Obes Metab 12:463–473
Cui ZG, Piao JL, Rehman MU, Ogawa R, Li P, Zhao QL, Kondo T, Inadera H (2014) Molecular mechanisms of hyperthermia-induced apoptosis enhanced by withaferin A. Eur J Pharmacol 723:99–107
Cunha DA, de Alves MC, Stoppiglia LF, Jorge AG, Módulo CM, Carneiro EM, Boschero AC, Saad MJ, Velloso LA, Rocha EM (2007) Extra-pancreatic insulin production in RAt lachrymal gland after streptozotocin-induced islet beta-cells destruction. Biochim Biophys Acta 1770:1128–1135
Dall'Asta M, Bayle M, Neasta J, Scazzina F, Renato B, Cros G, Rio DD, Oiry C (2015) Protection of pancreatic β-cell function by dietary polyphenols. Phytochem Rev 14:933–959
Dar NJ, Hamid A, Ahmad M (2015) Pharmacologic overview of Withania somnifera, the Indian Ginseng. Cell Mol Life Sci 72:4445–4460
Datta A, Bagchi C, Das S, Mitra A, Pati AD, Tripathi SK (2013) Antidiabetic and antihyperlipidemic activity of hydroalcoholic extract of Withania coagulans Dunal dried fruit in experimental rat models. J Ayurveda Integr Med 4:99–106
Dattilo S, Mancuso C, Koverech G, Di Mauro P, Ontario ML, Petralia CC, Petralia A, Maiolino L, Serra A, Calabrese EJ, Calabrese V (2015) Heat shock proteins and hormesis in the diagnosis and treatment of neurodegenerative diseases. Immun Ageing 12:20
Devi J, Rajkumar J (2013) Antihyperlipidemic effect of ambrex, a polyherbal formulation against experimentally induced hypercholesterolemia in rats. Afr J Pharm Pharmacol 7:1737–1743
Dey A, Chatterjee SS, Kumar V (2014) Comparison of the Adaptogenic efficacy of three different Withania somnifera extracts in mice. Indian J Pharm 46:S87–S87
Dey A, Chatterjee SS, Kumar V (2016a) Low dose effects of a Withania somnifera extract on marble burying behaviour in stressed mice. J Intercult Ethnopharmacol 5:274–277
Dey A, Chatterjee SS, Kumar V (2016b) Analgesic activity of a Withania somnifera extract in stressed mice. Orient Pharm Exp Med 16(4):295–302. Under review
Dhanani T, Shah S, Gajbhiye NA, Kumar S (2017) Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arab J Chem 10:S1193–S1199
Domingueti CP, Dusse LM, Md C, de Sousa LP, Gomes KB, Fernandes AP (2015) Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J Diabetes Complicat 30:738–745
Drewes AM, Søfteland E, Dimcevski G, Farmer AD, Brock C, Frøkjær JB, Krogh K, Drewes AM (2016) Brain changes in diabetes mellitus patients with gastrointestinal symptoms. World J Diabetes 7:14–26
Durg S, Dhadde SB, Vandal R, Shivakumar BS, Charan CS (2015) Withania somnifera (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: a systematic review and meta-analysis. J Pharm Pharmacol 67:879–899
Egede LE, Dismuke CE (2012) Serious psychological distress and diabetes: a review of the literature. Curr Psychiatry Rep 14:15–22
Egede LE, Gebregziabher M, Zhao Y, Dismuke CE, Walker RJ, Hunt KJ, Axon RN (2014) Impact of mental health visits on healthcare cost in patients with diabetes and comorbid mental health disorders. PLoS One 9:e103804
Farag YM, Gaballa MR (2010) Diabesity: an overview of a rising epidemic. Nephrol Dial Transplant 26:28–35

Farooqui AA (2013) Effect of dietary phytochemicals on metabolic syndrome and neurological disorders. In: Metabolic syndrome: an important risk factor for stroke, alzheimer disease, and depression. Springer, New York, pp p191–p234
Fatima N, Usharani P, Muralidhar N (2012) Randomised double blind placebo controlled study to evaluate the effect of cardipro on endothelial dysfunction and biomarkers in patients with type 2 diabetes mellitus. Innovative J Med Health Sci 2:122–128
Fernando IDNS, Abeysinghe DC, Dharmadasa RM (2013) Determination of phenolic contents and antioxidant capacity of different parts of Withania somnifera (L.) Dunal. from three different growth stages. Ind Crop Prod 50:537–539
Fiorentino TV, Prioletta A, Zuo P, Folli F (2013) Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. Curr Pharm Des 19:5695–5703
Flanagan RJ (2008) Side effects of clozapine and some other psychoactive drugs. Curr Drug Saf 3:115–122
Foster JA, Neufeld KAM (2013) Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 36:305–312
Gao R, Shah N, Lee JS (2014) Withanone-rich combination of ashwagandha Withanolides restricts metastasis and angiogenesis through hnRNP-K. Mol Cancer Ther 13:2930–2940
Gardner D, Shoback D (2011) Greenspan's basic & clinical endocrinology. LANGE Clinical Medicine, McGraw-Hill Medical, New York
Girish KS, Machiah KD, Ushanandini S, Harish Kumar K, Nagaraju S, Govindappa M, Vedavathi M, Kemparaju K (2006) Antimicrobial properties of a non-toxic glycoprotein (WSG) from Withania somnifera (Ashwagandha). J Basic Microbiol 46:365–374
Gorelick J, Rosenberg R, Smotrich A, Hanuš L, Bernstein N (2015) Hypoglycemic activity of Withanolides and elicitated Withania somnifera. Phytochemistry 116:283–289
Gormaz JG, Quintremil S, Rodrigo R (2015) Cardiovascular disease: a target for the pharmacological effects of quercetin. Curr Top Med Chem 15:1735–1742
Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 103:137–149
Hasnain SZ, Prins JB, McGuckin MA (2016) Oxidative and endoplasmic reticulum stress in β-cell dysfunction in diabetes. J Mol Endocrinol 56:R33–R54
Hemalatha S, Wahi AK, Singh PN, Chansouria JP (2006) Hypolipidemic activity of aqueous extract of Withania coagulans Dunal in albino rats. Phytother Res 20:614–617
Henschen F (1969) On the term diabetes in the works of Aretaeus and Galen. Med Hist 13:190–192
Henstridge DC, Whitham M, Febbraio MA (2014) Chaperoning to the metabolic party: the emerging therapeutic role of heat-shock proteins in obesity and type 2 diabetes. Mol Metab 3:781–793
Heyninck K, Lahtela-Kakkonen M, Van der Veken P, Haegeman G, Vanden Berghe W (2014) Withaferin A inhibits NF-kappaB activation by targeting cysteine 179 in IKKβ. Biochem Pharmacol 91:501–509
Hoda Q, Ahmad S, Akhtar M, Najmi AK, Pillai KK, Ahmad SJ (2010) Antihyperglycaemic and antihyperlipidaemic effect of poly-constituents, in aqueous and chloroform extracts, of Withania coagulans Dunal in experimental type 2 diabetes mellitus in rats. Hum Exp Toxicol 29:653–658
Hooper PL (2014) Induction of heat shock proteins in the therapy of type 2 diabetes and metabolic syndrome. EBio Med 1:14–15
Hooper PL, Hooper JJ (2005) Loss of defense against stress: diabetes and heat shock proteins. Diabetes Technol Ther 7:204–208
Hooper PL, Balogh G, Rivas E (2014) The importance of the cellular stress response in the pathogenesis and treatment of type 2 diabetes. Cell Stress Chaperones 19:447–464
Hosseini A, Abdollahi M (2013) Diabetic neuropathy and oxidative stress: therapeutic perspectives. Oxidative Med Cell Longev 2013:168039
Hostalek U, Gwilt M, Hildemann S (2015) Therapeutic use of metformin in prediabetes and diabetes prevention. Drugs 75:1071–1094

Houghton PJ (2009) Synergy and polyvalence: paradigms to explain the activity of herbal products. In: Houghton PJ, Mukherjee PK (eds) Evaluation of herbal medicinal products. Pharmaceutical Press, London, pp 85–94

Huqi A (2015) Antidiabetic treatment and cardiovascular events: all a matter of perspective. Heart Metab 68:39–40

Jatwa R, Kar A (2009) Amelioration of metformin-induced hypothyroidism by Withania somnifera and Bauhinia purpurea extracts in Type 2 diabetic mice. Phytother Res 23:1140–1145

Jonathan G, Rivka R, Avinoam S et al (2015) Hypoglycemic activity of withanolides and elicitated Withania somnifera. Phytochem 116:283–289

Kapoor A, Kaur G, Kaur R (2015) Antimicrobial activity of different herbal plants extracts: a review. World J Pharm Pharm Sci 4:422–459

Kawahito S, Kitahata H, Oshita S (2009) Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress. World J Gastroenterol 15:4137–4142

Kennedy DO (2014) Plants and the human brain. Oxford University Press, London

Khalili M (2009) The effect of oral administration of Withania somnifera root on formalin-induced pain in diabetic rats. Basic Clin Neurosci 1:29–31

Khan MA, Khan H, Ali T (2014) Withanolides isolated from Withania somnifera with α-glucosidase inhibition. Med Chem Res 23:2386–2390

Khan SA, Chatterjee SS, Kumar V (2016) Low dose aspirin like analgesic and anti-inflammatory activities of mono-hydroxybenzoic acids in stressed rodents. Life Sci 148:53–62

Khodaei M, Mehrana J, Noori M (2012) Remedial use of Withanolides from Withania coagolans (Stocks) Dunal. Adv Life Sci 2:6–19

Knutson KL, Spiegel K, Penev P, Van Cauter E (2007) The metabolic consequences of sleep deprivation. Sleep Med Rev 11:163–178

Kojima H, Fujimiya M, Terashima T, Kimura H, Chan L (2006) Extrapancreatic proinsulin/insulin-expressing cells in diabetes mellitus: is history repeating itself? Endocr J 53:715–722

Kowall B, Lehnich AT, Strucksberg KH, Führer D, Erbel R, Jankovic N, Moebus S, Jöckel KH, Stang A (2016) Associations among sleep disturbances, nocturnal sleep duration, daytime napping, and incident prediabetes and type 2 diabetes: the heinz nixdorf recall study. Sleep Med 21:35–41

Kuboyama T, Tohda C, Komatsu K (2014) Effects of Ashwagandha (roots of Withania somnifera) on neurodegenerative diseases. Biol Pharm Bull 37:892–897

Kulkarni SK, Dhir A (2008) Withania somnifera: an Indian ginseng. Prog Neuro-Psychopharmacol Biol Psychiatry 32:1093–1105

Kulkarni SK, Ninan I (1997) Inhibition of morphine tolerance and dependence by Withania somnifera in mice. J Ethnopharmacol 57:213–217

Kumar A, Kalonia H (2007) Protective effect of Withania somnifera Dunal on the behavioral and biochemical alterations in sleep-disturbed mice (Grid over water suspended method). Indian J Exp Biol 45:524–528

Kumar A, Kalonia H (2008) Effect of Withania somnifera on sleep-wake cycle in sleep-disturbed rats: possible GABAergic mechanism. Indian J Pharm Sci 70:806–810

Kumar S, Vinothkumar D, Kumar S, Aslam A, Shajahan A (2011) The phytochemical constituents of Withania somnifera and Withania obtusifolia by GCMS analysis. Int J Pharm Phytochem Res 3:31–34

Kumar R, Singh JK, Nath A, Ali M, Kumar A, Anshupriya SP (2013) Effect of Withania somnifera on estrogen, cholesterol and subcellular structure of ovary of chlorpyrifos exposed mice. Carib J SciTech 1:61–69

Kumar V, Dey A, Hadimani MB, Markovic TL, Emerald M (2015a) Chemistry and pharmacology of Withania somnifera: an update. TANG [Humanitas Medicine] 5:e1

Kumar CU, Pokuri VK, Pingali U (2015b) Evaluation of the analgesic activity of standardized aqueous extract of Terminalia chebula in healthy human participants using hot air pain model. J Clin Diagn Res 9:FC01–FC04

Kyathanahalli CN, Manjunath MJ, Muralidhara (2014) Oral supplementation of standardized extract of Withania somnifera protects against diabetes-induced testicular oxidative impairments in prepubertal rats. Protoplasma 251:1021–1029
Lee SWH, Ng KY, Chin WK (2016) The impact of sleep amount and sleep quality on glycaemic control in type 2 diabetes: a systematic review and meta-analysis. Sleep Med Rev 31:91–101
Lee-Kubli CA, Mixcoatl-Zecuatl T, Jolivalt CG, Calcutt NA (2014) Animal models of diabetes-induced neuropathic pain. In: Taylor BK, Finn D (eds) Behavioral neurobiology of chronic pain. Springer, Berlin/Heidelberg, pp p147–p170
Lehner C, Gehwolf R, Wagner A, Resch H, Hirzinger C, Augat P, Stephan D, Aigner L, Rivera FJ, Bauer HC, Tempfer H (2012) Tendons from non-diabetic humans and rats harbor a population of insulin-producing, pancreatic beta cell-like cells. Horm Metab Res 44:506–510
Leroith D, Vinik AI (2008) Controversies in treating diabetes: clinical and research aspects. (Contemporary endocrinology). Humana press, Totowa
Liu Q, Chen L, Hu L, Guo Y, Shen X (2010) Small molecules from natural sources, targeting signaling pathways in diabetes. Biochim Biophys Acta 1799:854–865
Maia AC, Braga Ade A, Brouwers A, Nardi AE, Oliveira e Silva AC (2012) Prevalence of psychiatric disorders in patients with diabetes types 1 and 2. Compr Psychiatry 53:1169–1173
Marrero D, Pan Q, Barrett-Connor E, de Groot M, Zhang P, Percy C, Florez H, Ackermann R, Montez M, Rubin RR (2014) Impact of diagnosis of diabetes on health-related quality of life among high-risk individuals: the diabetes prevention program outcomes study. Qual Life Res 23:75–88
Mary NK, Babu BH, Padikkala J (2003) Antiatherogenic effect of Caps HT2, a herbal Ayurvedic medicine formulation. Phytomedicine 10:474–482
Mattson MP, Cheng A (2006) Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses. Trends Neurosci 29:632–639
Maurya R, Akanksha J, Singh AB, Srivastava AK (2008) Coagulanolide, a withanolide from Withania coagulans fruits and antihyperglycemic activity. Bioorg Med Chem Lett 18:6534–6537
McMurray JJ, Gerstein HC, Holman RR, Pfeffer MA (2014) Heart failure: a cardiovascular outcome in diabetes that can no longer be ignored. Lancet Diabetes Endocrinol 2:843–851
Mendenhall E, Norris SA, Shidhaye R, Prabhakaran D (2014) Depression and type 2 diabetes in low-and middle income countries: a systematic review. Diabetes Res Clin Pract 103:276–285
Miele L, Giorgio V, Alberelli MA (2015) Impact of gut microbiota on obesity, diabetes, and cardiovascular disease risk. Curr Cardiol Rep 17:1–7
Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazón J (2009) Steroidal lactones from Withania somnifera, an ancient Plant for Novel Medicine. Molecules 14:2373–2393
Misico RI, Nicotra VE, Oberti JC, Barboza G, Gil RR, Burton G (2002) Induction of quinone reductase by Withanolides. J Nat Prod 65:677–680
Misico RI, Nicotra VE, Oberti JC et al (2011) Withanolides and related steroids. In: Kinghorn AD, Falk H, Kobayashi J (eds) Progress in the chemistry of organic natural products. Springer, Vienna, pp p127–p229
Mohan R, Bargagna-Mohan P (2016) The use of Withaferin A to study intermediate filaments. In: Handel M (ed) Methods in enzymology, vol 568. Elsevier, Amsterdam, pp p187–p218
Molina MN, Ferder L, Manucha W (2016) Emerging role of nitric oxide and heat shock proteins in insulin resistance. Curr Hypertens Rep 18:1–13
Nabavi SF, Russo GL, Daglia M, Nabavi SM (2015) Role of quercetin as an alternative for obesity treatment: you are what you eat! Food Chem 179:305–310
Navinder KM, Sehrawat R, Khatak S (2013) A comparative study: homoepathic medicine and a medicinal plant Withania somnifera for antidiabetic activity. J Pharmacogn Phytochem 2:109–112
Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S (2012) Host-gut microbiota metabolic interactions. Science 336:1262–1267
Nirupama R, Devaki M, Nirupama M et al (2014) In vitro and in vivo studies on the hypoglycaemic potential of Ashwagandha (Withania somnifera) root. Pharma Sci Monitor 5(3):45–58

Orrù A, Marchese G, Casu G, Casu MA, Kasture S, Cottiglia F, Acquas E, Mascia MP, Anzani N, Ruiu S (2014) Withania somnifera root extract prolongs analgesia and suppresses hyperalgesia in mice treated with morphine. Phytomedicine 21:745–752

Orrù A, Casu MA, Tambaro S, Marchese G, Casu G, Ruiu S (2016) Withania somnifera (L.) Dunal root extract alleviates formalin-induced nociception in mice: involvement of the opioidergic system. Behav Pharmacol 27:57–68

Parihar MS, Chaudhary M, Shetty R, Hemnani T (2004) Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera. J Clin Neurosci 11:397–402

Parihar P, Shetty R, Ghafourifar P, Parihar MS (2015) Increase in oxidative stress and mitochondrial impairment in hypothalamus of streptozotocin treated diabetic rat: Antioxidative effect of Withania somnifera. Cell Mol Biol (Noisy-le-Grand) 62:73–83

Petrak F, Baumeister H, Skinner TC, Brown A, Holt RI (2015) Depression and diabetes: treatment and health-care delivery. Lancet Diabetes Endocrinol 3:472–485

Pingali U, Pilli R, Fatima N (2013) Effect of Withania somnifera extract on mental stress induced changes in hemodynamic properties and arterial wave reflections in healthy subjects. Curr Topics Nutraceuticals Res 11:151

Pingali U, Pilli R, Fatima N (2014) Effect of standardized aqueous extract of Withania somnifera on tests of cognitive and psychomotor performance in healthy human participants. Pharm Res 6:12–18

Prabhakar VK, Jaidka A, Singh R (2013) In vitro study on α -amylase inhibitory activity and phytochemical screening of few Indian medicinal plant having anti-diabetic properties. Int J Sci Res Pub 3:1–6

Pradeep S, Prem Kumar N, Khajuria DK, Rao GS (2010) Preclinical evaluation of antinociceptive effect of Withania somnifera (ashwagandha) in diabetic peripheral neuropathic rat models. Pharmacol Ther 2:283–298

Pratte MA, Nanavati KB, Young V, Morley CP (2014) An alternative treatment for anxiety: a systematic review of human trial results reported for the Ayurvedic herb ashwagandha (Withania somnifera). J Altern Complement Med 20:901–908

Puranik A, Patwardhan B (2012) Ayurveda and metabolic diseases: the whole is greater than the sum of the parts. In: Bagchi D, Sreejayan N (eds) Nutritional and therapeutic interventions for diabetes and metabolic syndrome. Elsevier Inc./Academic, Amsterdam/Boston, pp 443–456

Rahola GJ (2012) Somatic drugs for psychiatric diseases: aspirin or simvastatin for depression? Curr Neuropharmacol 10:139–158

Rai M, Jogee PS, Agarkar G, dos Santos CA (2016) Anticancer activities of Withania somnifera: current research, formulations, and future perspectives. Pharm Biol 54:189–197

Rainsford KD (2013) Aspirin and the salicylates. Elsevier, Burlington

Raut AA, Rege NN, Tadvi FM, Solanki PV, Kene KR, Shirolkar SG, Pandey SN, Vaidya RA, Vaidya AB (2012) Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (Withania somnifera) in healthy volunteers. J Ayurveda Integr Med 3:111–114

Robertson RP, Harmon JS (2006) Diabetes, glucose toxicity, and oxidative stress: a case of double jeopardy for the pancreatic islet β-cell. Free Radic Biol Med 41:177–184

Rochette L, Zeller M, Cottin Y, Vergely C (2014) Diabetes, oxidative stress and therapeutic strategies. Biochim Biophys Acta 1840:2709–2729

Roughani M, Baluchnejadmojarad T, Khalili M, Salimi SFM (2006) The effect of chronic oral administration of Withania somnifera root on learning and memory in diabetic rats using passive avoidance test. Sci J Hamadan Univ Med Sci 13:10–17

Roughani M, Baluchnejadmojarad T, Khalili M, Salimi SFM (2007) Evaluation of the effect of chronic oral administration of Withania somnifera root on pain score in male diabetic rats. Jundishapur Sci Med J:168–174

Rumore MM, Kim KS (2010) Potential role of salicylates in type 2 diabetes. Ann Pharmacother 44:1207–1221

Safhi MM, Anwer T (2011) Comparative analysis of Withania somnifera and Rhus coriaria on hyperglycemia and insulin sensitivity in type 2 diabetic rats. J Jazan University 1:39–49
Samadi AK (2015) Chapter three-potential anticancer properties and mechanisms of action of Withanolides. Enzyme 37:73–94
Samadi Noshahr Z, Shahraki MR, Ahmadvand H, Nourabadi D, Nakhaei A (2015) Protective effects of Withania somnifera root on inflammatory markers and insulin resistance in fructose-fed rats. Rep Biochem Mol Biol 3:62–67
Sarangi A, Jena S, Sarangi AK, Swain B (2013) Anti-diabetic effects of Withania somnifera root and leaf extracts on streptozotocin induced diabetic rats. J Cell Tissue Res 13:3597–3601
Sarkar S, Balhara YPS (2014) Diabetes mellitus and suicide. Indian J Endocrinol Metabol 18:468–474
Saxena B (2010) Anti-hyperlipidemic activity of Withania coagulans in streptozotocin-induced diabetes: a potent anti-atherosclerotic agent. Drug Discov Ther 4:334–340
Scheen AJ, Paquot N (2015) Obesity: a new paradigm for treating obesity and diabetes mellitus. Nat Rev Endocrinol 11:196–198
Schmidt MI, Duncan BB (2003) Diabesity: an inflammatory metabolic condition. Clin Chem Lab Med 41:1120–1130
Sehgal N, Gupta A, Valli RK, Joshi SD, Mills JT, Hamel E, Khanna P, Jain SC, Thakur SS, Ravindranath V (2012) Withania somnifera reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. PNAS 109:3511
Serhiyenko VA, Serhiyenko AA (2015) Diabetic cardiac autonomic neuropathy: do we have any treatment perspectives? World J Diabetes 6:245–258
Sharma H, Chandola HM (2011a) Prameha in Ayurveda: correlation with obesity, metabolic syndrome, and diabetes mellitus. Part 1–Etiology, classification, and pathogenesis. J Altern Complement Med 17:491–496
Sharma H, Chandola HM (2011b) Ayurvedic concept of obesity, metabolic syndrome, and diabetes mellitus. J Altern Complement Med 17:549–552
Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14
Singh G, Kumar P (2011) Evaluation of antimicrobial efficacy of flavonoids of Withania somnifera L. Indian J Pharm Sci 73:473–478
Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand KK (2001) Adaptogenic activity of a novel, withanolide-free aqueous fraction from the roots of Withania somnifera Dun. Phytother Res 15:311–318
Singh B, Chandan BK, Gupta DK (2003) Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of Withania somnifera Dun. (Part II). Phytother Res 17:531–536
Singh A, Duggal S, Singh H, Singh J, Katekhaye S (2010) Withanolides: phytoconstituents with significant pharmacological activities. Int J Green Pharm 4:229–237
Singh R, Arif T, Khan I, Sharma P (2014) Phytochemicals in antidiabetic drug discovery. J Biomed Ther Sci 1:1–33
Snoek FJ, Bremmer MA, Hermanns N (2015) Constructs of depression and distress in diabetes: time for an appraisal. Lancet Diabetes Endocrinol 3:450–460
Son TG, Camandola S, Mattson MP (2008) Hormetic dietary phytochemicals. NeuroMolecular Med 10:236–246
Sonar VP, Cottiglia F, Ruiu S, Orru A, Anzani N, Floris C (2015) Withanolides and alkaloids from Withania somnifera roots with binding affinity to opioid, cannabinoids and GABAergic receptors. Planta Med 81:122
Spoormaker VI, van den Bout J (2005) Depression and anxiety complaints; relations with sleep disturbances. Eur Psychiatry 20:243–245
Thakur AK, Shakya A, Husain GM, Emerald M, Kumar V (2014) Gut-microbiota and mental health: current and future perspectives. J Pharmacol Clin Toxicol 2:1016–1031
Thakur AK, Dey A, Chatterjee SS, Kumar V (2015) Reverse ayurvedic pharmacology of Ashwagandha as an adaptogenic anti-diabetic plant: a pilot study. Curr Trad Med 1:51–61

Thomas RP, Hellmich MR, Townsend CM Jr, Evers BM (2003) Role of gastrointestinal hormones in the proliferation of normal and neoplastic tissues. Endocr Rev 24:571–599
Tiwari N, Thakur AK, Kumar V, Dey A, Kumar V (2014) Therapeutic targets for diabetes mellitus: an update. Clinic Pharmacol Biopharmaceut 3:117
Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF (2015) A natural solution for obesity: Bioactives for the prevention and treatment of weight gain. A review. Nutr Neurosci 18:49–65
Udayakumar R, Kasthurirengan S, Mariashibu TS, Rajesh M, Anbazhagan VR, Kim SC, Ganapathi A, Choi CW (2009) Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. Int J Mol Sci 10:2367–2382
Udayakumar R, Kasthurirengan S, Vasudevan A, Mariashibu TS, Rayan JJ, Choi CW, Ganapathi A, Kim SC (2010) Antioxidant effect of dietary supplement Withania somnifera L. reduce blood glucose levels in alloxan-induced diabetic rats. Plant Foods Hum Nutr 65:91–98
Ugurlucan M, Caglar IM, Caglar FN, Ziyade S, Karatepe O, Yildiz Y, Zencirci E, Ugurlucan FG, Arslan AH, Korkmaz S, Filizcan U, Cicek S (2012) Aspirin: from a historical perspective. Recent Pat Cardiovasc Drug Discov 7:71–76
Usharani P, Nalini P, Manjunath NK, Reddy KSK (2013) Evaluation of the analgesic activity of standardized aqueous extract of Withania somnifera in healthy human volunteers using hot air pain model. Res J Life Sci 1:1–6
Usharani P, Fatima N, Kumar CU, Kishan PV (2015) Evaluation of a highly standardized Withania somnifera extract on endothelial dysfunction and biomarkers of oxidative stress in patients with type 2 diabetes mellitus: a randomized, double blind, placebo controlled study. Int J Ayur Pharma Res 2:22–32
Vaishnavi K, Saxena N, Shah N, Singh R, Manjunath K, Uthayakumar M, Kanaujia SP, Kaul SC, Sekar K, Wadhwa R (2012) Differential activities of the two closely related Withanolides, Withaferin A and Withanone: bioinformatics and experimental evidences. PLoS One 7:e44419
Vanden Berghe W, Sabbe L, Kaileh M, Haegeman G, Heyninck K (2012) Molecular insight in the multifunctional activities of Withaferin A. Biochem Pharmacol 84:1282–1291
Vincent RP, Ashrafian H, le Roux CW (2008) Mechanisms of disease: the role of gastrointestinal hormones in appetite and obesity. Nat Clin Pract Gastroenterol Hepatol 5:268–277
Visavadiya NP, Narasimhacharya AV (2007) Hypocholesteremic and antioxidant effects of Withania somnifera (Dunal) in hypercholesteremic rats. Phytomedicine 14:136–142
Visavadiya NP, Narasimhacharya AV (2011) Ameliorative effects of herbal combinations in hyperlipidemia. Oxidative Med Cell Longev 2011:160408
Wadhwa R, Singh R, Gao R, Shah N, Widodo N, Nakamoto T, Ishida Y, Terao K, Kaul SC (2013) Water extract of Ashwagandha leaves has anticancer activity: identification of an active component and its mechanism of action. PLoS One 8:e77189
Wadhwa R, Konar A, Kaul SC (2015) Nootropic potential of Ashwagandha leaves: beyond traditional root extracts. Neurochem Int 95:109–118
White JR Jr (2014) A brief history of the development of diabetes medications. Diabetes Spectr 27:82–86
WHO (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. WHO, Geneva, p 21
Wijeratne EM, Xu YM, Scherz-Shouval R, Marron MT, Rocha DD, Liu MX, Costa-Lotufo LV, Santagata S, Lindquist S, Whitesell L, Gunatilaka AA (2014) Structure–activity relationships for Withanolides as inducers of the cellular heat-shock response. J Med Chem 57:2851–2863
Winston D, Maimes S (2007) Adaptogens: herbs for strength, stamina, and stress relief. Inner Traditions/Bear & Co/Healing Arts Press, Rochester
Xu X, Guo L, Tian G (2013) Diabetes cognitive impairments and the effect of traditional chinese herbs. Evid Based Complement Alternat Med 2013:649396
Zajac J, Shrestha A, Patel P, Poretsky L (2010) The main events in the history of diabetes mellitus. In: Principles of diabetes mellitus. Springer, New York
Zhang H, Tan C, Wang H, Xue S, Wang M (2010) Study on the history of traditional Chinese medicine to treat diabetes. Eur J Integr Med 2:41–46

Zhang H, Samadi AK, Cohen MS, Timmermann BN (2012) Antiproliferative Withanolides from the Solanaceae: a structure–activity study. Pure Appl Chem 84:1353–1367
Zhang H, Cao CM, Gallagher RJ, Timmermann BN (2014) Antiproliferative Withanolides from several solanaceous species. Nat Prod Res 28:1941–1951
Zimmet P, Thomas CR (2003) Genotype, obesity and cardiovascular disease – has technical and social advancement outstripped evolution. J Intern Med 254:114–125
Zimmet PZ, Magliano DJ, Herman WH, Shaw JE (2014) Diabetes: a 21st century challenge. Lancet Diabetes Endocrinol 2:56–64