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REVIEW

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Withania somnifera (Indian ginseng) in diabetes mellitus: A systematic review and meta-analysis of scientific evidence from experimental research to clinical application

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Withania somnifera Dunal, also known as Indian ginseng, has been in use since ancient times in the management of diabetes mellitus (DM). This systematic review and meta-analysis evaluated the efficacy/effectiveness, safety and tolerability of *W. somnifera* in managing DM. Literature search (published/unpublished) was performed from inception to April 2019 in guidelines recommended databases. A total of 6 in-vitro, 13 pre-clinical and 5 clinical studies were included for systematic evaluation. *W. somnifera* treatment in DM significantly restored the altered levels of blood glucose (experimental data; mean difference, −196.27; 95% confidence interval [−220.96, −171.58]; $p < .00001$) glycosylated haemoglobin (HbA1c), insulin, lipid profile, serum and oxidative stress markers with no safety concerns. The results suggest the potential role of *W. somnifera* in managing DM. However, the available clinical data are not considerably enough to provide novel and sufficiently robust evidence for the use of *W. somnifera* in managing DM. To further strength the antidiabetic profile of *W. somnifera*, well-designed randomized-controlled trial(s) with a larger sample size and longer duration is warranted with evaluation of its effect primarily on blood glucose, HbA1c and insulin. Future research also needs to elucidate the molecular mechanism(s) of *W. somnifera* including its active principles in DM.

KEYWORDS

diabetes mellitus, Indian ginseng, meta-analysis, oxidative stress, systematic review

1 | INTRODUCTION

Diabetes mellitus (DM), a chronic and progressive heterogeneous metabolic disorder, is characterized by elevated levels of blood glucose—a common effect if uncontrolled, may gradually lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves (Guariguata et al., 2014; World Health Organization, 2016). Due to diabetes, in 2012, there were 1.5 million deaths, and elevated blood glucose additionally led to 2.2 million deaths due to increased risks of cardiovascular (CV) and other diseases (World Health Organization, 2016). The latest WHO report states that the global estimates of diabetes prevalence, in adult population, had nearly doubled from 1980 to 2014, rising from 4.7% (108 million) to 8.5% (422 million; World Health Organization, 2016). Further, as per 2013 estimate (in millions), China (98.4) had the highest number of adult population with diabetes, followed by India (65.1) and the United States (24.4): the projections for 2035 also showed continuing increase in patients with diabetes in these three countries (in millions); 142.7, 109.0 and 29.7, respectively (Guariguata et al., 2014). With the increasing burden of diabetes, effective preventive measures and management are required to reduce an individual's risk of developing diabetes and its complications. Scientific evidence shows that lifestyle interventions such as healthy diet and physical

Dedication: The authors dedicate this work to his holiness Dr Sri Shivakumar Swamiji who is an inspiration to millions of lives to serve the humanity.

activity are more effective than medication in delaying or preventing diabetes, especially in whose who are overweight or with impaired glucose tolerance (Diabetes Prevention Program Research Group, 2009, 2015; Knowler et al., 2002; Lim et al., 2011; Tuomilehto et al., 2001). The concept of ancient Indian System of Medicine (ISM; Ayurveda) also emphasizes healthy eating with regular physical and psychological exercises for preventing and managing diabetes (Sharma & Chandola, 2011).

Withania somnifera Dunal (in Sanskrit, Hayagandhā/Vājigandhā; Family, Solanaceae), commonly known as Indian ginseng, winter cherry and ajagandha, is a dietary supplement with various nutrients and phytochemicals in its composition (Department of AYUSH, 1989; Facciola, 1998; Udayakumar et al., 2010). The ISM describes *W. somnifera* as *Rasayanas* (rejuvenating tonic), which promote health and longevity, and create a sense of mental well-being (Dar, Hamid, & Ahmad, 2015; Durg, Dhadde, Vandal, Shivakumar, & Charan, 2015; Durg, Shivaram, & Bavage, 2018; Kuppurajan et al., 1980; D. B. A. Narayana, Durg, Manohar, Mahapatra, & Aramya, 2017; Weiner & Weiner, 1994). The European Commission recognizes *W. somnifera* as a food or food ingredient, and its access to the market is not subject to the Novel Food Regulation (EC) No. 258/97 (http://ec.europa.eu/food/safety/novel_food/catalogue/search/public/index.cfm: Product Name—*W. somnifera*). In the USA, *W. somnifera* is freely available as a dietary supplement under Dietary Supplement Health and Education Act (DSHEA of 1994); however, it does not have generally recognized as safe (GRAS) status (Mills, Simon Mills, & Bone, 2004).

The whole plant of *W. somnifera*, although dried mature roots most widely, has been in use for its medicinal properties, such as anti-inflammatory, anti-microbial, anti-tumour, anti-stress, antioxidant, immunomodulatory, haemopoietic and rejuvenating properties besides its positive influence on the endocrine, cardiopulmonary and central nervous system (Durg et al., 2015, 2018; Kaul & Wadhwa, 2017; Kulkarni & Dhir, 2008; Weiner & Weiner, 1994). *W. somnifera*, generally in the form of tea, used to enhance immunity, CV system and promote detoxification which helps to maintain good health (Bhat et al., 2010; Reuland et al., 2013).

Experimental (in-vitro/pre-clinical) and clinical studies have demonstrated anti-diabetic effects of *W. somnifera* extracts. The active constituents of *W. somnifera*, viz. withanone, withaferin A, withanolide and sitoindosides, are reported to possess antioxidant activity which may contribute to the aforementioned beneficial effects of the title herb (Durg et al., 2015; Kaul & Wadhwa, 2017; Kulkarni & Dhir, 2008).

W. somnifera, though, has been well documented with scientific reports for its anti-diabetic activity; however, till now, there is no systematic scientific assessment exploring its therapeutic efficacy in clinical research/effectiveness in clinical practice, safety and tolerability in managing DM. Constructing on these, the present study aimed for systematic evaluation and meta-analysis of *W. somnifera* effects in managing DM. The study also updates the information of *W. somnifera* extract and its isolated marker compounds used in the experimental and clinical studies.

2 | MATERIALS AND METHODS

2.1 | Literature search

A systematic literature search of published and unpublished studies reporting anti-diabetic activity of *W. somnifera* was performed following the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Liberati et al., 2009); complete details are provided in the Supplementary material.

2.2 | Inclusion and exclusion criteria

Experimental (in-vitro/pre-clinical) and clinical studies which evaluated anti-diabetic activity of *W. somnifera* were included; complete details are provided in the Supplementary material.

2.3 | Data extraction and quality (risk of bias) assessment

The included studies were systematically reviewed, and data extraction was performed, if the parameter of interest was reported in at least two studies; complete details are provided in the Supplementary material besides quality (risk of bias) assessment details.

2.4 | Data analysis

The meta-analysis was performed using Review Manager (RevMan; Computer program), version 5.3.5, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014; complete details are provided in the Supplementary material. In all analyses, a p-value <.05 (two-tailed test) was considered statistically significant.

3 | RESULTS

3.1 | Selection of studies

Figure 1, PRISMA flow-chart of study selection, depicts the overall process of study selection. A total of 24 studies met the inclusion criteria; in-vitro (n = 6; Huerta, Mihalik, Becket, Maitin, & Vattem, 2010; Jonathan, Rivka, Avinoam, Lumír, & Nirit, 2015; Khan, Khan, & Ali, 2014; Nirupama, Devaki, Nirupama, & Yajurvedi, 2014; Shah, Khan, Vakil, & Qureshi, 2017; Singh, Joshi, & Jatwa, 2013); pre-clinical (n = 13 [mice model, n = 2; rat model, n = 11]; Anwer et al., 2017; Anwer, Sharma, Pillai, & Iqbal, 2008; Anwer, Sharma, Pillai, & Khan, 2012; Jain, Pandhi, Singh, & Malhotra, 2006; Kiasalari, Khalili, & Aghaei, 2009; Kyathanahalli, Manjunath, & Muralidhara, 2014; M. S. Parihar, Chaudhary, Shetty, & Hemnani, 2004; P. Parihar, Shetty, Ghafourifar, & Parihar, 2016; Sarangi, Jena, Sarangi, & Swain, 2013; Tekula, Khurana, Anchi, & Godugu, 2018; Thakur, Dey, Shyam

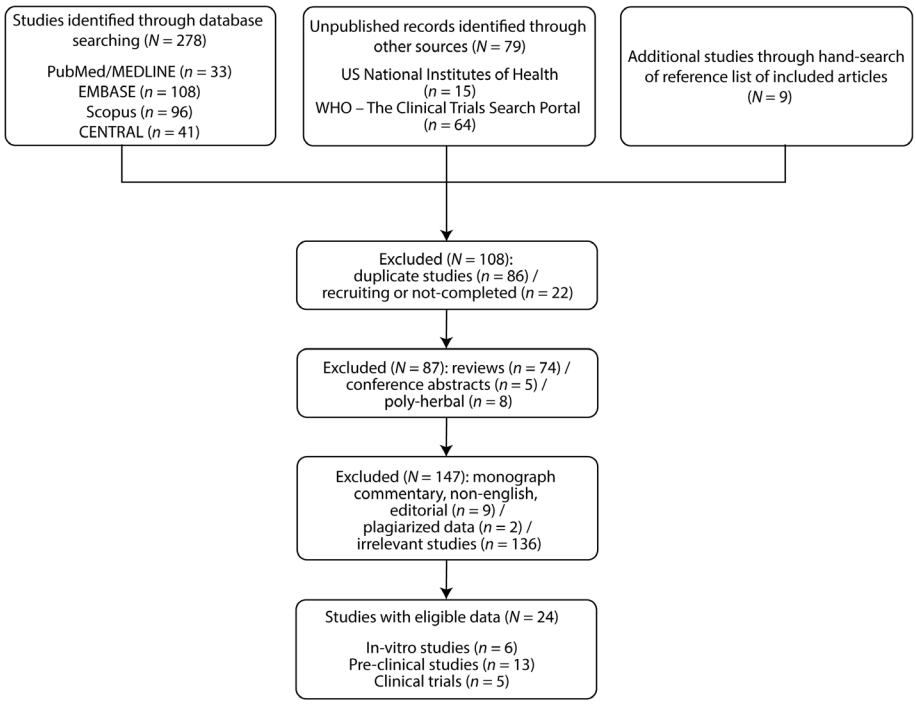

FIGURE 1 Preferred reporting items for systematic reviews and meta-analyses flow-chart of study selection

Chatterjee, & Kumar, 2015; Udayakumar et al., 2009, 2010); clinical (*n* = 5; Agnihotri, Sontakke, Thawani, Saoji, & Goswami, 2013; Andallu & Radhika, 2000; Nayak, Nayak, Panda, & Das, 2015; Usharani, Fatima, Kumar, & Kishan, 2014; Usharani, Kishan, Fatima, & Kumar, 2014). The duration of included pre-clinical studies ranged from 10 to 56 days, and clinical trials from 30 to 84 days which used authenticated extract of *W. somnifera*. The descriptions of included studies are summarized in Tables 1, 2 and 3.

3.2 | In-vitro studies

Jonathan and colleagues assessed anti-diabetic activity of *W. somnifera* leaf and root extracts besides the following purified withanolides: withaferin A, withanone, withanolide A/B and withanoside IV. A dose-dependent increase in glucose uptake in myotubes and adipocytes was noted by both leaf and root extracts. Further, leaf extract enhanced insulin secretion in basal pancreatic β-cells, but not in stimulated cells. Among all tested purified withanolides, withaferin A in skeletal myotubes increased glucose uptake by 54%, signifying its role for the observed anti-diabetic activity of *W. somnifera* (Jonathan et al., 2015). Huerta et al. reported *W. somnifera* effect on reducing oxidative stress (OS), inhibition of carbohydrate and lipid digestion in the gastrointestinal tract. The low and high molecular weight *W. somnifera* fraction, respectively, neutralized ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) radical formation (>40 and <20%), decreased thiobarbituric acid reactive substances (TBARS) formation (<20 and <15%), inhibited rat intestinal maltase activity (<20% each) and reduced lipase activity (<10 and 32%). However, both the fractions of *W. somnifera* did not exhibit any α-amylase or sucrase inhibition activity (Huerta et al., 2010). Khan et al. reported that three withanolides (1–3), isolated from chloroform soluble fraction of *W. somnifera* whole plant, showed concentration-dependent potent α-glucosidase inhibitory activity. The maximum inhibition was observed at 100 μg/ml; 69.74, 96.07 and 94.33%, respectively (Khan et al., 2014). Chloroform and ethanolic root extracts of *W. somnifera* were found to be more effective in inhibiting α-amylase activity, elevating glucose uptake by yeast cells and increasing glucose adsorption and decreasing diffusion compared to other extracts such as petroleum ether, benzene, cold and hot water, sodium hydroxide and aqueous crude extracts (Nirupama et al., 2014). *W. somnifera* leaf and root extracts were evaluated against 3T3F442A fibroblast (3T3 adipocyte) cell line, using glucose uptake assay, in presence of insulin. Leaf extract was active at above 10 μg/ml and root extract showed good glucose promoting activity at 40 μg/ml; the isolated fractions, withaferin A and withanolide A, were active at 20 μg/ml (Shah et al., 2017). Singh et al. (2013) noted that methanolic extract of *W. somnifera* root powder showed significant ($p < .001$) dipeptidyl peptidase-IV (DPP-IV) enzyme inhibition (69.7%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging (61.1%), antioxidant activity (47%) and reducing power (0.19%). Kyathanahalli et al. measured *W. somnifera* (dried powder; 0–500 μg) potential in scavenging DPPH, hypochlorous acid (HOCl) and hydroxyl radicals when added to the methanolic solution of DPPH or HOCl–taurine–KI mixture. The IC_{50} of *W. somnifera* extract inhibited the stable-free radical DPPH (365 μg/ml) and HOCl (100 μg/ml) as well significant dose-dependent (440 μg/ml) deoxyribose inhibition (Kyathanahalli et al., 2014).

3.3 | Body weight

Single intraperitoneal (ip) injection of streptozotocin (STZ; 90 mg/kg) decreased body weight in experimental prepubertal rats (Kyathanahalli et al., 2014). Alloxan monohydrate (AM) injection

TABLE 1 Description of included in-vitro studies

Study	Description of *W. somnifera*/its derivatives	Outcomes
Jonathan et al. (2015)	*W. somnifera* leaf and root extracts, and six purified withanolides were prepared from dried plant material according to the published literature	Anti-diabetic activity based on glucose uptake in myotubes and adipocytes, insulin secretion in basal pancreatic β-cells Effect of elicitors (methyl salicylate and chitosan) on anti-diabetic activity
Huerta et al. (2010)	*W. somnifera* was obtained from Taj imports (Austin, TX); the extraction of low and high molecular weight *W. somnifera* fraction according to the published literature	ABTS radical and TBARS formation, α-amylase inhibition, and maltase, sucrase and lipase activities
Khan et al. (2014)	Withanolides isolated from the shade-dried whole *W. somnifera* plant material according to the published literature	α-Glucosidase inhibitory assay with IC_{50} values
Nirupama et al. (2014)	*W. somnifera* petroleum ether, benzene, chloroform, alcohol, cold and hot water, NaOH, and aqueous crude root extract were prepared according to the published literature	Glucose uptake/transport by yeast cells, α-amylase inhibitory activity, glucose adsorption and diffusion
Shah et al. (2017)	*W. somnifera* (10 g) leaf and root powder extracted according to the published literature	Glucose uptake assay (via 3T3 F442A fibroblast [adipocyte] cells)
Singh et al. (2013)	Methanolic extract of *W. somnifera* root powder (herb: extract 5:1 w/w) was gifted (voucher specimens, WS -1,142) by Amasar Pvt. Ltd, Indore, India	DPP-IV enzyme assay DPPH scavenging assay Total phenolics content Antioxidant activity Reducing power

Abbreviations: ABTS, azinobis 3-ethyl-benzothiazoline-6-sulfonic acid; DPP-IV, dipeptidyl peptidase-IV; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TBARS, thiobarbituric acid reactive substances.

(150 mg/kg, ip) too showed continuous body weight loss in diabetic rats (Udayakumar et al., 2009). Also, STZ (65 mg/kg) and nicotinamide (120 mg/kg, 15 min after STZ) ip injection demonstrated decrease in body weight in type-2 diabetic mice (Thakur et al., 2015). The meta-analysis data confirmed that oral supplementation of *W. somnifera* (root/leaf extract) significantly (Table 4; Figure S1) protected body weight loss in diabetic rats/mice. Tekula and colleagues assessed the effect of withaferin A on body weight in type-1 diabetes mice. The study noted insignificant decrease in body weight in diabetic control mice; supplementation of withaferin A (2 and 10 mg/kg) showed insignificant body weight improvement in diabetic mice (Tekula et al., 2018).

3.4 | Blood glucose and glycosylated haemoglobin

In experimental rats, AM/STZ injection produced significant increase in blood glucose level depicting diabetes. The meta-analysis of *W. somnifera* (root/leaf extract) effect in diabetic rats reported significant ($p < .00001$; Figure 2a) reduction in blood glucose level. Parihar and colleagues also assessed the dietary effect of *W. somnifera* on blood glucose level in STZ-induced diabetic mice and rats, respectively. *W. somnifera* treated diabetic rats and mice showed significant (43.44%; $p < .05$) and numerical (31.70%) decrease in blood glucose level (M. S. Parihar et al., 2004; P. Parihar et al., 2016). Thakur et al. evaluated three different doses (25/50/100 mg/kg) of *W. somnifera* in diabetic mice and all three doses significantly ($p < .05$) lowered (27.32%/44.76%/55.81%) plasma glucose levels (Thakur et al., 2015). Withaferin A (2 and 10 mg/kg) supplementation, in 3 and 4 weeks, also statistically ($p < .001$) reduced the elevated blood glucose levels in type-1 diabetes mice (Tekula et al., 2018). Clinical studies reported significant reduction in fasting blood glucose level ($p = .001$; Figure 2b) on *W. somnifera* administration. Similarly, *W. somnifera* supplementation (root/leaf extract) significantly ($p < .00001$; Figure 3) reduced elevated HbAlc levels to near normal in diabetic rats. Further, a clinical study also observed with significant reduction in post prandial blood glucose (PPBS) and HbAlc levels in patients who were on oral hypoglycaemic agents (metformin, 500 mg + glimepride, 1 mg) with or without *W. somnifera*; however, the reduction was higher (PPBS, 29.66 vs. 25.51%; HbA1c, 13.73 vs. 9.31%) in patients receiving oral hypoglycaemic agents with *W. somnifera* (Nayak et al., 2015).

3.5 | Insulin level/sensitivity and homeostasis model assessment of insulin resistance

Anwer and colleagues reported the effect of *W. somnifera* on insulin level in STZ-induced type-2 diabetic rats. STZ injection resulted in hyperinsulinemia and treatment with *W. somnifera* (200 and 400 mg/kg) statistically ($p < .001$) reduced (17.71 and 30.21%) the elevated levels of insulin (Anwer et al., 2008). On the contrary, Thakur et al. reported significantly lower levels of insulin in mice injected with STZ (65 mg/kg) and nicotinamide (120 mg/kg, 15 min after STZ). Oral supplementation of *W. somnifera* (50 and 100 mg/kg) in a dose-dependent manner increased ($p < .05$; 32.99 and 52.57%) plasma insulin levels (Thakur et al., 2015). Multiple low doses of STZ (40 mg kg^{-1} day^{-1}, for 5 consecutive days) ip injection significantly ($p < .001$) decreased plasma and tissue insulin levels, indicating type-1

TABLE 2 Description of included pre-clinical studies

Study ID	Diabetes inducing agent (dose mg/kg b.w, route)	Description of *W. somnifera*/its derivatives	Dose (route)	Duration of treatment	Experimental design	Parameters studied
Anwer et al. (2008)	STZ (100 mg/kg, ip)	Standardized powdered, aqueous root extract of *W. somnifera* (batch no. WS/05002; withanolides, 3.9% w/w) was gifted by Natural Remedies, Bangalore, India	200 and 400 mg/kg (oral)	5 weeks	Albino Wistar rats (*N* = 30); five groups (each containing six rats) Group 1; normal control (citrate buffer 0.1 ml/10 g, ip): Group 2; diabetic control (single dose of STZ): Group 3; single dose of STZ + *W. somnifera* (200 mg/kg): Group 4; single dose of STZ + *W. somnifera* (400 mg/kg): Group 5; only *W. somnifera* (400 mg/kg)	Blood glucose, HbA_1c, insulin level, oral glucose tolerance test and insulin sensitivity
Anwer et al. (2012)	STZ (100 mg/kg, ip)	Standardized powdered, aqueous root extract of *W. somnifera* (batch no. WS/05002; withanolides, 3.9% w/w) was gifted by Natural Remedies, Bangalore, India	200 and 400 mg/kg (oral)	5 weeks	Albino Wistar rats (*N* = 24); four groups (each containing six rats) Group 1; normal control (citrate buffer): Group 2; diabetic control (single dose of STZ): Group 3; single dose of STZ + *W. somnifera* (200 mg/kg): Group 4: Single dose of STZ + *W. somnifera* (400 mg/kg)	Blood glucose, oxidative stress markers (MDA [a secondary product of LPO], GSH, GPx, GR, GST, SOD and CAT) and histopathological examination of pancreas
Anwer et al. (2017)	STZ (100 mg/kg, ip)	Aqueous root extract of *W. somnifera* (batch no. WS/05002; withanolides, 3.9% w/w) was gifted by natural remedies, Bangalore, India	200 and 400 mg/kg	5 weeks	Albino Wistar rats (*N* = 30); five groups (each containing six rats) Group 1; normal control (citrate buffer, 0.1 ml/kg, ip): Group 2; diabetic control (single dose of STZ); Group 3; only *W. somnifera* (400 mg/kg): Group 4; single dose of STZ + *W. somnifera* (200 mg/kg): Group 5; single dose of STZ + *W. somnifera* (400 mg/kg)	Blood glucose, lipid profile (TC, TG, HDL-C, LDL-C, and VLDL-C), serum markers (LDH and CK), oxidative stress markers (MDA [a secondary product of LPO], GSH, GPx, GR, GST, SOD and CAT), and protein (in heart)
Jain et al. (2006)	STZ (60 mg/kg, ip)	Alcoholic root extract of *W. somnifera* (1.5% withanolides) procured from Sanat Products, New Delhi, India	20 mg/kg	3 weeks	Sprague–Dawley rats (150–200 g) of either sex (*N* = 42); seven groups (each containing six rats) Group 1; diabetic control: Group 2; *W. somnifera* (20 mg/kg): Group 3; *A. sativum* (100 mg/kg): Group 4; *G. sylvestre* (100 mg/kg): Group 5; *F. foetida* (100 or 200 mg/kg):	Fasting blood glucose Blood glucose

(Continues)

TABLE 2 (Continued)

Study ID	Diabetes inducing agent (dose mg/kg b.w, route)	Description of *W. somnifera*/its derivatives	Dose (route)	Duration of treatment	Experimental design	Parameters studied
					Group 6; *M. koenigii* (200 mg/kg): Group 7; insulin	
Kiasalari et al. (2009)	STZ (60 mg/kg, ip)	*W. somnifera* root was procured from the local market, then powdered and mixed in pelleted food at ratio of 6.25%	*W. somnifera* powdered root-mixed pelleted food at ratio of 6.25%	4 weeks	Adult male (195–220 g) Wistar rats (*N* = 39); four groups Group 1 (*n* = 8); normal control (*W. somnifera*, 4 weeks): Group 2 (*n* = 11); sham: Group 3 (*n* = 9); diabetic control: Group 4 (*n* = 11); single dose of STZ + *W. somnifera*	Serum sexual hormone levels (estrogen, progesterone, FSH, LH and testosterone), serum glucose, cholesterol and triglyceride levels
Kyathanahalli et al. (2014)	STZ (90 mg/kg, ip)	Standard root extract of *W. somnifera* powder (batch number: C81015; anolides, 2.57%; aferin A, 2.38%) was procured from M/s Sami Labs Ltd., Bangalore, India The major active principles: Anolides (steroidal alkaloids and steroidal lactones) with glycosides, starch, and variety of amino acids in trace quantities	500 mg/kg (oral)	15 days	Four weeks old prepubertal male (40 ± 5 g) CFT-Wistar rats (*N* = 18); three groups (each containing six rats) Group 1; normal control: Group 2; diabetic control (single dose of STZ): Group 3; single dose of STZ + *W. somnifera* (500 mg/kg)	Total polyphenol and flavonoid content analysis In-vitro: Anti-oxidant and free radical scavenging activity (DPPH, HOCl and hydroxyl radicals) In-vivo: Body weight, organ weights (testis and liver), plasma glucose, testicular oxidative stress markers (ROS, LPO, GSH, SOD, GPx, GST, GR, G6PDH, LDH, CAT and 3β-HSD), testis glutathione, total thiol and nonprotein thiol
M. S. Parihar et al. (2004)	STZ (60 mg/kg, ip)	Methanolic fraction of *W. somnifera* root extract was prepared according to the published research study	20 mg/kg (oral)	1 month	Female (25 g) Swiss albino mice (*N* = 40); five groups (each containing eight mice) Group 1; normal control: Group 2; diabetic control (single dose of STZ): Group 3; single dose of STZ + *W. somnifera* (20 mg/kg): Group 4; single dose of STZ + *Aloe vera* (32 mg/kg): Group 5; single dose of STZ + combination of *W. somnifera* and aloe vera	LPO and protein carbonyl in hippocampus and cerebral cortex, blood glucose, total protein content in brain homogenates, iron reducing activity of extracts and behavioral test (motor and memory performance)
P. Parihar et al. (2016)	STZ (60 mg/kg, ip)	Same as M. S. Parihar (2004)	35 mg/kg (oral)	30 days	Adult (250–300 g) male Wistar rats (*N* = 40); four groups (each containing 10 rats) Group 1; normal control: Group 2; *W. somnifera* (35 mg/kg): Group 3; diabetic control (single dose of STZ): Group 4; single dose of STZ + *W. somnifera* (35 mg/kg)	Total anti-oxidant activity of extract (ferric [Fe^{3+}] reducing anti-oxidant power and ferrozine assay), total protein content of hypothalamus tissue homogenates, LPO and protein carbonyl in hippocampus, GSH, GPx, mitochondrial permeability transition, blood glucose

(Continues)

TABLE 2 (Continued)

Study ID	Diabetes inducing agent (dose mg/kg b.w, route)	Description of *W. somnifera*/its derivatives	Dose (route)	Duration of treatment	Experimental design	Parameters studied
Sarangi et al. (2013)	STZ (150 mg/kg, ip)	Root and leaf extract of *W. somnifera* were prepared according to the published research study	200 mg/kg (oral)	8 weeks	Adult (150–180 g) male albino Wistar rats (N = 24); four groups (each containing six rats) Group 1; normal control (distilled water): Group 2; diabetic control (single dose of STZ): Group 3; single dose of STZ + *W. somnifera* root extract (200 mg/kg): Group 4; single dose of STZ + *W. somnifera* leaf extract (200 mg/kg)	Blood glucose, lipid profile (TC, TG, HDL-C, and LDL-C), serum enzymes (AST, ALT, ALP and LDH), total protein, albumin, globulin and A:G ratio
Tekula et al. (2018)	STZ (40 mg/kg, ip)	Withaferin A (>98% pure) was purchased from Aptus therapeutics, Hyderabad, India (characterized by NMR and FTIR spectroscopy for integrity)	2 and 10 mg/kg (oral)	4 weeks	Male Swiss albino mice (*N* = 40); five groups (each containing eight mice) Group 1; normal control: Group 2; diabetic Control (multiple low dose of STZ) + vehicle: Group 3; drug control (WA per se) which were treated with 10 mg/kg WA alone: Group 4; MLD-STZ + WA (2 mg/kg): Group 5: MLD-STZ + WA (10 mg/kg)	Body weight, blood glucose, intraperitoneal glucose tolerance test, pancreatic oxidative and nitrosative stress Plasma and tissue inflammatory cytokine levels, plasma and tissue insulin estimation Nuclear p65-NFκB levels in pancreatic tissues, histopathology and insulitis scoring Immunohistochemistry, TUNEL assay
Thakur et al. (2015)	STZ (65 mg/kg, ip)	Methanolic extract prepared from root of *W. somnifera* according to the published research study	500 mg/kg	10 days	Adult male (25 ± 5 g) albino mice (*N* = 30); five groups (each containing six mice) Group 1: Normal control; Group 2: Diabetic control (single dose of STZ + vehicle only); Group 3: Single dose of STZ + *W. somnifera* (25 mg/kg); Group 4: Single dose of STZ + *W. somnifera* (50 mg/kg); Group 5: Single dose of STZ + *W. somnifera* (100 mg/kg)	Body weight, basal rectal temperature, foot shock stress-triggered transient hyperthermia, organ weights (adrenal glands and spleen), plasma glucose, insulin, cortisol levels
Udayakumar et al. (2009)	Alloxan (150 mg/kg, ip)	Root and leaf extract of *W. somnifera* were prepared according to the published research study	100 and 200 mg/kg (oral)	8 weeks	Male (150–180 g) albino Wistar rats (*N* = 42) seven groups (each containing six rats) Group 1; normal control (distilled water): Group 2; diabetic control	Body weight, urine sugar, blood glucose, Hb, HbA1c, liver glycogen, lipid profile (TC, TG, PL, HDL-C, LDL-C and VLDL-C), tissue lipids (heart, liver and kidney; TC, TG and PL), serum protein (total protein, albumin

(Continues)

TABLE 2 (Continued)

Study ID	Diabetes inducing agent (dose mg/kg b.w, route)	Description of *W. somnifera*/its derivatives	Dose (route)	Duration of treatment	Experimental design	Parameters studied
					(single dose of alloxan): Group 3; single dose of alloxan + *W. somnifera* root extract (100 mg/kg): Group 4; single dose of alloxan + *W. somnifera* root extract (200 mg/kg): Group 5; single dose of alloxan + *W. somnifera* leaf extract (100 mg/kg): Group 6; single dose of alloxan + *W. somnifera* leaf extract (200 mg/kg): Group 7; single dose of alloxan + glibenclamide (0.6 mg/kg)	and globulin), tissue protein (heart, liver and kidney), liver G6P, serum enzymes (AST, ALT, ACP and ALP)
Udayakumar et al. (2010)	Alloxan (150 mg/kg, ip)	Root and leaf extract of *W. somnifera* were prepared according to the published research study	100 and 200 mg/kg (oral)	8 weeks	Male (150–180 g) albino Wistar rats (*N* = 42); seven groups (each containing six rats) Group 1; normal control (distilled water): Group 2; diabetic control (single dose of alloxan): Group 3; single dose of alloxan + *W. somnifera* root extract (100 mg/kg): Group 4; single dose of alloxan + *W. somnifera* root extract (200 mg/kg): Group 5; single dose of alloxan + *W. somnifera* leaf extract (100 mg/kg): Group 6; single dose of alloxan + *W. somnifera* leaf extract (200 mg/kg): Group 7; single dose of alloxan + glibenclamide (0.6 mg/kg)	Urine sugar, blood glucose, liver glycogen, liver, kidney and heart antioxidants (SOD, LPO, CAT, GPx, GSH, GST), plasma vitamin C and E

Note: N = total number of animals in the study; *n* = total number of animals in a group.

Abbreviations: A:G ratio, albumin:globulin; 3β-HSD, 3 β-hydroxysteroid dehydrogenase; ACP, acid phosphatase; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; CK, creatinine kinase; DPPH, diphenyl-2-picryl-hydrazil; FSH, follicle stimulating hormone; FTIR, fourier-transform infrared; GP_X, glutathione peroxidise; G6PDH, glucose 6-phosphate dehydrogenase, GSH, glutathione; GST, glutathione-*S*-transferase; GR, glutathione reductase; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; HOC1, hypochlorous acid; HOMA-IR, homeostasis model assessment of insulin resistance; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LH, luteinizing hormone; LPO, lipid peroxidation; MDA, malondialdehyde; MLD, multiple low dose; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMR, nuclear magnetic resonance; PL, phospholipids; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; VLDL, very low-density lipoprotein.

diabetic profile. The intervention of withaferin A in type-1 diabetes mice demonstrated significant increase ($p < .001$) to near normal in plasma (2 and 10 mg/kg) and tissue (10 mg/kg) insulin levels (Tekula et al., 2018). Further, the levels of homeostasis model assessment of insulin resistance (HOMA-IR; an index of hepatic insulin resistance) and K_{ITT} (an index of peripheral insulin resistance) were assessed; *W. somnifera* (200 and 400 mg/kg) significantly restored the HOMA-IR ($p < .001$; 61.37 and 73.87%) and K_{ITT} ($p < .001$; 33.34 and 75.56%) levels in diabetic rats (Anwer et al., 2008).

3.6 | Lipid profile

Induction of diabetes by AM/STZ in experimental rats produced elevated lipid profile such as total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) besides decrease in high-density lipoprotein (HDL) levels. Administration of *W. somnifera* root/leaf extract significantly restored altered lipid profile to near normal (Table 4; Figures S2–S6). AM-induced diabetes also noted with elevated levels of TC, TG and phospholipids in heart, liver and kidney which were lowered to near normal on *W. somnifera* treatment (Figure S7; Udayakumar et al., 2009). Clinical evidence also corroborated with experimental data showing significantly improved lipid profile with *W. somnifera* treatment (Figure 4a; vs. active control/placebo: Figure 4b; vs. pre-treatment; Andallu & Radhika, 2000; Nayak et al., 2015; Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014).

3.7 | Serum markers

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were elevated in experimental diabetic rats which were reduced to near normal with *W. somnifera* treatment (Table 4; Figures S8–S10). Similarly, *W. somnifera* root (28.93%) and leaf (31.53%) extracts significantly decreased the elevated levels of acid phosphatase (Udayakumar et al., 2009). Induction of diabetes in experimental rats significantly reduced serum total protein, albumin, albumin:globulin (A:G) ratio and liver glycogen which were statistically improved after administration of *W. somnifera* root/leaf extract (Table 4; Figures S11–S14).

3.8 | OS markers

Injection of AM/STZ in experimental rats significantly reduced endogenous antioxidant levels, such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), glutathione *S*-transferase (GST) and glutathione reductase (GR) in the myocardial, hepatic and kidney as well pancreatic (only GPx, GST and GR) tissues and administration of *W. somnifera* root/leaf extract restored decreased levels close to normal. Further, increased lipid peroxidation (LPO)/TBARS (*n* mole malondialdehyde [MDA]/mg protein) level in the myocardial, hepatic, pancreatic and kidney tissues of diabetic rats reduced statistically with *W. somnifera* treatment (Table 4; Figures S15–S21).

Induction of diabetes in prepubertal rats significantly elevated; reactive oxidative species (ROS) generation (120%; in both testis cytosol and mitochondria), membrane LPO (66%; in both testis cytosol and mitochondria), GSH (cytosol, less altered; mitochondria, 22%), SOD (cytosol, less altered; mitochondria, 53%), GST and GR (in both testis cytosol and mitochondria): decreased total thiol concentration (cytosol, 43%; mitochondria, 16%) and CAT (Kyathanahalli et al., 2014). Supplementation of *W. somnifera* (15 days) statistically restored diabetes-induced testicular oxidative impairments. Further, *W. somnifera* administration significantly restored the altered activities of lactate dehydrogenase (LDH), glucose 6-phosphate dehydrogenase (G6PDH) and β-hydroxysteroid dehydrogenase (3β-HSD) to near normal. Two studies noted with significant decrease in LDH levels after *W. somnifera* root/leaf extract treatment (Anwer et al., 2017; Sarangi et al., 2013). Diabetic rats also observed with statistical decrease in elevated levels of serum marker enzyme, creatinine kinase (CK), when treated with *W. somnifera* root extract (Anwer et al., 2017). Parihar et al. noticed that induction of diabetes in mice/rat significantly ($p < .05$) increased LPO and protein carbonyl (PC) in hippocampus/hypothalamus and cerebral cortex brain regions (M. S. Parihar et al., 2004; P. Parihar et al., 2016). Supplementation of *W. somnifera* in diabetic mice/rat significantly ($p < .05$) reduced LPO and PC in the analyzed brain regions. Further, *W. somnifera* also significantly ($p < .05$) stabilized GSH and GPx levels to near normal in the hypothalamus (P. Parihar et al., 2016). AM injection significantly ($p < .05$) increased liver glucose-6-phosphatase (G6P; 220%) and ALP (124%) levels and treatment with *W. somnifera* root/leaf extract significantly restored these (G6P; 55%/52%: ALP; 84%/85%) altered levels (Udayakumar et al., 2009). Tekula et al. also noted increased ($p < .05$) MDA and nitrosative stress levels in type-1 diabetes mice (Tekula et al., 2018). Supplementation with withaferin A: documented with 3 and 4.1 folds decrease in MDA levels at 2 and 10 mg/day, respectively; prevented (both doses) the elevation of tissue nitrosative stress; lowered tissue nitrite levels (2 mg/kg, $p < .001$; 10 mg/kg, $p < .01$). In two RCTs, supplementation of *W. somnifera* root extract (250 and 500 mg) in patients with type-2 diabetes significantly ($p \leq .05$) increased GSH and nitric oxide (NO) levels and decreased MDA and high-sensitivity C-reactive protein (hs-CRP; an inflammatory biomarker) levels (Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014).

3.9 | Other biochemical parameters

W. somnifera root extract was evaluated for oral glucose tolerance test (OGTT) in experimental rats after oral supplementation of glucose (2 g/kg), Figure S22 (Anwer et al., 2008). In type-2 diabetes rats, there was significant ($p < .001$) increase in blood glucose level from baseline to 120 min. *W. somnifera* treatment (200 and 400 mg/kg) statistically ($p < .001$) decreased blood glucose level at all intervals during OGTT. Experimental rats treated with only *W. somnifera* showed no

significant difference in blood glucose level at 60 and 120 min (Anwer et al., 2008). Anwer et al. observed the histopathological changes in diabetic and control rats. Experimental rats treated with citrate buffer (pH 4.5) showed normal pancreatic acini and β-cells of islets of Langerhans. However, STZ-induced type-2 diabetes rats observed with degenerative and lytic changes in the pancreatic islets of Langerhans and depletion of β-cells in addition to reduced dimension and, shrunken of the islet. Supplementation of *W. somnifera* restored the histological changes of pancreas to near normal—as evident from increased size and number of β-cells (Anwer et al., 2012).

Withaferin A (2 and 10 mg/kg) significantly ($p < .05$) reduced inflammatory cytokines, that is, IL-6 and TNF-α in type-1 diabetes mice (Tekula et al., 2018). Furthermore, withaferin A (10 mg/kg) statistically decreased cytosolic ($p < .05$) and nuclear ($p < .001$) levels of p65-NFκB. Withaferin A was also observed with improved insulitis scoring and pancreatic islets morphology, in addition to reduced DNA fragmentation and apoptosis due to diabetes. Supplementation of type-1 diabetes mice with withaferin A ($p < .001$; 10 mg/kg) additionally noted with enhanced/reduced expression of Nrf2/NFκB, further indicating its contributory antioxidant role in *W. somnifera* activity.

W. somnifera root extract supplementation for 10 days significantly inhibited (25 mg, −7.14%; 50 mg, −17.53%; 100 mg, −30.52%) the elevated cortisol levels in diabetic mice (Thakur et al., 2015). Induction of diabetes in rats was observed with significant reduction in plasma vitamins C and E levels which were restored to near normal after treatment with *W. somnifera* root (73.19%) and leaf (54.35%) extracts, indicating its convincing antioxidant potential (Udayakumar et al., 2010).

Usharani and colleagues, in patients with type-2 diabetes, measured the effect of *W. somnifera* in endothelial dysfunction (measured as a change in reflection index [RI] of more than 6%) at 12 weeks of treatment. *W. somnifera* (250 and 500 mg) showed significant ($p < .001$) change in RI (Usharani, Fatima, et al., 2014); in another study, *W. somnifera* at 500 mg significantly ($p < .001$) improved RI (Usharani, Kishan, et al., 2014).

3.10 | Safety and tolerability

Of five included clinical studies, three measured the safety and tolerability of *W. somnifera* in patients with type-2 diabetes and reported no safety concerns from evaluated laboratory parameters (i.e., haematological, renal and hepatic; Nayak et al., 2015; Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014). Further, Raut et al. assessed the safety and tolerability of *W. somnifera* in 18 healthy volunteers, and noted significant reduction in TC and LDL with decreasing trend in TG, and increase in muscle strength activity (Raut et al., 2012). The organ function tests were normal after *W. somnifera* intervention and escalated doses (750–1,250 mg/day) were well tolerated. Similarly, in two RCTs, supplementation of *W. somnifera* in healthy young adults (of either sex; 500 mg/day) and aging overweight males (two tablets/day; each tablet containing 10.5 mg of withanolide glycosides) for 8 weeks noted to be safe with well tolerance (Lopresti, Drummond, & Smith, 2019; Sandhu et al., 2010). The systematic evaluation of *W. somnifera* by Mishra, Venkatachalapathy, & Khanli (2017) also reported with no significant safety concerns. Furthermore, the control group (experimental animals)—from 13 pre-clinical included studies, showed no adverse effects. Additionally, a sub-acute toxicity study with *W. somnifera* having 4.5% of withaferin A (2,000 mg $kg^{-1}day^{-1}$) confirmed no adverse effects in experimental rats (Patel, Rao, & Hingorani, 2016). Besides all these, *W. somnifera* has a long history of safe use (HoSU) data (D. Narayana & Durg, 2019).

4 | DISCUSSION

To the facts, this is the first systematic review and meta-analysis evaluating the efficacy/effectiveness, safety and tolerability of *W. somnifera* treatment in DM. Of 13 pre-clinical studies, four studies investigated effect of *W. somnifera* in type-2 diabetes rats (Anwer et al., 2017, 2008, 2012; Thakur et al., 2015), three studies in type-1 diabetes rats (Jain et al., 2006; Kiasalari et al., 2009; Tekula et al., 2018) and the remaining studies did not mention about the type of diabetes condition (Kyathanahalli et al., 2014; M. S. Parihar et al., 2004; P. Parihar et al., 2016; Sarangi et al., 2013; Udayakumar et al., 2009, 2010). However, by virtue of their pancreatic β-cell reduction/toxicity by AM/STZ, these studies mimicked either type-1 or type-2 diabetes or both conditions (Durg, Veerapur, Neelima, & Dhadde, 2017). Clinical trials mainly evaluated the role of *W. somnifera* root extract in controlling type-2 diabetes and its related symptoms.

Induction of diabetes by AM leads to selective necrosis of pancreatic β-cells via inhibition of glucokinase resulting in insulin resistance and generation of ROS (Lenzen, 2008). In case of STZ, there is liberation of NO leading to aconitase inhibition thus causing pancreatic β-cell toxicity (via alkylation of DNA) which ultimately inhibits insulin secretion causing diabetes (Lenzen, 2008; Szkudelski, 2001). These actions, together, lead to impaired antioxidant defense mechanism in the pancreatic β-cells, and thus causing OS which lead to β-cell secretory pathway dysfunction and insulin resistance (Hasnain, Prins, & McGuckin, 2016; Tangvarasittichai, 2015). It is also implicated as the underlying cause of both micro- and macro-vascular diabetes complications (Giacco & Brownlee, 2010). Therapeutic interventions that intercept OS may delay and/or prevent the progression and onset of DM (Hasnain et al., 2016; Tangvarasittichai, 2015).

W. somnifera, an adaptogen, as *Rasayanas* (rejuvenating tonic) with a potent antioxidant activity which promote health and longevity by resisting the stress related to physical, chemical or biological. In-vitro studies apparently demonstrated antioxidant activity of *W. somnifera* by its potential in scavenging DPPH, HOCl and hydroxyl radicals (Khan et al., 2014; Kyathanahalli et al., 2014). Further, the experimental studies showed protective action of *W. somnifera* against OS by significantly increasing the levels of antioxidant enzymes such as SOD, CAT, GSH, GPx, GST and GR in diabetic rats (Anwer et al., 2017; Udayakumar et al., 2010). *W. somnifera* supplementation also significantly elevated decreased levels of vitamin C (a major water-

TABLE 3 Description of included clinical studies

Study ID	Participants/inclusion criteria	Age (years; mean ± SD)	Study design Total sample size (gender; M/F)	Description of *W. somnifera*	Treatment(s) groups and dosage	Outcomes
Andallu & Radhika, (2000)	Mild hyperglycemic (NIDDM) and hypercholesterolemic	40–60[a]	Observational study *N* = 12[b]	Dried roots of *W. somnifera* were collected from IMPCOPS (Indian medical Practitioner's cooperative society), powdered and filled in capsules to make 500 mg	*W. somnifera* group (*n* = 6): 500 mg capsule (6 capsules/day, 2 after every meal) for 30 days Control group (*n* = 6): Oral hypoglycemic drug (daonil) for 30 days	Blood glucose, sodium and potassium in serum and urine, urine volume, and lipid profile (TC, TG, HDL-C, LDL-C and VLDL-C)
Agnihotri et al. (2013)	Schizophrenia patients, suffering from metabolic syndrome, on second-generation Antipsychotics for 6 months or more, with FBG level >100 mg/dl, serum TGs >150 mg/dl, HDL-C <40 mg/dl in men and <50 mg/dl in women	>18[a]	Randomised, double-blind, placebo-controlled trial *N* = 25[b]	*W. somnifera* extract (cap Strelaxin, manufactured by M/s Pharmanza Herbal Pvt. Ltd. Gujarat, India) containing 400 mg of *W. somnifera* extract per capsule	*W. somnifera* extract group (*n* = 12): 400 mg of *W. somnifera* extract per capsule, one capsule TID for 1 month Placebo group (*n* = 13): Matching placebo to *W. somnifera* extract, one capsule TID for 1 month	Body weight, FBG and lipid profile (TG and HDL-C), blood pressure
Nayak et al. (2015)	T2DM patients treated with fixed OHAs (metformin, 500 mg + glimepride, 1 mg), FBS ≥126 and ≤180 mg/dl, PPBS ≤240 mg/dl, HbA1C ≥7%, and mean total DDS scoring ≥3	21–70[a]	Randomised clinical trial *N* = 55[b]	Soft gelatine *W. somnifera* capsules containing 300 mg of root extract in ground nut oil base manufactured by Dabur India Ltd.	*W. somnifera* group (*n* = 28): Capsule of 300 mg root extract in ground nut oil base, one capsule BID with a cup of Luke warm milk for 6 weeks Control group (*n* = 27): Soft gelatine capsule of only ground nut oil, one capsule BID with a cup of Luke warm milk for 6 weeks another 6 weeks, the patients were followed-up	DDS17 score (emotional burden, physician related distress, regimen related distress, and interpersonal distress), FBS, PPBS, HbA1c, lipid profile (TC) and adverse events
Usharani, Fatima, et al. (2014)	T2DM patients with FBG between 110–126 mg/dl, HbA1c between 6.5 and 8.0%, on oral hypoglycemic agents for last 8 weeks (metformin, 1,500–2,000 mg/day) prior to screening visit, endothelial dysfunction defined as ≤6% change in reflection index (RI) on post salbutamol challenge test	*W. somnifera* (250 mg): 55.40 ± 8.07 *W. somnifera* (500 mg): 57.30 ± 9.40 Placebo: 57.45 ± 8.85	Randomised, double-blind, placebo-controlled, parallel design trial *N* = 60 *W. somnifera* (250 mg): *n* = 20; 14/6 *W. somnifera* (500 mg): *n* = 20; 13/7 Placebo: *n* = 20; 12/8	SENSORIL®: An aqueous root extract of *W. somnifera*, containing not less than 10% with anolide glycosides, not more than 0.5% of withaferin-A and not less than 32% of oligosaccharides	*W. somnifera* group (*n* = 20): Capsule of 250 mg root extract BID for 12 weeks *W. somnifera group* (*n* = 20): Capsule of 500 mg root extract BID for 12 weeks Placebo (*n* = 20): Identical matching capsule to *W. somnifera* BID for 12 weeks	Endothelial dysfunction (RI), oxidative stress biomarkers (NO, MDA, GSH and hs-CRP), lipid profile (TC, TG, HDL-C, LDL-C and VLDL-C), safety and tolerability
Usharani, Kishan,	T2DM patients with FBG ≥110 mg/dl, HbA1c between 6.5 and 8.0%, on oral	*somnifera* (250 mg): 60.10 ± 6.47	Prospective, randomised, double-blind, parallel group trial	Same as Usharani, Fatima, et al. (2014)	*W. somnifera* (*n* = 10): Capsule of 250 mg root extract BID for 12 weeks	Endothelial dysfunction (RI), oxidative stress biomarkers (NO,

(Continues)

TABLE 3 (Continued)

Study ID	Participants/inclusion criteria	Age (years; mean ± SD)	Study design: Total sample size (gender; M/F)	Description of *W. somnifera*	Treatment(s) groups and dosage	Outcomes
et al. (2014)	hypoglycemic agents for last 8 weeks (metformin, 1,500–2,000 mg/day) prior to screening visit, endothelial dysfunction defined as ≤6% change in reflection index (RI) on post salbutamol challenge test	*Phyllanthus emblica* (250 mg): 58.60 ± 10.54 Combination of *Phyllanthus emblica* + *W. somnifera*: 57.30 ± 9.23	*N* = 30 *W. somnifera* (250 mg): *n* = 20; 8/2 *Phyllanthus emblica* (250 mg): *n* = 10; 9/1 Combination of *Phyllanthus emblica* + *W. somnifera* (*n* = 10): *n* = 10; 9/1		*Phyllanthus emblica* (*n* = 10): Capsule of 500 mg BID for 12 weeks Combination of *Phyllanthus emblica* + *W. somnifera* (*n* = 10): Capsule of 250 mg + 250 mg BID for 12 weeks	MDA, GSH and hs-CRP), lipid profile (TC, TG, HDL-C, and LDL-C), and safety and tolerability

Note: *N* = total number of subjects in the study; *n* = total number of subjects in a group.

Abbreviations: BID, two times daily; DDS, diabetes distress scale; FBS, fasting blood; GSH, glutathione; $HbA1_C$, glycosylated haemoglobin; HDL, high-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; LDL, low-density lipoprotein; M/F, male/female; MDA, malondialdehyde; NIDDM, non-insulin dependent diabetes mellitus; NO, nitric oxide; OHAs, oral hyoglycemic agents; PPBS, post prandial blood sugar; RI, reflection index; T2DM, type-II diabetes mellitus; TC, total cholesterol; TG, triglyceride; TID: three times daily; VLDL, very low-density lipoprotein.

[a]Mean ± *SD* values were not mentioned.

[b]The distribution of male and female were not mentioned.

soluble non-enzymatic antioxidant) and E (a chain-breaking antioxidant) in diabetic rats (Udayakumar et al., 2010), thus showing strong antioxidant defense mechanism of *W. somnifera*. Likewise, the antioxidant effects of *W. somnifera* were also evidenced in clinical studies; increase in NO and GSH levels (Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014). The environment of OS causes excessive generation of ROS, viz., superoxide anion, hydrogen peroxide and hydroxyl radicals which induce LPO (toxic peroxide product) of cell membrane lipids leading to cellular and molecular damage (Durg et al., 2015). In the present analysis, diabetic rats were noted with increased LPO levels in heart, liver, pancreas and kidney tissues which were decreased after supplementation of *W. somnifera*. Treatment of hypercholesteremic rats with *W. somnifera* were also noted with significant decrease in LPO levels (Visavadiya & Narasimhacharya, 2007). Similarly, *W. somnifera* decreased MDA (a secondary product of LPO) levels in patients with type-2 diabetes (Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014). The observed antioxidant activities of *W. somnifera* might be, majorly, attributed to its active constituent withaferin A—a steroidal lactone (Aliper et al., 2017; Lee et al., 2016) as well other bioactive elements—steroidal alkaloids/lactones such as withanine, somniferine, withanone sitoindosides VII–X and withanolide A, D and G (Durg et al., 2015; Mirjalili, Moyano, Bonfill, Cusido, & Palazón, 2009).

Induction of diabetes by AM/STZ in experimental rats significantly increased blood glucose and HbA1c levels. The meta-analysis of *W. somnifera* supplementation in these diabetic rats significantly reduced blood glucose and HbA1c; similarly, in patients with type-2 diabetes, *W. somnifera* significantly reduced fasting blood glucose level (Agnihotri et al., 2013; Nayak et al., 2015). Additionally, isolated constituents of *W. somnifera* root extract noticed to inhibit stress-induced hyperglycemia by blocking corticosterone hypersecretion in experimental stressed rats (Sarjan & Yajurvedi, 2018). The supportive mechanism(s) of action(s) of *W. somnifera* for the observed anti-diabetic activity are depicted in Figure 5. The observed anti-hyperglycaemic activity of *W. somnifera* might be attributed to multiple mechanisms such as β-cell protection (preserve the size and number of β-cells) from OS (Anwer et al., 2012), rejuvenation and activation of pancreatic β–cells leading to insulin release (Udayakumar et al., 2010). *W. somnifera* also acts by inhibiting α-glucosidase, α-amylase and DPP-IV enzymes (Huerta et al., 2010; Khan et al., 2014; Singh et al., 2013). It also stimulates glucose transports which further accelerates transport of glucose into cells (Nirupama et al., 2014). Further, the bioactive constituents from *W. somnifera* extract, that is, withanolides reported to increase glucose uptake in myotubes and adipocytes (Jonathan et al., 2015). *W. somnifera* also showed glucose lowering activity through insulinotropic effects which improves insulin sensitivity by reducing hyperinsulinemia and insulin resistant besides improving glucose tolerance (Anwer et al., 2008). The recent *in-silico* screening revealed that withaferin A mimics the anti-aging properties of mTOR inhibitors, that is, metformin and rapamycin (Aliper et al., 2017); the two FDA-approved drugs which tops the list of drugs mimicking caloric restriction (Ingram et al., 2004). Further, Lee et al. demonstrated withaferin A

TABLE 4 Summary of meta-analyses results

Outcome	Studies	*N* (total; *W. somnifera*/control)	Effect measure (95% CI)	I^2% (*p* value)
Body weight	Kyathanahalli et al. (2014) Thakur et al. (2015) Udayakumar et al. (2009)	24/24	MD; 32.29 (3.06, 61.53)	98 (.03)
Lipid profile				
Total cholesterol	Anwer et al. (2017) Kiasalari et al. (2009) Sarangi et al. (2013) Udayakumar et al. (2009)	41/39	MD; −64.57 (−88.12, −41.01)	97 (<.00001)
Triglyceride			MD; −27.32 (−47.62, −7.01)	96 (.008)
Low density lipoprotein	Anwer et al. (2017) Sarangi et al. (2013) Udayakumar et al. (2009)	30/30	MD; −35.60 (−47.56, −23.63)	96 (<.00001)
Very low density lipoprotein	Anwer et al. (2017) Udayakumar et al. (2009)	18/18	MD; −7.45 (−11.56, −3.34)	90 (.0004)
High density lipoprotein	Anwer et al. (2017) Sarangi et al. (2013) Udayakumar et al. (2009)	30/30	MD; 5.23 (3.72, 6.74)	53 (<.00001)
Serum markers				
Aspartate aminotransferase	Sarangi et al. (2013) Udayakumar et al. (2009)	24/24	MD; −68.62 (−75.31, −61.93)	74 (<.00001)
Alanine aminotransferase			MD; −56.27 (−63.72, −48.82)	82 (<.00001)
Alkaline phosphatase			MD; −3.00 (−4.15, −1-85)	0 (<.00001)
Total protein			MD; 1.66 (1.19, 2.13)	66 (<.00001)
Albumin			MD; 1.48 (1.30, 1.66)	0 (<.00001)
Albumin:Globulin ration			MD; 0.69 (0.56, 0.82)	58 (<.00001)
Liver glycogen	Udayakumar et al. (2009) Udayakumar et al. (2010)	24/24	MD; 9.33 (6.85, 11.80)	55 (<.00001)
Oxidative stress markers				
Superoxide dismutase	Anwer et al. (2017) Udayakumar et al. (2010)	42/42	MD; 3.13 (2.03, 4.22)	48 (<.00001)
Catalase			SMD; 2.26 (1.53, 2.98)	26 (<.00001)
Glutathione	Anwer et al. (2012) Anwer et al. (2017) Udayakumar et al. (2010)	48/48	SMD; 1.98 (1.12, 2.84)	58 (<.00001)
Glutathione peroxidase			SMD; 1.90 (1.24, 2.56)	33 (<.00001)
Glutathione *S*-transferase	Anwer et al. (2017) Udayakumar et al. (2010)	48/48	SMD; 2.92 (2.24, 3.60)	4 (<.00001)
Glutathione reductase	Anwer et al. (2012) Anwer et al. (2017)	12/12	MD; 61.56 (41.91, 73.21)	0 (<.00001)
Lipid peroxidation	Anwer et al. (2012) Anwer et al. (2017) Udayakumar et al. (2010)	48/48	SMD; −2.58 (−3.49, −1.67)	51 (<.00001)

Abbreviations: MD, mean difference; SMD, standardized mean difference.

as a potential leptin sensitizer with great anti-diabetic effects in mice (Lee et al., 2016).

Lipid profile abnormality is evident in diabetes which increases the risks of atherosclerosis and coronary heart diseases (Krauss, 2004). The elevated lipid levels are result of altered oxidative metabolism which leads to increased LPO in diabetes (de Souza Bastos et al., 2016; Tangvarasittichai, 2015). Furthermore, impaired lipid metabolism and LPO leads to release of proinflammatory markers such as IL-1β, IL-6, IL-8 and TNF-α; these markers cause complications such as retinopathy and nephropathy (de Souza Bastos et al., 2016). In addition, hyper-sensitive C-reactive protein (hs-CRP), another inflammatory marker, can be a risk factor for endothelial dysfunction and cardio-cerebrovascular events (Fan et al., 2018; Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014).

Induction of diabetes in experimental animals significantly increased TC, TG, LDL, and VLDL, and decreased HDL levels which were restored to near normal with *W. somnifera* treatment. Similar observations were validated in clinical trials in patients with type-2 diabetes. The hypolipidemic activity of *W. somnifera* can be explained by its activity to mobilize LDL for synthesizing bile acid to remove excess body cholesterol (Visavadiya & Narasimhacharya, 2007).

Furthermore, *W. somnifera* supplementation decreased pro-inflammatory cytokines such as IL-6 and TNF-α in fructose-fed male rats (Samadi Noshahr, Shahraki, Ahmadvand, Nourabadi, & Nakhaei, 2015;

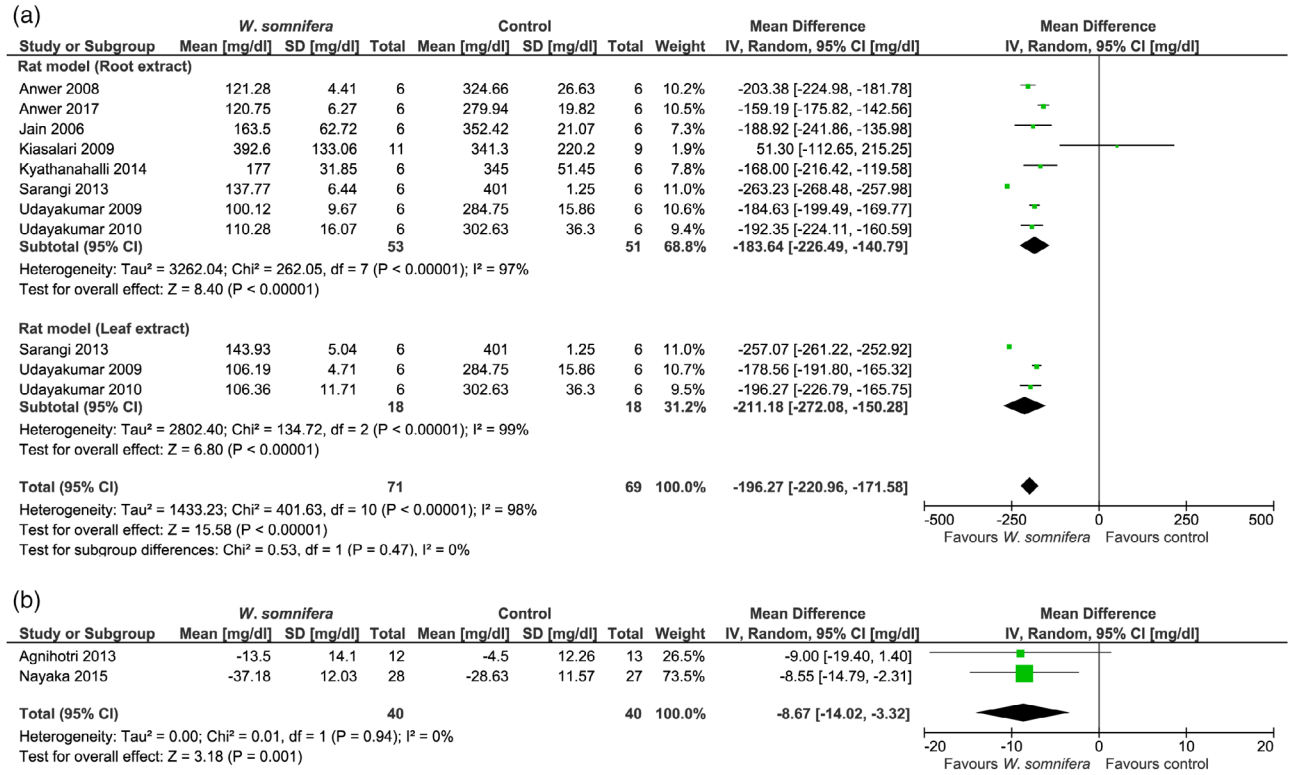

FIGURE 2 Effect of *Withania somnifera* on (a) blood glucose; (b) on fasting blood glucose [Colour figure can be viewed at wileyonlinelibrary.com]

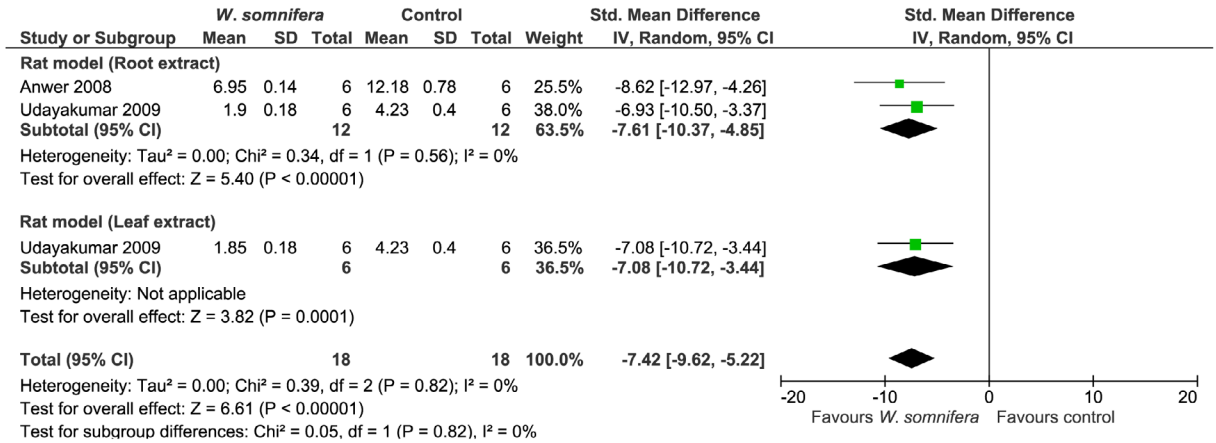

FIGURE 3 Effect of *Withania somnifera* on glycosylated haemoglobin [Colour figure can be viewed at wileyonlinelibrary.com]

Shahraki, Samadi Noshahr, Ahmadvand, & Nakhaie, 2016). In two RCTs, *W. somnifera* treatment significantly improved endothelial dysfunction in patients with type-2 diabetes by decreasing RI and OS markers such MDA and inflammatory markers—hs-CRP (Usharani, Fatima, et al., 2014; Usharani, Kishan, et al., 2014). The incidence of diabetes also causes micro- and macro-vascular complications leading to CV disorder which ultimately alters heart rate (HR) and blood pressure (BP; Wagner, Martijnez-Rubio, Ordonez-Llanos, & Perez-Perez, 2002). Gupta et al. (2004) reported that *W. somnifera* treatment decreased HR and BP in isoproterenol-induced myocardial infarction rat model, thus suggesting cardio-protective potential against diabetes-related CV risk. Further, a recent study by Chukwuma and colleagues found that *W. somnifera* has concomitant anti-diabetic and anti-hypertensive actions via blood glucose-lowering and diuretic effects in humans (Chukwuma et al., 2019).

Diabetes and hyperlipidaemia, via disturbance in the cell membrane architecture, cause cell damage—resulting in impaired liver function (Harris, 2005; Salmela, Sotaniemi, Niemi, & Mäentausta, 1984; Udayakumar et al., 2009). Incidence of diabetes by AM/STZ in experimental animals observed with elevated levels of AST, ALT, CK and ALP. Further, induction of diabetes also resulted in decreased levels of total protein, albumin and A:G ratio probably because of albuminurea and microproteinurea and/or might also be due to

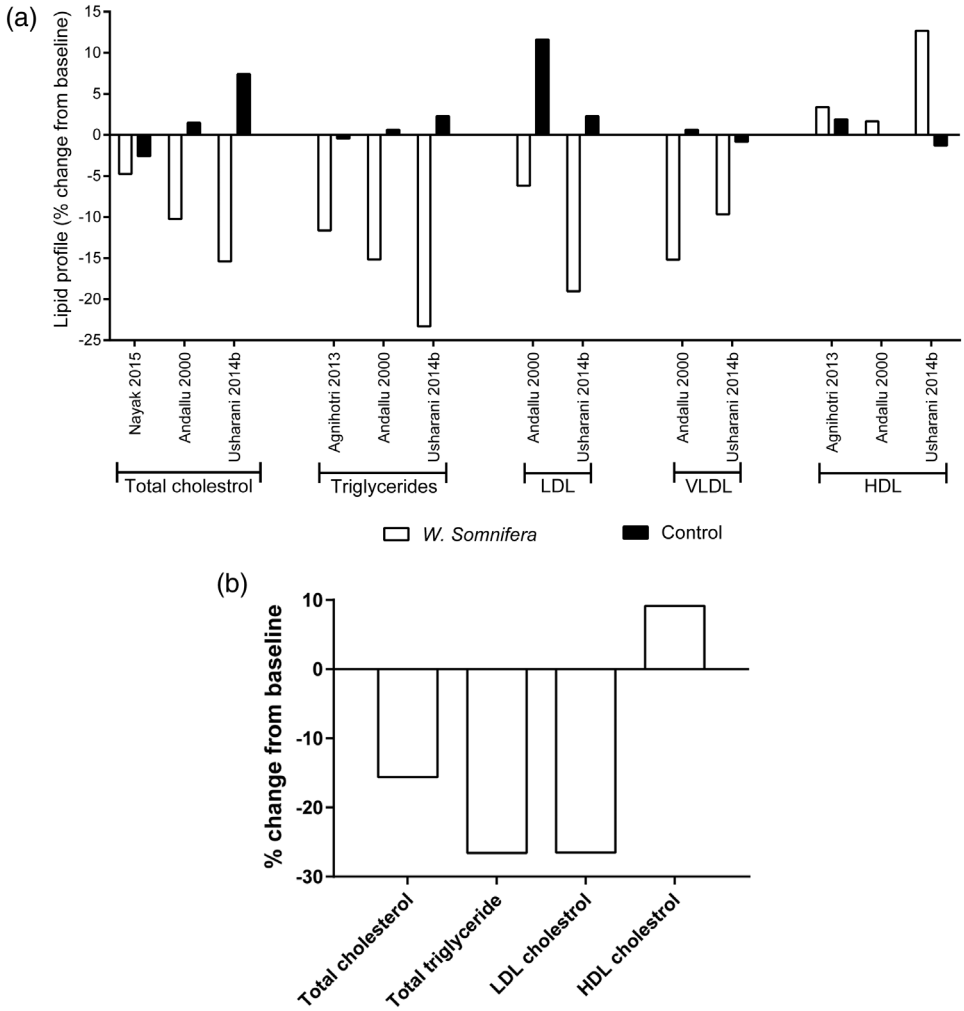

FIGURE 4 Effect of *Withania somnifera* on lipid profile: (a) versus active control/placebo; (b) versus pre-treatment

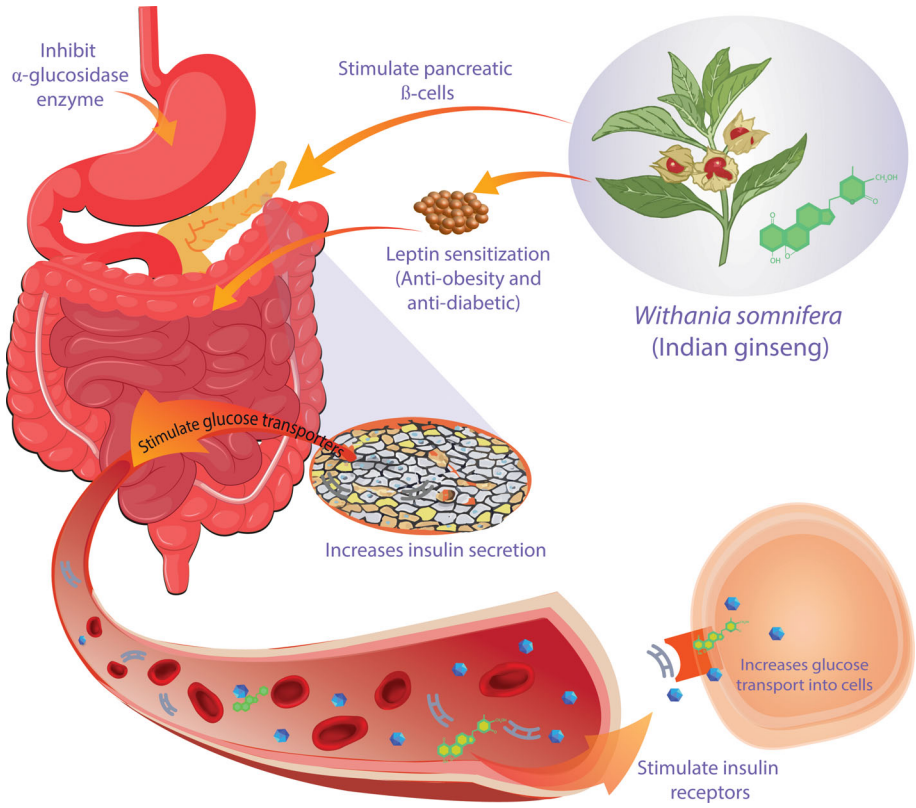

FIGURE 5 Supportive mechanism(s) of action(s) of *Withania somnifera* in diabetes mellitus [Colour figure can be viewed at wileyonlinelibrary.com]

increased protein catabolism (Almdal & Vilstrup, 1988). *W. somnifera* treatment restored AST, ALT, CK and ALP levels to near normal besides elevated the reduced levels of total protein, albumin and A:G ratio; these findings support hepato- and reno-protective activities of *W. somnifera* in diabetes.

The current meta-analysis was observed with low to significant heterogeneity in both experimental and clinical studies which can be attributed to variation in *W. somnifera* extracts; experimental studies majorly used aqueous or alcoholic root extract, and leaf extract in few studies, with different doses. Whereas clinical studies used only root extract, but not of same concentration (dose) and strength. Another key factor can be use of different diabetes-inducing agents and different doses of same agent in pre-clinical studies. In clinical studies, deviation in diabetes status with or without comorbid condition, age of included diabetic participants, some patients in addition to *W. somnifera* treated with other drugs/supplementation for diabetic condition, and duration of the treatment are major key factors in the noted heterogeneity. Apart from aforementioned factors, the following also play an important role in pre-clinical studies: experimental animals mimicked either type-1 or type-2 diabetes or both conditions.

5 | STRENGTHS

The major strength of this study is systematically incorporating the mechanistic (in-vitro and pre-clinical) and clinical studies to assess the anti-diabetic activity of *W. somnifera* together with its biomarkers. The literature was searched according to the guideline recommended databases to identify both published and unpublished (grey) data, besides hand-searching the references of included articles for additional studies, if any, which resulted in inclusion of seven studies; three in-vitro, and two each pre-clinical and clinical studies. To minimize the potential of bias, during selection process, rigid inclusion/exclusion criteria were applied.

6 | LIMITATIONS

Clinical data were limited by both quantity and data-type availability and accessibility. Of five included clinical studies, four were RCTs; however, the statistical pooling from these RCTs was not significant for the analyzed parameters. The included diabetes population varied from mild hyperglycemia with hypercholesterolemia or schizophrenia and type-2 diabetes. Further, only Nayaka et al. evaluated the effect of *W. somnifera* on HbA1c (Nayak et al., 2015), and the participants in this study in addition to *W. somnifera* treatment were receiving fixed dose regime of metformin (500 mg) + glimepride (1 mg); considering this, the effect of *W. somnifera* on HbAlc in patients with type-2 diabetes does not hold the importance. HbA1c, an important test for accessing the long-term glycemic control, which also echo the cumulative glycemic history of the past 2–3 months (Sherwani, Khan, Ekhzaimy, Masood, & Sakharkar, 2016). A recent case report of *W. somnifera* root usage (400 mg BID for 3 months), in an old woman with pre-diabetes (class III obesity, BMI; 49.4 kg/m^2), reduced HbA1c and body weight, respectively, from 6.4 to 5.9% and 135 to 131.8 kg with no further changes in lifestyle (Simon, Inoue, Fenteany, Bahtiyar, & Sacerdote, 2018). The clinical studies evaluating the effect of *W. somnifera* in patients with type-2 diabetes must measure its effect on HbA1c as monotherapy, not in combination with allopathic treatment. The five included clinical studies, in the present systematic evaluation, were conducted in India, which might limit the generalization of results to other countries. Of 13 included pre-clinical studies, six studies did not mention the type of diabetes; however, by virtue of diabetes inducing agents the experimental animals in these studies partly mimicked either type-1, type-2 diabetes or both (Durg et al., 2017). For assessing the efficacy and safety of *W. somnifera*, appropriate standardization and validation is imperative to mimic the type of diabetes. Another important concern while performing meta-analysis was plagiarized data. We were able to trace the plagiarism in two experimental studies; this cautions the authenticity of experimental studies, although both studies were excluded from meta-analysis.

7 | CONCLUSIONS

The collective experimental data in this study look modest. However, clinical data of five studies are too limited to provide novel and sufficiently robust evidence of the benefits of *W. somnifera* to recommend in managing DM. To further strength and validate anti-diabetic profile of *W. somnifera* in clinical application, at least one RCT with a larger sample size and longer duration is warranted with evaluation majorly for HbA1c, blood glucose and insulin. Future research also needs to elucidate the molecular mechanism(s) of *W. somnifera* besides its active principles in DM.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Agnihotri, A. P., Sontakke, S. D., Thawani, V. R., Saoji, A., & Goswami, V. S. S. (2013). Effects of *Withania somnifera* in patients of schizophrenia: A randomized, double blind, placebo controlled pilot trial study. *Indian Journal of Pharmacology*, *45*(4), 417–418. https://doi.org/10.4103/0253-7613.115012

Aliper, A., Jellen, L., Cortese, F., Artemov, A., Karpinsky-Semper, D., Moskalev, A., … Zhavoronkov, A. (2017). Towards natural mimetics of metformin and rapamycin. *Aging*, *9*(11), 2245–2268. https://doi.org/10.18632/aging.101319

Almdal, T. P., & Vilstrup, H. (1988). Strict insulin therapy normalises organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. *Diabetologia*, *31*(2), 114–118. https://doi.org/10.1007/BF00395558

Andallu, B., & Radhika, B. (2000). Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*, Dunal) root. *Indian Journal of Experimental Biology*, *38*(6), 607–609.

Anwer, T., Sharma, M., Khan, G., Alam, M. F., Alam, N., Ali, M. S., & Alam, M. S. (2017). Preventive role of *Withania somnifera* on hyperlipidemia and cardiac oxidative stress in streptozotocin induced type 2 diabetic rats. *Tropical Journal of Pharmaceutical Research*, *16*(1), 119. https://doi.org/10.4314/tjpr.v16i1.15

Anwer, T., Sharma, M., Pillai, K. K., & Iqbal, M. (2008). Effect of *Withania somnifera* on insulin sensitivity in non-insulin-dependent diabetes mellitus rats. *Basic & Clinical Pharmacology & Toxicology*, *102*(6), 498–503. https://doi.org/10.1111/j.1742-7843.2008.00223.x

Anwer, T., Sharma, M., Pillai, K. K., & Khan, G. (2012). Protective effect of *Withania somnifera* against oxidative stress and pancreatic beta-cell damage in type 2 diabetic rats. *Acta Poloniae Pharmaceutica*, *69*(6), 1095–1101.

Bhat, J., Damle, A., Vaishnav, P. P., Albers, R., Joshi, M., & Banerjee, G. (2010). In vivo enhancement of natural killer cell activity through tea fortified with Ayurvedic herbs. *Phytotherapy Research*, *24*(1), 129–135. https://doi.org/10.1002/ptr.2889

Chukwuma, C. I., Matsabisa, M. G., Ibrahim, M. A., Erukainure, O. L., Chabalala, M. H., & Islam, M. S. (2019). Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: A review. *Journal of Ethnopharmacology*, *235*, 329–360. https://doi.org/10.1016/j.jep.2019.02.024

Dar, N. J., Hamid, A., & Ahmad, M. (2015). Pharmacologic overview of *Withania somnifera*, the Indian ginseng. *Cellular and Molecular Life Sciences*, *72*(23), 4445–4460. https://doi.org/10.1007/s00018-015-2012-1

de Souza Bastos, A., Graves, D. T., de Melo Loureiro, A. P., Júnior, C. R., Corbi, S. C. T., Frizzera, F., … Orrico, S. R. P. (2016). Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients. *Journal of Diabetes and its Complications*, *30*(8), 1593–1599. https://doi.org/10.1016/j.jdiacomp.2016.07.011

Department of AYUSH. (1989). *The ayurvedic pharmacopoeia*, *part-I*, *volume-I* (Vol. 1). Government of India: Ministry of Health and Family Welfare.

Diabetes Prevention Program Research Group. (2009). 10-year follow-up of diabetes incidence and weight loss in the diabetes prevention program outcomes study. *The Lancet*, *374*(9702), 1677–1686. https://doi.org/10.1016/S0140-6736(09)61457-4

Diabetes Prevention Program Research Group. (2015). Long-term effects of lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: The diabetes prevention program outcomes study. *The Lancet Diabetes & Endocrinology*, *3*(11), 866–875. https://doi.org/10.1016/S2213-8587(15)00291-0

Durg, S., Dhadde, S. B., Vandal, R., Shivakumar, B. S., & Charan, C. S. (2015). *Withania somnifera* (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: A systematic review and meta-analysis. *Journal of Pharmacy and Pharmacology*, *67*(7), 879–899. https://doi.org/10.1111/jphp.12398

Durg, S., Shivaram, S. B., & Bavage, S. (2018). *Withania somnifera* (Indian ginseng) in male infertility: An evidence-based systematic review and meta-analysis. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *50*, 247–256. https://doi.org/10.1016/j.phymed.2017.11.011

Durg, S., Veerapur, V. P., Neelima, S., & Dhadde, S. B. (2017). Antidiabetic activity of *Embelia ribes*, embelin and its derivatives: A systematic review and meta-analysis. *Biomedicine and Pharmacotherapy*, *86*, 195–204. https://doi.org/10.1016/j.biopha.2016.12.001

Facciola, S. (1998). *Cornucopia II: A source book of edible plants*. Facciola, New York: Kampong Publications.

Fan, D., Li, L., Li, Z., Zhang, Y., Ma, X., Wu, L., & Qin, G. (2018). Effect of hyperlipidemia on the incidence of cardio-cerebrovascular events in patients with type 2 diabetes. *Lipids in Health and Disease*, *17*(1), 102. https://doi.org/10.1186/s12944-018-0676-x

Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, *107*(9), 1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, *103*(2), 137–149. https://doi.org/10.1016/j.diabres.2013.11.002

Gupta, S. K., Mohanty, I., Talwar, K. K., Dinda, A., Joshi, S., Bansal, P., … Arya, D. S. (2004). Cardioprotection from ischemia and reperfusion injury by *Withania somnifera*: A hemodynamic biochemical and histopathological assessment. *Molecular and Cellular Biochemistry*, *260*(1–2), 39–47. https://doi.org/10.1023/B:MCBI.0000026051.16803.03

Harris, E. H. (2005). Elevated liver function tests in type 2 diabetes. *Clinical Diabetes*, *23*(3), 115–119. https://doi.org/10.2337/diaclin.23.3.115

Hasnain, S. Z., Prins, J. B., & McGuckin, M. A. (2016). Oxidative and endoplasmic reticulum stress in β-cell dysfunction in diabetes. *Journal of Molecular Endocrinology*, *56*(2), R33–R54. https://doi.org/10.1530/JME-15-0232

Huerta, V., Mihalik, K., Becket, K., Maitin, V., & Vattem, D. (2010). Antidiabetic and anti-energy harvesting properties of common traditional herbs, spices and medicinal plants from India. *Journal of Natural Remedies*, *10*(2), 123–135. https://doi.org/10.18311/jnr/2010/253

Ingram, D. K., Anson, R. M., De Cabo, R., Mamczarz, J., Zhu, M., Mattison, J., … Roth, G. S. (2004). Development of calorie restriction mimetics as a prolongevity strategy. *Annals of the New York Academy of Sciences*, *1019*, 412–423. https://doi.org/10.1196/annals.1297.074

Jain, S., Pandhi, P., Singh, A., & Malhotra, S. (2006). Efficacy of standardised herbal extracts in type 1 diabetes-an experimental study. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(4), 23–33.

Jonathan, G., Rivka, R., Avinoam, S., Lumír, H., & Nirit, B. (2015). Hypoglycemic activity of withanolides and elicitated *Withania somnifera*. *Phytochemistry*, *116*(1), 283–289. https://doi.org/10.1016/j.phytochem.2015.02.029

Kaul, S. C., & Wadhwa, R. (Eds.). (2017). *Science of Ashwagandha: Preventive and therapeutic potentials*. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-59192-6

Khan, M. A., Khan, H., & Ali, T. (2014). Withanolides isolated from *Withania somnifera* with α-glucosidase inhibition. *Medicinal Chemistry Research*, *23*(5), 2386–2390. https://doi.org/10.1007/s00044-013-0838-3

Kiasalari, Z., Khalili, M., & Aghaei, M. (2009). Effect of *Withania somnifera*on levels of sex hormonesin the diabetic male rats. *Iranian Journal of Reproductive Medicine*, *7*(4), 163–168.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., … Diabetes Prevention Program Research Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine*, *346*(6), 393–403. https://doi.org/10.1056/NEJMoa012512

Krauss, R. M. (2004). Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care*, *27*(6), 1496–1504.

Kulkarni, S. K., & Dhir, A. (2008). *Withania somnifera*: An Indian ginseng. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(5), 1093–1105. https://doi.org/10.1016/j.pnpbp.2007.09.011

Kuppurajan, K., Rajagopalan, S., Sitaraman, R., Rajagopalan, V., Janaki, K., Revathi, R., & Venkataraghavan, S. (1980). Effects of Ashwagandha on the process of ageing on human volunteers. *Journal of Research in Ayurveda & Siddha*, *1*, 247–258.

Kyathanahalli, C. N., Manjunath, M. J., & Muralidhara. (2014). Oral supplementation of standardized extract of *Withania somnifera* protects against diabetes-induced testicular oxidative impairments in

prepubertal rats. *Protoplasma*, *251*(5), 1021–1029. https://doi.org/10.1007/s00709-014-0612-5

Lee, J., Liu, J., Feng, X., Hernández, M. A. S., Mucka, P., Ibi, D., … Ozcan, U. (2016). Withaferin a is a leptin sensitizer with strong antidiabetic properties in mice. *Nature Medicine*, *22*(9), 1023–1032. https://doi.org/10.1038/nm.4145

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216–226. https://doi.org/10.1007/s00125-007-0886-7

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., … Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*, *6*(7), e1000100. https://doi.org/10.1371/journal.pmed.1000100

Lim, E. L., Hollingsworth, K. G., Aribisala, B. S., Chen, M. J., Mathers, J. C., & Taylor, R. (2011). Reversal of type 2 diabetes: Normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia*, *54*(10), 2506–2514. https://doi.org/10.1007/s00125-011-2204-7

Lopresti, A. L., Drummond, P. D., & Smith, S. J. (2019). A randomized, double-blind, placebo-controlled, crossover study examining the hormonal and vitality effects of Ashwagandha (*Withania somnifera*) in aging, overweight males. *American Journal of Men's Health*, *13*(2), 1–15. https://doi.org/10.1177/1557988319835985

Mills, S. Y., Simon Mills, M. F. M. A., & Bone, K. (2004). *The essential guide to herbal safety*. St. Louis, Missouri: Elsevier Health Sciences.

Mirjalili, M. H., Moyano, E., Bonfill, M., Cusido, R. M., & Palazón, J. (2009). Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Molecules*, *14*(7), 2373–2393. https://doi.org/10.3390/molecules14072373

Mishra, S. K., Venkatachalapathy, B. A., & Khanli, H. M. (2017). Safety and efficacy of Ashwagandha (*Withania somnifera*). In *Neuroprotective natural products: Clinical aspects and mode of action* (pp. 313–319). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA. https://doi.org/10.1002/9783527803781.ch12

Narayana, D., & Durg, S. (2019). Approaches to safety evaluation of botanicals and processed botanicals known in traditional knowledge. In D. Ghosh & P. Mukherjee (Eds.), *Natural medicines: Clinical efficacy, safety and quality* (pp. 187–214). Boca Raton, FL: CRC Press. https://doi.org/10.1201/9781315187853-10

Narayana, D. B. A., Durg, S., Manohar, P. R., Mahapatra, A., & Aramya, A. R. (2017). Chyawanprash: A review of therapeutic benefits as in authoritative texts and documented clinical literature. *Journal of Ethnopharmacology*, *197*, 52–60. https://doi.org/10.1016/j.jep.2016.07.078

Nayak, S., Nayak, S., Panda, B., & Das, S. (2015). A clinical study on management of stress in type-2 diabetes mellitus (Madhumeha) with ashwagandha (*Withania somnifera*). *Ayushdhara*, *2*(6), 413–417.

Nirupama, R., Devaki, M., Nirupama, M., & Yajurvedi, H. (2014). In vitro and in vivo studies on the hypoglycaemic potential of Ashwagandha (*Withania somnifera*) root. *Pharma Science Monitor*, *5*(3), 45–58.

Parihar, M. S., 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*. *Journal of Clinical Neuroscience*, *11*(4), 397–402. https://doi.org/10.1016/j.jocn.2003.09.008

Parihar, P., Shetty, R., Ghafourifar, P., & Parihar, M. S. (2016). Increase in oxidative stress and mitochondrial impairment in hypothalamus of streptozotocin treated diabetic rat: Antioxidative effect of *Withania somnifera*. *Cellular and Molecular Biology*, *62*(1), 73–83. https://doi.org/10.14715/cmb/2016.62.1.15

Patel, S. B., Rao, N. J., & Hingorani, L. L. (2016). Safety assessment of *Withania somnifera* extract standardized for Withaferin A: Acute and sub-acute toxicity study. *Journal of Ayurveda and Integrative Medicine*, *7*(1), 30–37. https://doi.org/10.1016/j.jaim.2015.08.001

Raut, A., Rege, N., Shirolkar, S., Pandey, S., Tadvi, F., Solanki, P., … Kene, K. (2012). Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (*Withania somnifera*) in healthy volunteers. *Journal of Ayurveda and Integrative Medicine*, *3*(3), 111–114. https://doi.org/10.4103/0975-9476.100168

Reuland, D. J., Khademi, S., Castle, C. J., Irwin, D. C., McCord, J. M., Miller, B. F., & Hamilton, K. L. (2013). Upregulation of phase II enzymes through phytochemical activation of Nrf2 protects cardiomyocytes against oxidant stress. *Free Radical Biology and Medicine*, *56*, 102–111. https://doi.org/10.1016/j.freeradbiomed.2012.11.016

Salmela, P. I., Sotaniemi, E. A., Niemi, M., & Mäentausta, O. (1984). Liver function tests in diabetic patients. *Diabetes Care*, *7*(3), 248–254. https://doi.org/10.2337/diacare.7.3.248

Samadi Noshahr, Z., Shahraki, M. R., Ahmadvand, H., Nourabadi, D., & Nakhaei, A. (2015). Protective effects of *Withania somnifera* root on inflammatory markers and insulin resistance in fructose-fed rats. *Reports of Biochemistry & Molecular Biology*, *3*(2), 62–67.

Sandhu, J. S., Shah, B., Shenoy, S., Chauhan, S., Lavekar, G. S., & Padhi, M. M. (2010). Effects of *Withania somnifera* (Ashwagandha) and *Terminalia arjuna* (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults. *International Journal of Ayurveda Research*, *1*(3), 144–149. https://doi.org/10.4103/0974-7788.72485

Sarangi, A., Jena, S., Sarangi, A. K., & Swain, B. (2013). Anti-diabetic effects of *Withania somnifera* root and leaf extracts on streptozotocin induced diabetic rats. *Journal of Cell and Tissue Research*, *13*(1), 3597–3601.

Sarjan, H., & Yajurvedi, H. (2018). Efficacy of an active compound of the herb, Ashwagandha in prevention of stress induced hyperglycemia. *International Journal of Pharmacy and Pharmaceutical Sciences*, *10*(10), 44. https://doi.org/10.22159/ijpps.2018v10i10.28717

Shah, R. A., Khan, S., Vakil, M., & Qureshi, M. T. (2017). Evaluation of antidiabetic potential of *Withania somnifera* by glucose uptake assay on3T3 F442A fibroblast (adipocyte) cells. *International Journal of Pharmaceutical Sciences and Research*, *8*(1), 120–125. https://doi.org/10.13040/IJPSR.0975-8232.8(1).120-25

Shahraki, M. R., Samadi Noshahr, Z., Ahmadvand, H., & Nakhaie, A. (2016). Anti-nociceptive and anti-inflammatory effects of *Withania somnifera* root in fructose fed male rats. *Journal of Basic and Clinical Physiology and Pharmacology*, *27*(4), 387–391. https://doi.org/10.1515/jbcpp-2015-0053

Sharma, H., & Chandola, H. M. (2011). Prameha in ayurveda: Correlation with obesity, metabolic syndrome, and diabetes mellitus. Part 1–etiology, classification, and pathogenesis. *The Journal of Alternative and Complementary Medicine*, *17*(6), 491–496. https://doi.org/10.1089/acm.2010.0396

Sherwani, S. I., Khan, H. A., Ekhzaimy, A., Masood, A., & Sakharkar, M. K. (2016). Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker Insights*, *11*, 95–104. https://doi.org/10.4137/BMI.S38440

Simon, K., Inoue, T., Fenteany, G., Bahtiyar, G., & Sacerdote, A. (2018). Ashwagandha root in the treatment of prediabetes. *Endocrine Reviews*, *39*(2) Supplement 1, Page i. https://doi.org/10.1093/edrv/39.supp_2.1

Singh, A. K., Joshi, J., & Jatwa, R. (2013). Dipeptidyl peptidase IV (CD26/DPP-IV) inhibitory and free radical scavenging potential of W somnifera and T foenum-graecum extract. *International Journal of Phytomedicine*, *5*(4), 503–509. https://doi.org/10.5138/ijpm.v5i4.1212

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537–546.

Tangvarasittichai, S. (2015). Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World Journal of Diabetes*, *6*(3), 456–480. https://doi.org/10.4239/wjd.v6.i3.456

Tekula, S., Khurana, A., Anchi, P., & Godugu, C. (2018). Withaferin-A attenuates multiple low doses of streptozotocin (MLD-STZ) induced type 1 diabetes. *Biomedicine & Pharmacotherapy*, *106*, 1428–1440. https://doi.org/10.1016/j.biopha.2018.07.090

Thakur, A., Dey, A., Shyam Chatterjee, S., & Kumar, V. (2015). Reverse ayurvedic pharmacology of Ashwagandha as an adaptogenic antidiabetic plant: A pilot study. *Current Traditional Medicine*, *1*(1), 51–61. https://doi.org/10.2174/2215083801999150527115205

Tuomilehto, J., Lindström, J., Eriksson, J. G., Valle, T. T., Hämäläinen, H., Ilanne-Parikka, P., … Uusitupa, M. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine*, *344*(18), 1343–1350. https://doi.org/10.1056/NEJM200105033441801

Udayakumar, R., Kasthurirengan, S., Mariashibu, T. S., Rajesh, M., Anbazhagan, V. R., Kim, S. C., … Choi, C. W. (2009). Hypoglycaemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan-induced diabetic rats. *International Journal of Molecular Sciences*, *10*(5), 2367–2382. https://doi.org/10.3390/ijms10052367

Udayakumar, R., Kasthurirengan, S., Vasudevan, A., Mariashibu, T. S., Rayan, J. J. S., Choi, C. W., … Kim, S. C. (2010). Antioxidant effect of dietary supplement *Withania somnifera* L. reduce blood glucose levels in Alloxan-induced diabetic rats. *Plant Foods for Human Nutrition*, *65*(2), 91–98. https://doi.org/10.1007/s11130-009-0146-8

Usharani, P., Fatima, N., Kumar, C., & Kishan, P. (2014). 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. *International Journal of Ayurveda and Pharma Research*, *2*(3), 22–32.

Usharani, P., Kishan, P., Fatima, N., & Kumar, C. (2014). A comparative study to evaluate the effect of highly standardised aqueous extracts of *Phyllanthus emblica*, *Withania somnifera* and their combination on endothelial dysfunction and biomarkers in patients with type II diabetes mellitus. *International Journal of Pharmaceutical Sciences and Research*, *5*(7), 2687–2697. https://doi.org/10.13040/IJPSR.0975-8232.5(6).2687-97

Visavadiya, N. P., & Narasimhacharya, A. V. R. L. (2007). Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*, *14*(2–3), 136–142. https://doi.org/10.1016/j.phymed.2006.03.005

Wagner, A. M., Martijnez-Rubio, A., Ordonez-Llanos, J., & Perez-Perez, A. (2002). Diabetes mellitus and cardiovascular disease. *European Journal of Internal Medicine*, *13*(1), 15–30.

Weiner, M., & Weiner, J. (1994). Ashwagandha (Indian ginseng). In M. Weiner & J. Weiner (Eds.), *Herbs that heal. Mill Valley, California: Quantum Books*, (pp. 70–72).

World Health Organization. (2016). *Global report on diabetes*. ISBN: 978 92 4 156525 7.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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