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Review

Withania somnifera (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: a systematic review and meta-analysis

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Abstract

Objectives *Withania somnifera* has been in use for several thousand years in Ayurveda to treat various neurological disorders. There is, however, not much scientific data on its protective role in neuronal pathology specifically against brain oxidative stress. Hence, an attempt is made in this work for systematic review and meta-analysis of *W. somnifera* on neurobehavioural disorders induced by brain oxidative stress in rodents.

Methods A systematic search of the effect of *W. somnifera* on brain oxidative stress-induced neuronal pathology was performed using electronic databases. The systematic review was performed on neurobehavioural parameters, whereas meta-analysis of *W. somnifera* effect was done on oxidative stress markers (superoxide dismutase, catalase, glutathione peroxidase, glutathione and lipid peroxidation), nitrite, protein carbonyl, AchE, ChAT and Ach of rodent brain. Data were analysed using Review Manager Software.

Key findings Twenty-eight studies were selected based upon the inclusion and exclusion criteria. *W. somnifera* appreciably inhibited the neurological abnormalities due to oxidative stress in rodent brain produced by different physical and chemical stimuli. *W. somnifera* also significantly restored the altered oxidative and other stress markers in different parts of rodent brain.

Summary The systematic review provides scientific evidence for the traditional claim of *W. somnifera* use in different neurological aliments. However, future clinical trials are mandated to establish the therapeutic efficacy and safety in human beings.

Introduction

Withania somnifera Dunal (*Ashwagandha*; Family-Solanaceae), popularly known as Indian ginseng, winter cherry and *ajagandha*, has been in use for more than 2500 years in Ayurvedic medicine to treat several neurological disorders.[1] In India, *W. somnifera* is found in waste land, cultivated field and open grounds, and is widely cultivated in certain areas of Madhya Pradesh and Rajasthan. Various parts of the herb have been used for centuries, but dried mature roots are mostly used to treat a variety of ailments.[1]

The root extract contains steroidal lactones with ergostane, which include withanone, withaferin, withanolides, withanolide C, sitoindosides and about 0.2% alkaloids, and thus shows similarity to the active constituents of *Panax ginseng*.[2] The traditional Indian system of medicine categorized *W. somnifera* as *rasayana*, which are reputed to promote health and longevity by arresting the ageing process, augmenting defence against disease, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental effects and creating a sense of mental well-being.[3] *W. somnifera* has been known for its potent antioxidant and free radical quenching properties in various disease conditions.[4] The title herb is reported to possess beneficial effects in a wide range of central nervous system (CNS) pathology in rodents. These include catalepsy,[5] cognitive and memory impairment,[6–9] orofacial dyskinesia,[10] stress,[4,11–13] Parkinson's disease (PD),[14–17] Huntington's

disease (HD),[18–20] Alzheimer's disease (AD),[6,21] cerebral stroke,[22] epilepsy,[23] excitotoxicity,[24] in sleep disturbed mice,[25] chronic fatigue syndrome,[26] streptozotocin-induced oxidative stress,[8,27] copper-induced oxidative stress[28] and rotenone-induced oxidative stress.[29]

The herb is also known to modulate the brain oxidative stress makers, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), lipid peroxidation (LPO), and non-enzymatic antioxidants like glutathione (GSH).[4,6] The root extract of *W. somnifera* could induce axon and dendrite outgrowth, suggesting its potential effect on neuronal regeneration.[30,31]

Presently, there is no scientific documentation exploring the therapeutic role of *W. somnifera* on neurobehavioural disorders induced by brain oxidative stress. Hence, this study aims at the systematic evaluation and meta-analysis of *W. somnifera* literature on CNS disorders in rodents, mainly brain oxidative stress. The review also updates the information of *W. somnifera* extract and its isolated marker compounds used in the selected research studies.

Methodology

Literature search

The research publications reporting the activity of *W. somnifera* on brain oxidative stress were searched for systematic review and meta-analysis. The literature search was performed using the keywords like '*Withania somnifera*, *Indian ginseng*, *Ashwagandha and Winter cherry*' in combination with '*stress*, *oxidative stress*, *adaptogenic*, *antioxidant*, *nervous tension*, *neurological disorders* and *anxiety*' in the following databases: PubMed/Medline, Scifinder, SciVerse Scopus, SciVerse Science Direct and Google Scholar for studies published up to October 2013. The literature search was not restricted to any part or isolated marker of *W. somnifera*, dose, route and duration of administration, language, and animal and human studies. The authors, however, did not find any specific human studies reporting the treatment of neuronal disorders using *W. somnifera* alone or its isolated marker compounds. From here onwards, the authors of the study concentrated only on animal (rodent) models. The commentaries and conference proceedings were however, excluded. The research articles obtained with the above said databases and keywords were analysed by interpreting the 'abstracts' or 'full copy'. All studies reporting oxidative stress markers in rodent brain and behavioural parameters, regardless of their inducing agents or neurological disorders, were retained. To obtain additional data, cross-references in papers or reviews were also searched. The literature search was performed by Durg S., but the final selection, inclusion and exclusion of articles for systematic review and meta-analysis were confirmed after concerning all the co-authors.

Data extraction and analysis

A systematic review and meta-analysis of research evaluating the activity of *W. somnifera* on oxidative stress in rodent brain was performed if the parameter of interest was reported in two or more studies. The systematic review was done on neurobehavioural parameters, whereas meta-analysis was performed on the means of brain oxidative stress markers (SOD, catalase, GPx, GSH and LPO), nitrite, protein carbonyl, as well as neuronal stress markers like acetyl choline esterase, choline acetyl transferase and acetyl choline, by analysing between the pathological control and *W. somnifera* treatment group. In the studies where the results were only graphically represented, the numerical values from graphs were extracted using Adobe Acrobat's XI inbuilt measuring tool (Adobe Systems Incorporated, San Jose (California), The United States). In studies that used more than one dose of *W. somnifera* or its isolated marker, the most effective dose as stated by the authors was considered. The random statistical model was used for meta-analysis of all selected parameters. Separate graphs were plotted for mice and rat studies, as well as for different expressing units representing single parameter. The amount of heterogeneity during meta-analysis was assessed by calculating I^2 statistic (which represents the percentage of variation between the studies that is due to heterogeneity rather than chance). Potential sources of heterogeneity were investigated by comparing the study designs. The studies that expressed results in standard error mean were converted to standard deviations as per the published formula.[32] Data were analysed using Review Manager Software (RevMan; computer programme; version 5.3.5., The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, 2012). In all analyses, a P-value < 0.05 was considered statistically significant.

Results

Selection of articles

Electronic searches resulted in the following number (n) of articles in the databases: PubMed/Medline ($n = 45$), Scifinder ($n = 34$), SciVerse Scopus ($n = 31$), SciVerse Science Direct ($n = 49$) and Google Scholar ($n = 43$). Four articles documented by cross-references in papers were also considered for the study. Out of 206 articles, based on the inclusion and exclusion criteria, 28 articles were selected for the review. The duration of included studies varied from single day to multiple (63) days and were conducted in mice ($n = 12$) or rat ($n = 16$) models. All the studies were conducted using authenticated parts of *W. somnifera* extract (root extract was used in 24 studies, leaf extract in one study and the remaining three studies did not mention the part of herb used). The search strategy and descriptions of

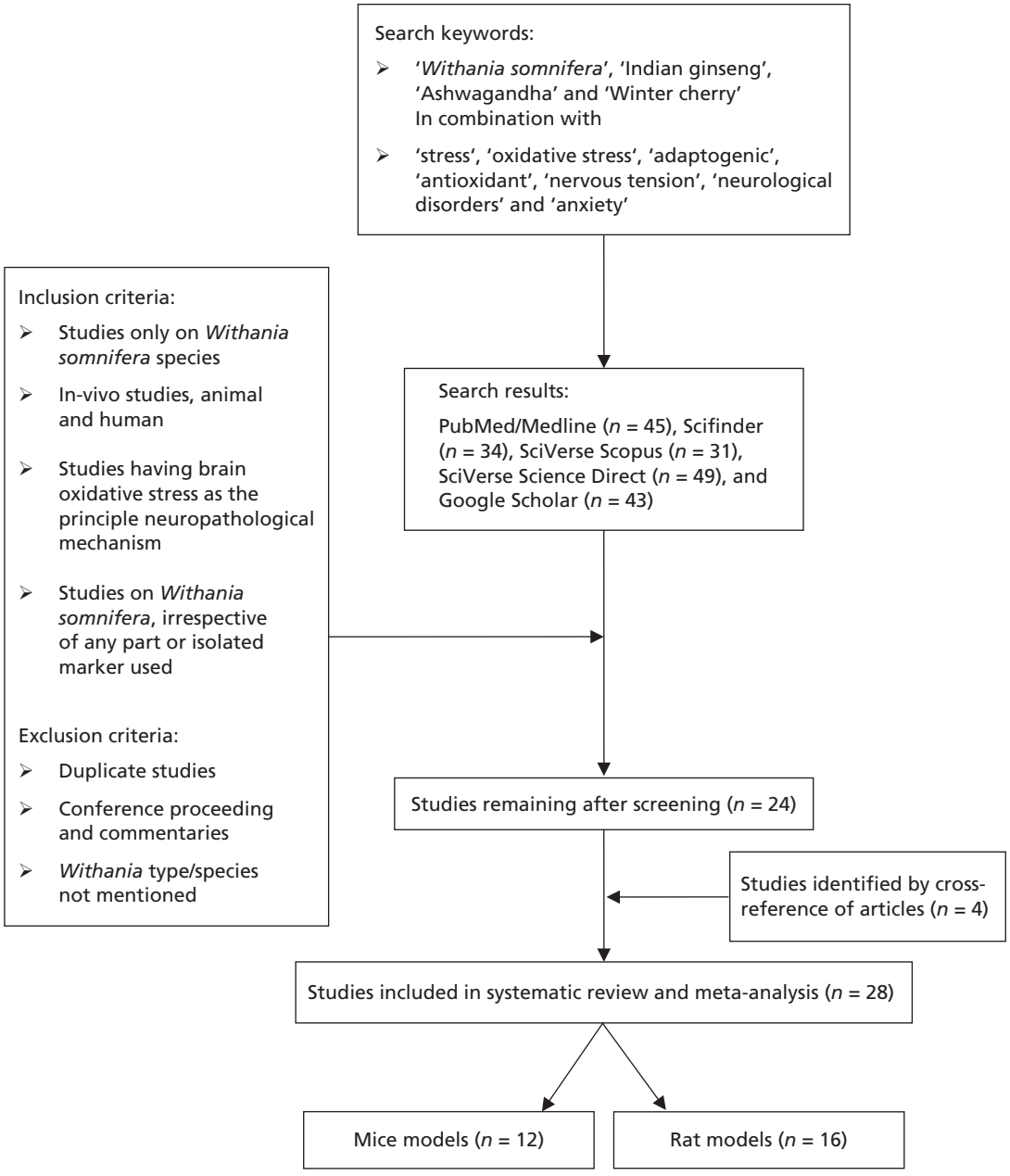


Figure 1 Search strategy.

the studies including *W. somnifera* extract were summarized in Figure 1 and Table 1, respectively.

Effect of *Withania somnifera* on body weight changes

Reduction in the body weight is one of the parameters of interest in 3-NP (3-nitropropionic acid)-induced HD. *W. somnifera* treatment for 14 days in animals with 3-NP-induced HD showed protective effect. Both the tested doses of *W. somnifera* (100 and 200 mg/kg) protected the animals well from decrease in body weight induced by 3-NP. The metabolic impairment and hypoactivity, induced by 3-NP, was the responsible factor for reducing the body weight in these animals. The protective action of the herb against 3-NP-induced decrease in body weight might be due to improvement in metabolic process or decreased hypoactivity in these animals.[19,25]

Table 1 Description of the studies included

Authors	Description of *Withania somnifera*	Dose – mg/kg (route of administration)	Study description	Parameters studied
Ahmed *et al*.[8]	Authenticity and quality of *W. somnifera* (the authors did not mention the part used), alcoholic extract, was checked by fingerprinting and quantification of Withaferin A using the CAMAG HPTLC system	100, 200 and 300 (oral)	Male Wistar rats ($N = 80$); STZ induced oxidative damage associated cognitive decline; control ($n = 10$) and *W. somnifera* ($n = 10$); eight groups; duration – 21 days	Morris water maze test; brain oxidative stress markers (LPO, GSH, GPx, GR and CAT in hippocampus and cerebral cortex) and AchE (in hippocampus) estimation; caspase-3 activity; H&E staining; immunohistochemistry
Baitharu *et al*.[9]	Authenticated *W. somnifera* root powder was provided by Ambe Phytochemicals, (Delhi), India. The extract was quantified by glycowithanolides and withanones using HPLC system for withanolide A and D, withanone and withaferin A	200 (oral)	Male Sprague Dawley rats (phase I; $N = 40$ and phase II; $N = 84$); hypobaric hypoxia induced memory impairment; phase I – control ($n = 10$) and *W. somnifera* ($n = 10$); four groups; duration 21 days; phase II – control ($n = 12$) and *W. somnifera* ($n = 12$); duration – 7 days	Plasma and hippocampal tissue corticosterone; Ach, AchE and nitric oxide in hippocampal tissue; nNOS, L-type calcium channels, NCAM, BDNF, Bcl_2, Bax and synaptophysin in hippocampus by western blotting; oxidative stress markers (SOD, GSH, ROS and LPO) in the hippocampus of brain; morphological analysis of hippocampal neurons using crystal violet staining; Morris water maze
Bhatnagar *et al*.[33]	The purified extract of the root of *W. somnifera* was purchased from R and D Center, Indian Herbs Research and Supply Co. Ltd. Saharanpur (UP), India	40 (oral)	Adult Swiss albino mice of both sexes ($N = 24$); restraint stress induced neurodegeneration; control ($n = 6$) and *W. somnifera* ($n = 6$); four groups; duration – 30 days	NADPH-d histochemistry; ChAT immunohistochemistry; serum corticosterone and brain hippocampus serotonin estimation; hippocampal nNOS positive cell number
Bhattacharya *et al*.[6]	Aqueous root extract of *W. somnifera* was quantified by sitoindosides VII-X and withaferin (collectively called as glycowithanolides) using CAMAG HPTLC system	20 and 50 (oral)	Adult male Wistar rats ($N = 54$; learned active avoidance task, $N = 44$; cortical and hippocampal Ach concentrations, AchE activity and muscarinic cholinergic receptor binding); ibotenic acid induced lesioning of the nucleus basalis magnocellularis in Alzheimer's disease model; control ($n = 10$) and *W. somnifera* ($n = 8$); six groups; duration – 7 and 14 days	Learned active avoidance task; frontal cortical and hippocampal Ach concentrations, ChAT activity and muscarinic cholinergic receptor binding
Bhattacharya *et al*.[11]	Aqueous root extract of *W. somnifera* was quantified by sitoindosides VII-X and withaferin (collectively called as glycowithanolides) using CAMAG HPTLC system	10 and 20 (i.p.)	Adult male Wistar rats ($N = 108$); control ($n = 14$) and *W. somnifera* ($n = 8$); four groups; duration – 7, 14 and 21 days	Oxidative stress markers (SOD, CAT and GPx) in frontal cortex and striatum of brain
Bhattacharya *et al*.[4]	Standardized alcoholic root extract of *W. somnifera* was quantified by sitoindosides and withaferin A using CAMAG HPTLC system	10, 20 and 50 (oral)	Adult male Wistar rats ($N = 40$); chronic foot shock stress induced oxidative stress; control ($n = 8$) and *W. somnifera* ($n = 8$); five groups; duration – 21 days	Oxidative stress markers (SOD, CAT, GPx and LPO) in frontal cortex and striatum of brain

Chaudhary *et al*.[22]	A standardized hydroalcoholic extract of *W. somnifera* (the authors did not mention the part used) was obtained from Dabur Research Foundation, Ghaziabad (UP), India	100 (oral)	Male Wistar rats ($N = 48$); middle cerebral artery induced occlusion induced stroke; control ($n = 12$) and *W. somnifera* ($n = 12$); four groups; duration – 15 and 30 days	Motor performance tests; grip test; rota-rod test; foot fault test; MRI; brain MDA levels
Gupta *et al*.[28]	The purified extract of *W. somnifera* (the authors did not mention the part used) was supplied by Lakshmi Natural Products Pvt. Ltd., Bombay (Maharashtra), India	50, 100 and 150 (i.p.)	Male albino rats ($N = 20$); aged spinal cord induced LPO and protein oxidation; control ($n = 5$) and *W. somnifera* ($n = 5$); four groups; duration – 30 days	Oxidative stress markers (GPx and MDA) in spinal cord
Khan and Ghosh[12]	*W. somnifera* root extract was standardized using HPLC system with withaferin A as a standard biomarker	i) *W. somnifera*: 50, 100, 200 and 500 (oral) ii) Withaferin A: 10, 20, 30, 40 and 50 (i.p.)	Male Wistar rats ($N = 69$); stress induced neurobehavioural changes; control ($n = 8$), *W. somnifera* ($n = 8$) and withaferin A ($n = 8$); ten groups; duration – 1 day	Elevated plus maze test; open field test; brain NOx activity
Khan and Ghosh[13]	HPLC system was used for standardization and quantification of *W. somnifera* root extract with withaferin A as a standard biomarker	Withaferin A: 20, 30, 40 and 50 (i.p.)	Naive young (2 months) and old (16 months) male Wistar rats ($N = 256$); restraint stress on neurobehavioural and brain oxidative/nitrosative stress markers and their modulation by withaferin A; control ($n = 8$) and withaferin A ($n = 8$); 10 groups; duration – 1 day	Elevated plus maze test; open field test; brain oxidative stress markers (SOD, CAT, GSH and LPO) and Nox activity
Kumar and Kalonia[25]	The purified root extract of *W. somnifera* was supplied by Himalayan Drugs, Bangalore (Karnataka), India	100 and 200 (oral)	Male Laca mice ($N = 36$); grid over water suspended sleep disturbance; control ($n = 6$) and *W. somnifera* ($n = 6$); six groups; duration – 5 days	Body weight; elevated plus maze, locomotor activity (actophotometer); brain oxidative stress markers (CAT, GSH, LPO and nitrite)
Kumar and Kumar[18]	The purified root extract of *W. somnifera* was supplied by Himalayan Drugs, Bangalore (Karnataka), India	100 and 200 (oral)	Male Wistar rats ($N = 50$); 3-NP induced cognitive dysfunction and oxidative damage; control ($n = 10$) and *W. somnifera* ($n = 10$); five groups; duration – 14 days	Morris water maze test; elevated plus maze test; oxidative stress markers (GSH and AchE) in striatum, cortex and hippocampus of brain
Kumar and Kumar[19]	The purified root extract of *W. somnifera* was supplied by Himalayan Drugs, Bangalore (Karnataka), India	100 and 200 (oral)	Male Wistar rats ($N = 50$); 3-NP induced behavioural, biochemical, mitochondrial dysfunction in Huntington's disease; control ($n = 10$) and *W. somnifera* ($n = 10$); five groups; duration – 14 days	Locomotor activity (actophotometer); limb withdrawal test; oxidative stress markers (SOD, CAT, LDH and LPO) and nitrite in striatum and cortex of brain; mitochondrial complex estimation
Manjunath and Muralidhara[29]	Standard root extract powder of *W. somnifera* (batch number: C81015, Withanolides, 2.57%; Withaferin A, 2.38%) was obtained from M/s Sami Labs Ltd., Bangalore (Karnataka), India	400 (oral)	Prepubertal male mice ($N = 18$); rotenone induced oxidative stress and neurotoxicity; control ($n = 6$) and *W. somnifera* ($n = 6$); three groups; duration – 28 days	Stride length; landing foot spread distance; total thiols, oxidative stress markers (SOD, CAT, GPx and GSH) in striatum and cerebellum of brain; neuronal function markers (AchE and DA) in striatum and cerebellum of brain; ETC enzymes; MTT in brain regions

Table 1 *Continued*

Authors	Description of *Withania somnifera*	Dose – mg/kg (route of administration)	Study description	Parameters studied
Naidu *et al*.[10]	The purified *W. somnifera* root extract was obtained from Gufic Labs, Mumbai (Maharashtra), India	100, 200 and 300 (oral)	Male Wistar rats ($N = 30$); haloperidol induced orofacial dyskinesia; control ($n = 6$) and *W. somnifera* ($n = 6$); four groups; duration – 22 days	Vacuous chewing movements and tongue protrusions counted; brain oxidative stress markers (SOD, CAT, GPx and LPO in forebrain)
Naidu *et al*.[7]	The purified *W. somnifera* root extract was obtained from Gufic Labs, Mumbai (Maharashtra), India	50 and 100 (oral)	Male Wistar rats ($N = 24$); reserpine induced orofacial dyskinesia and cognitive dysfunction; control ($n = 6$) and *W. somnifera* ($n = 6$); six groups; duration – 28 days	Behavioural assessment of orofacial dyskinesia; transfer latency on elevated plus-maze; brain oxidative stress markers (SOD, CAT, GSH and LPO)
Nair *et al*.[5]	Standardized water root extract of *W. somnifera* (Withanolides, 2.1% w/w, quantified by gravimetric method) was supplied by Natural Remedies Pvt. Ltd., Bangalore (Karnataka), India	1.7, 4.25 and 8.5 (oral)	Adult male Swiss albino mice ($N = 30$); haloperidol induced catalepsy; control ($n = 6$) and *W. somnifera* ($n = 6$); five groups; duration – 7 days	Catalepsy; brain oxidative stress marker (SOD)
Parihar and Hemnani[24]	Powdered root of *W. somnifera* was extracted in acetone and then fractioned using methanol (90%): hexane (1:1) liquid–liquid extraction method from a published research study	20 (oral)	Female Swiss albino mice ($N = 72$); kainic acid induced excitotoxicity; control ($n = 8$) and *W. somnifera* ($n = 8$); nine groups; duration – 21 days	Brain oxidative stress markers (GPx, GSH and LPO) and protein carbonyl estimation
Parihar *et al*.[27]	Powdered root of *W. somnifera* was extracted in acetone and then fractioned using methanol (90%): hexane (1:1) liquid–liquid extraction method from a published research study	20 (oral)	Female Swiss albino mice ($N = 40$); STZ induced oxidative damage; control ($n = 8$) and *W. somnifera* ($n = 8$); five groups; duration – 30 days	T-maze; active avoidance test; blood glucose; LPO and protein carbonyl content assay in hippocampus and cerebral cortex of brain
Parkash *et al*.[34]	Ethanolic root extract of *W. somnifera* was used for study. *W. somnifera* plants were obtained from the Institute of Medical Science, Banaras Hindu University, Varanasi (UP), India	100 (oral)	Male Swiss albino mice ($N = 54$); Maneb–Paraquat induced Parkinsonism; control ($n = 6$) and *W. somnifera* ($n = 6$); three groups; duration – 21, 42 and 63 days	Neurobehavioural parameters – hang test, narrow beam walking test, foot printing test; Tyrosine hydroxylase (TH)-immunoreactivity; brain oxidative stress markers (LPO, CAT and nitrite) in nigrostriatal tissues
Preeti *et al*.[20]	*W. somnifera* root extract was used for the study, but the authors did not give the information regarding its source	100 (oral)	Male Wistar rats ($N = 36$); 3-NP induced neuronal damage in Wistar rats; control ($n = 6$) and *W. somnifera* ($n = 6$); six groups; duration – 7 days	Locomotor activity; creatine kinase assay; comet assay
Rajasankar *et al*.[15]	The purified *W. somnifera* root extract was obtained from Indian Medical Practitioners Co-operative Society, Adyar, Madras (Tamil Nadu), India	100 (oral)	Male albino mice ($N = 18$); MPTP induced Parkinson's disease; control ($n = 6$) and *W. somnifera* ($n = 6$); three groups; duration – 7 and 28 days	Rota-rod test; hang test; HPLC analysis of DA, DOPAC and HVA; oxidative stress markers (GSH, GPx and MDA) in the striatum of brain

Rajasankar *et al.*[16]	The purified *W. somnifera* leaf extract was used for the study, but the authors did not mention the source	100 (oral)	Male Swiss albino mice ($N = 18$); MPTP induced Parkinson's disease; control ($n = 6$) and *W. somnifera* ($n = 6$); three groups; duration – 7 days	Open field test; narrow beam walking; stride length measurement; oxidative stress markers (SOD, CAT, GPx, GSH and MDA) in corpus striatum and mid brain
Sankar *et al.*[14]	The purified *W. somnifera* root extract was obtained from Indian Medical Practitioners Co-operative Society, Adyar, Madras (Tamil Nadu), India	100 (oral)	Male albino mice ($N = 36$); MPTP induced Parkinsonism; control ($n = 6$) and *W. somnifera* ($n = 6$); four groups; duration – 28 days	Rota-rod test; hang test; stride length; brain oxidative stress markers (SOD, CAT and TBARS in mid brain)
Sehgal *et al.*[21]	The powdered root of *W. somnifera* was obtained from an authenticated source (Arya Vaidya Sala). Quality of *W. somnifera* extract was checked by fingerprinting and quantification of withanolides (75%) and withanosides (20%) using LC-MS	1000 (oral)	Transgenic mice ($N = 16$); *W. somnifera* reveres Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver; control ($n = 8$) and *W. somnifera* ($n = 8$); two groups; duration – 30 days	Radial maze task; Morris water maze test; amyloid beta; expression studies of low-density lipoprotein receptor-related protein and neprilysin
Singh *et al.*[26]	The purified *W. somnifera* root extract was obtained from Gufic Ltd., Mumbai (Maharashtra), India	100 (oral)	Male Laca mice ($N = 42$); forced swim induced chronic fatigue syndrome; control ($n = 6$) and *W. somnifera* ($n = 6$); seven groups; duration – 15 days	Measurement of immobility period; brain oxidative stress markers (LPO, GSH SOD and CAT)
Soman *et al.*[23]	Roots of *W. somnifera* were obtained from Kerala Ayurveda Ltd., Aluva (Kerala), India. The water extract was used for the study	150 (oral)	Male Wistar rats ($N = 30$ to 40); pilocarpine induced oxidative stress leads to NMDA receptor alterations and spatial memory deficits in temporal lobe epilepsy; control ($n = 6$ to 8) and *W. somnifera* ($n = 6$ to 8); five groups; duration – 7 days	Radial arm maze test; TOPRO-3 staining; NMDA receptor binding studies using [3H] MK-801, immunohistochemistry; RT-PCR for total RNA estimation in hippocampus; oxidative stress markers (SOD, CAT and MDA) in hippocampus of brain
Trigunayat *et al.*[35]	The standardized ethanolic root extract of *W. somnifera* was quantified by biomarkers, sitoindosides and withaferin A, using CAMAG HPTLC system	50 (oral)	Charles-Foster male albino rats ($N = 24$); cerebral ischaemia-reperfusion and long-term hypoperfusion induced alteration; control ($n = 6$) and *W. somnifera* ($n = 6$); four groups; duration – 15 days	Morris water maze test; histopathology; open field test; oxidative stress markers (LPO and SOD) in forebrain regions, T-SH and cAMP estimation

N = total number of animals in the study; n = total number of animals in a group. Ach, acetylcholine; AchE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CAT, catalase; ChAT, choline acetyltransferase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; ETC, electron transport chain; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; LPO, lipid peroxidation; MDA, malondialdehyde; MRI, magnetic resonance imaging; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADPH-d, reduced nicotinamide adenine dinucleotide phosphate; NCAM, neural cell adhesion molecule; NMDA, N-methyl-D-aspartic acid; nNOS, neuronal nitric oxide synthase; Nox, total nitrates and nitrites; RNA, ribonucleic acid; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

Behavioural parameters

Effect of Withania somnifera *on locomotor activity*

The effect of *W. somnifera* on hypoactivity in 3-NP-induced HD model in rats was studied using actophotometer chamber.[19,25] The locomotor activity was expressed in terms of total photo beams counts/5 min/animal. In both the studies, administration of *W. somnifera* root extract (100 and 200 mg/kg) significantly shifted hypoactivity induced by 3-NP towards normal as compared with 3-NP alone treated rats. However, co-administration of diazepam, a benzodiazepine, with *W. somnifera* could not significantly further improve the locomotor activity.[25] These data reveal the beneficial effects of *W. somnifera* upon HD-induced hypoactivity in rodents.

Effect of Withania somnifera *on motor coordination*

Motor incoordination in CNS pathology, stimulated by chemical and physical inducing agents, was studied by different parameters like narrow beam walking test,[16,36] limb withdrawal test,[19] T-maze and foot fall test.[16,22,27]

Narrow beam walking test was used to access the motor coordination (the walking posture of the experimental animal) requiring balance and equilibrium. Animals were made to train to walk on a stationary wooden narrow flat beam (L100-cm × W1-cm) placed at a height of 100 cm from the floor. The time to walk the beam from one end to the other and the number of foot errors were counted. Narrow beam walking errors were increased in PD mice when compared with control treated mice. *W. somnifera* (100 mg/kg) pretreatment decreased the walking errors in PD mice as compared with *W. somnifera* untreated PD mice.[16,36]

Parihar and colleagues (2004) used T-maze apparatus to evaluate the motor performance of diabetic mice. Mice were placed in a T-maze box, and the time taken to cross the serpentine path(s) was evaluated from the front-view images. A significant impairment of locomotory activity was observed in diabetic mice. This locomotory impairment was attenuated by supplementation of *W. somnifera* root extract in diabetic mice. A decline in latency to cross the serpentine path was observed in mice supplemented with *W. somnifera* (20 mg/kg) root extract when compared with diabetic control.[27]

Limb withdrawal test is considered to be an important parameter to measure functional abnormalities of the hind limbs, which is indicative of the extent of striatal degeneration. Intraperitoneal administration of 3-NP (10 mg/kg) to rats caused a delay in retraction time of the hind limb, which was dose-dependently improved by *W. somnifera* (100 and 200 mg/kg) pretreatment.[19]

Chaudhary and colleagues (2003) used foot fault error test to evaluate the muscular integrity of middle cerebral artery occluded (MCAO) rats and is calculated by the formula '% foot fault error = (no. foot fault steps/no. paired steps) × 100'. The mean foot fault error was significantly increased in vehicle-treated MCAO rats (69 ± 8%). *W. somnifera* (30 days) pretreated MCAO rats showed a significant decrease in foot fault errors (30 ± 9%; $P < 0.05$) compared with vehicle-treated MCAO rats.[22] In another study, foot-slipping errors were increased in PD mice as compared with vehicle control. *W. somnifera* coadministration decreased foot-slipping errors in PD mice when compared with *W. somnifera* untreated PD mice.[16]

Effect of Withania somnifera *on anxiety*

The effect of *W. somnifera* on sleep disturbed mice anxiety was studied using plus maze apparatus.[25] Pretreatment with *W. somnifera* (100 and 200 mg/kg) root extract significantly increased the number of entries and duration of open arm as compared with control (sleep-deprived). Besides, there was decrease in the duration of closed arm. Beneficial effect of withaferin A, an isolated biomarker of *W. somnifera*, in plus maze was also reported.[12,13] Trigunayat and colleagues (2007) studied the effect of *W. somnifera* using open field test on anxiety in cerebral ischaemia-reperfusion and long-term induced hypoperfusion in rats. *W. somnifera* at 50 mg/kg dose reversed the reduced number of ambulation, rearing and grooming induced by ischaemia-reperfusion to normal.[35] Rajasankar and team (2009b) also used open field test to study the effect of *W. somnifera* on anxiety (100 mg/kg) in mouse model of PD and found significant beneficial effects against anxiety by *W. somnifera* treatment.[16] Khan and Ghosh (2010) used *W. somnifera* and its isolated biomarker withaferin A in restraint stress-induced rats taking open field test.[12] Interestingly, withaferin A displayed reduction in the number of ambulation, rearing and the latency at very low dose compared with *W. somnifera* alone. Outcome of the above studies indicates the potent anxiolytic activity of *W. somnifera* and withaferin A.

Effect of Withania somnifera *on muscle grip strength*

Rota-rod test was used to study the effect of *W. somnifera* on muscle grip strength of rodents in various CNS pathological models, such as 3-NP-induced HD, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced AD and cerebral stroke, etc. In all the tested models, *W. somnifera* significantly improved muscle grip strength compared with the disease controls. In case of MCAO model of stroke, *W. somnifera* pretreatment for 15 days did not show any significant effect, but regular treatment for 30 days

showed statistically significant results when compared with *W. somnifera* untreated rats.[14,19,22]

Some of the researchers used hanging test to study the effect of *W. somnifera* on muscle grip strength in rodents during neuropathological conditions. *W. somnifera* elucidated significant reduction in the hang time of the MPTP-injected animals as compared with the controls. The administration of *W. somnifera* to animals with lesions significantly increased the retention time. The group treated with *W. somnifera* alone, however, did not show any difference from the control group.[14] Administration of *W. somnifera* in the PD mice for 7 days improved motor function as determined by hang time test compared with the *W. somnifera* untreated PD mice.[15] Parkash and colleagues (2013) also performed the hanging experiment to evaluate the motor changes occurring in PD mice and *W. somnifera* treated mice. Behaviour analysis showed that the time period for gripping and hanging was significantly less in PD mice when compared with vehicle control.[35] Data from rota-rod and hanging tests further suggest that *W. somnifera* improves the altered muscle grip strength in various CNS pathological conditions.

Effect of Withania somnifera *on spatial reference memory and cognitive behaviour*

Morris water maze test was used to study the effect of *W. somnifera* supplementation on spatial reference memory deficit induced by hypoxia in rats,[9,34] and streptozotocin in rats and mice.[8,21] In the above-mentioned test models, pretreatment with *W. somnifera* caused significant alterations towards normal as compared with disease controls. Kumar and Kumar (2008) used plus maze test along with Morris water maze test to study the effect of *W. somnifera* on 3-NP-induced HD.[18] In both the tests, *W. somnifera* (100 and 200 mg/kg) treatment for 14 days significantly restored the memory loss caused by 3-NP. The effect of title herb on cognitive behaviour using plus maze learning task against reserpine-induced cognitive dysfunction was assessed.[7] *W. somnifera* root extract (50 and 100 mg/kg/day for four weeks) in reserpine-treated animals significantly improved the long-term memory and learning ability compared with reserpine alone treated animals. These data indicate that *W. somnifera* has a potent protective action against memory loss caused in various neurodegenerative disorders and on the capacity of improving learning ability.

Effect of Withania somnifera *on gait abnormalities*

Effect of *W. somnifera* on gait abnormalities was studied by stride length measurement method.[14,15] Stride length was determined by measuring the distance between each step on the same side of the body, measuring from the middle toe of the first step to the heel of the second step. An average of at least four clear steps was calculated. Mice treated with MPTP showed reduced grooming and stride length hind paw compared with the control. These abnormalities were significantly improved in the PD mice treated with *W. somnifera* (100 mg/kg) when compared with *W. somnifera* untreated PD mice.

Brain oxidative stress markers

Super oxide dismutase

Exposure of rodents to different physical and chemical stimuli significantly increased free radicals level, as SOD activity was significantly decreased compared with normoxic group. Coadministration of *W. somnifera* showed significant reversal of decreased SOD activity induced by various neuropathological conditions in the striatum (inverse variance (IV); 5.87, 95% confidence interval (CI), 3.56 to 8.18, $I^2 = 75\%$, $P < 0.00001$) and cerebellum (IV; 3.78, 95% CI, 2.42 to 5.14, $I^2 = 0\%$, $P < 0.00001$) of mice brain (Figure 2a). *W. somnifera* also exhibited significant elevation of decreased SOD content in striatum and frontal cortex (IV; 11.84, 95% CI, 8.51 to 15.17, $I^2 = 33\%$, $P < 0.00001$), hippocampus (IV; 6.81, 95% CI, 4.24 to 9.37, $I^2 = 0\%$, $P < 0.00001$) and rat brain homogenate (IV; 1.10, 95% CI, 0.12 to 2.08, $I^2 = 61\%$, $P = 0.03$; Figure 2b). Some studies reported SOD level in terms of percentage change; meta-analysis of the same also showed significant elevation of SOD level in different parts of rat and mice brain (IV; 26.09, 95% CI, 16.94 to 35.24, $I^2 = 74\%$, $P < 0.00001$, Figure 2c).

In some studies, higher concentration of SOD was reported in the neuropathological conditions compared with normal control animals. *W. somnifera* pretreatment normalized the increased SOD levels in striatum, frontal cortex and forebrain (IV; −7.92, 95% CI, −17.20 to 1.35, $I^2 = 98\%$, $P = 0.09$, Figure 2d) of rat brain, as well as midbrain, striatum and brain homogenate in mice (IV; −1.10, 95% CI, −1.56 to −0.64, $I^2 = 94\%$, $P < 0.00001$, Figure 2e). The meta-analysis of *W. somnifera* effect on SOD indicates the protective effect of *W. somnifera* against brain SOD changes induced in CNS pathology.

Catalase

Catalase activity was diminished significantly in different parts of rodents' brain by physical (chronic swimming, hypobaric hypoxia, chronic fatigue syndrome, sleep deprivation) and chemical (3-NP, streptozotocin) stimuli. Pretreatment with *W. somnifera* significantly reversed catalase activity in different parts of rat brain, such as striatum (IV; 11.34, 95% CI, 5.49 to 17.20, $I^2 = 84\%$, $P = 0.0001$), cortex (IV; 10.66, 95% CI, 6.02 to 15.31, $I^2 = 74\%$, $P < 0.0001$),

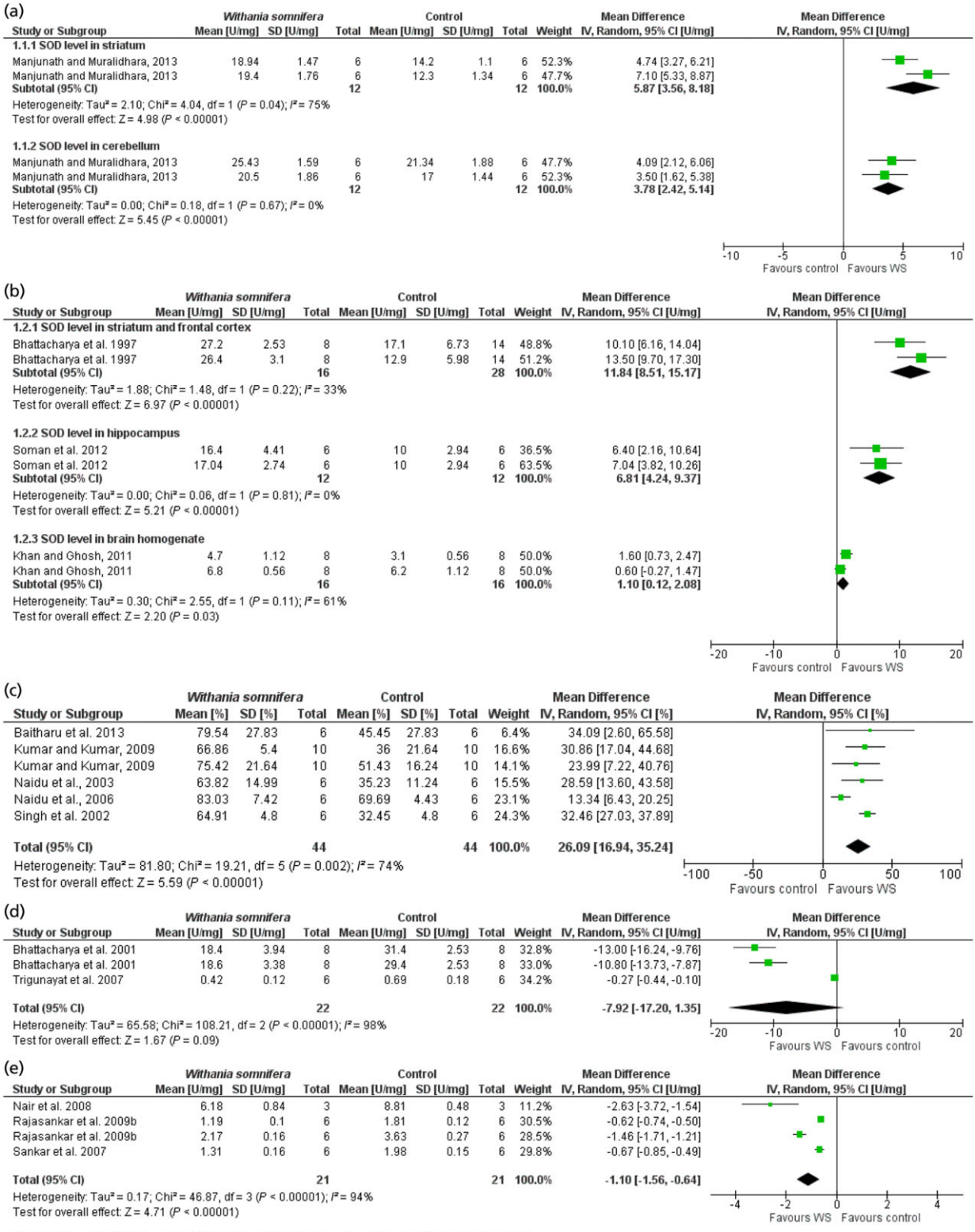


Figure 2 Forest plots showing superoxide dismutase levels in (a) striatum and cerebellum of mice brain; (b) striatum, frontal cortex, hippocampus and brain homogenate of rat; (c) hippocampus, striatum, cortex and brain homogenate of mice and rat (percentage change); (d) striatum, frontal cortex and brain homogenate of rat; and (e) midbrain, striatum and brain homogenate of mice.

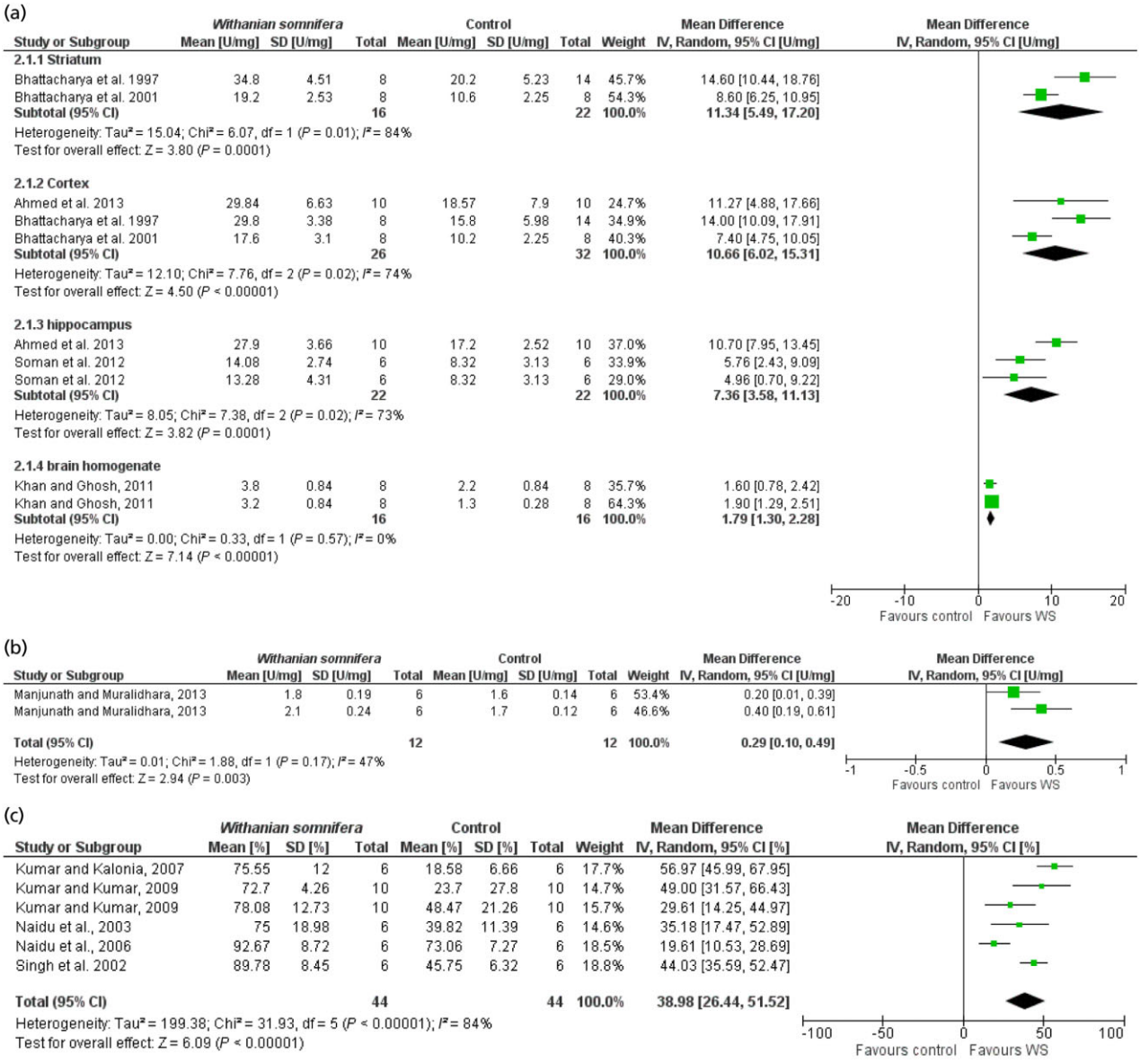


Figure 3 Forest plots showing catalase levels in (a) striatum, cortex, hippocampus and brain homogenate of rat; (b) striatum of mice brain; and (c) striatum, cortex and brain homogenate of mice and rat (percentage change).

hippocampus (IV; 7.36, 95% CI, 3.58 to 11.13, $I^2 = 73\%$, $P < 0.0001$) as well as whole brain (IV; 1.79, 95% CI, 1.30 to 2.28, $I^2 = 0\%$, $P < 0.0001$; Figure 3a). Similar statistically significant results were observed in cerebellum and striatum parts of mice brain (IV; 0.29, 95% CI, 0.10 to 0.49, $I^2 = 47\%$, $P = 0.003$, Figure 3b). The random statistic forest plot of catalase data depicted as percentage change also presented significant (IV; 38.98, 95% CI, 26.44 to 51.52, $I^2 = 84\%$, $P < 0.0001$, Figure 3c) elevation of catalase content by *W. somnifera* treatment. Decreased level of catalase was also observed in the nigrostriatum of the PD mouse (787.67 ± 134.23 μmol/mg protein) induced by Maneb–Paraquat. The treatment of PD mice with *W. somnifera* significantly (69.56%) increased (1335.61 ± 193.55 μmol/mg protein) the reduced levels of catalase in the nigrostriatum.[34]

Glutathione peroxidase

GPx activity was significantly reduced from oxidative damage and also its biochemical function to reduce lipid hydroperoxide action. *W. somnifera* administration

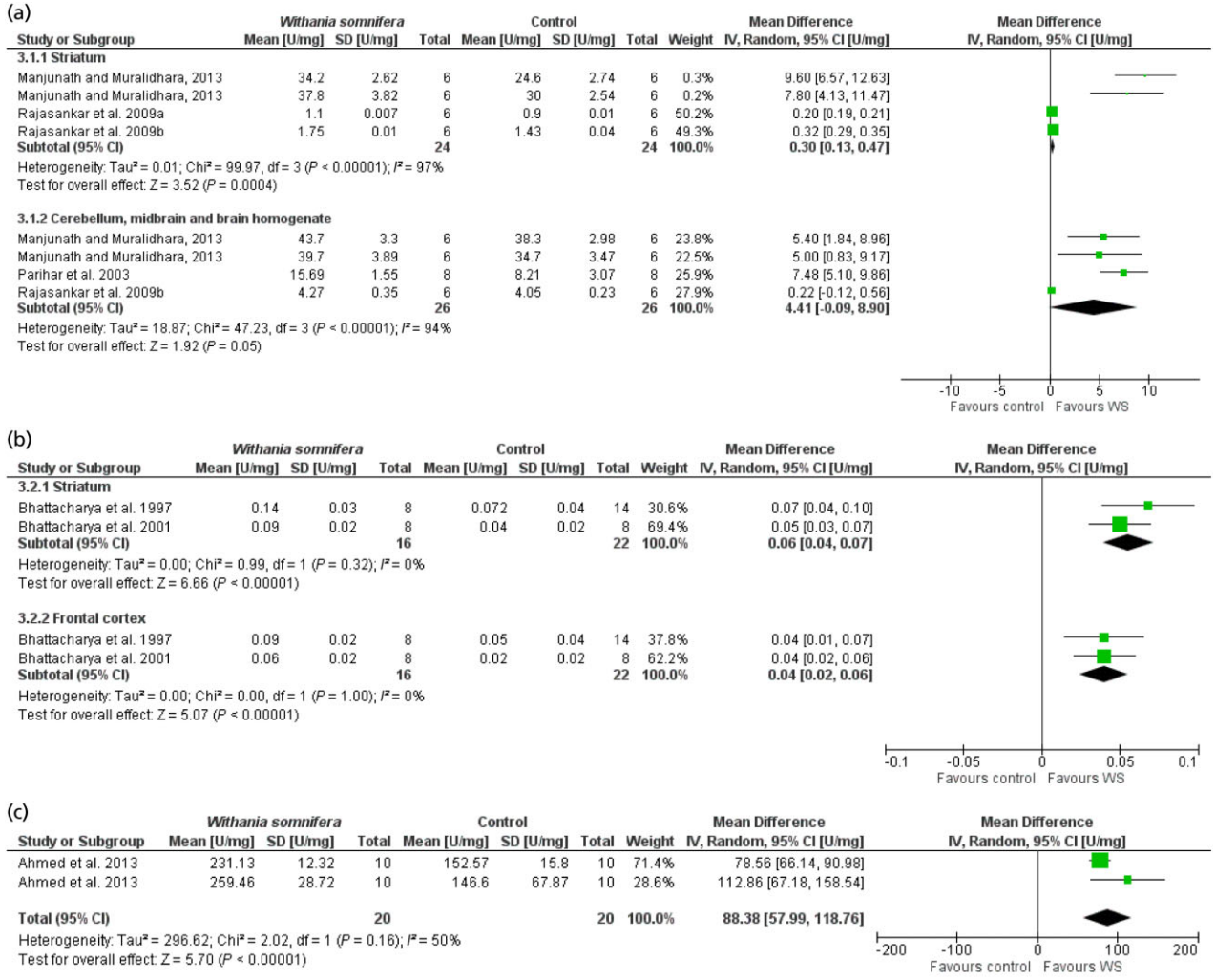


Figure 4 Forest plots showing glutathione peroxidase levels in (a) striatum, cerebellum, midbrain and brain homogenate of mice; (b) striatum and frontal cortex of rat brain; and (c) hippocampus and cerebral cortex of rat brain.

displayed increased effects in GPx activity in striatum (IV; 0.30, 95% CI, 0.13 to 0.47, $I^2 = 97\%$, $P = 0.0004$), cerebellum and midbrain of mice (IV; 4.41, 95% CI, −0.09 to 8.90, $I^2 = 94\%$, $P = 0.005$; Figure 4a). Similar outcomes were observed in striatum (IV; 0.06, 95% CI, 0.04 to 0.07, $I^2 = 0\%$, $P < 0.00001$), frontal cortex (IV; 0.04, 95% CI, 0.02 to 0.06, $I^2 = 0\%$, $P < 0.00001$), and in the hippocampus and cerebral cortex (IV; 88.38, 95% CI, 57.99 to 118.76, $I^2 = 50\%$, $P < 0.00001$) of rat brain (Figure 4b and 4c). Gupta and colleagues (2003) investigated the effects of *W. somnifera* on copper-induced GPx level in ageing spinal cord of Wistar rats. GPx activity decreased (3.83 ± 0.66 µmol/mg protein) significantly in the spinal cord from adult to aged mice. Treatment with *W. somnifera* significantly (81.46%) attenuated GPx (6.95 ± 1.18 µmol/mg protein) activity.[28]

Reduced glutathione

The content of GSH was significantly decreased in various parts of mice and rat brain by physical and chemical stimuli as compared with the vehicle-treated group. The random effects meta-analysis of GSH illustrated a significant increase in the reduced levels of GSH by coadministration of *W. somnifera* root and leaf extract. The statistically significant results were observed in rat brain hippocampus and cerebral cortex (IV; 0.71, 95% CI, 0.42 to 1.01, $I^2 = 0\%$, $P < 0.00001$, Figure 5a), rat brain homogenate (IV; 5.12, 95% CI, 4.69 to 5.56, $I^2 = 0\%$, $P < 0.00001$, Figure 5b), and in the striatum, midbrain and frontal cortex (IV; 0.14, 95% CI, 0.09 to 0.20, $I^2 = 99\%$, $P < 0.00001$, Figure 5c) of mice brain. Similar findings were obtained from the studies that reported GSH level in terms of percentage change (IV;

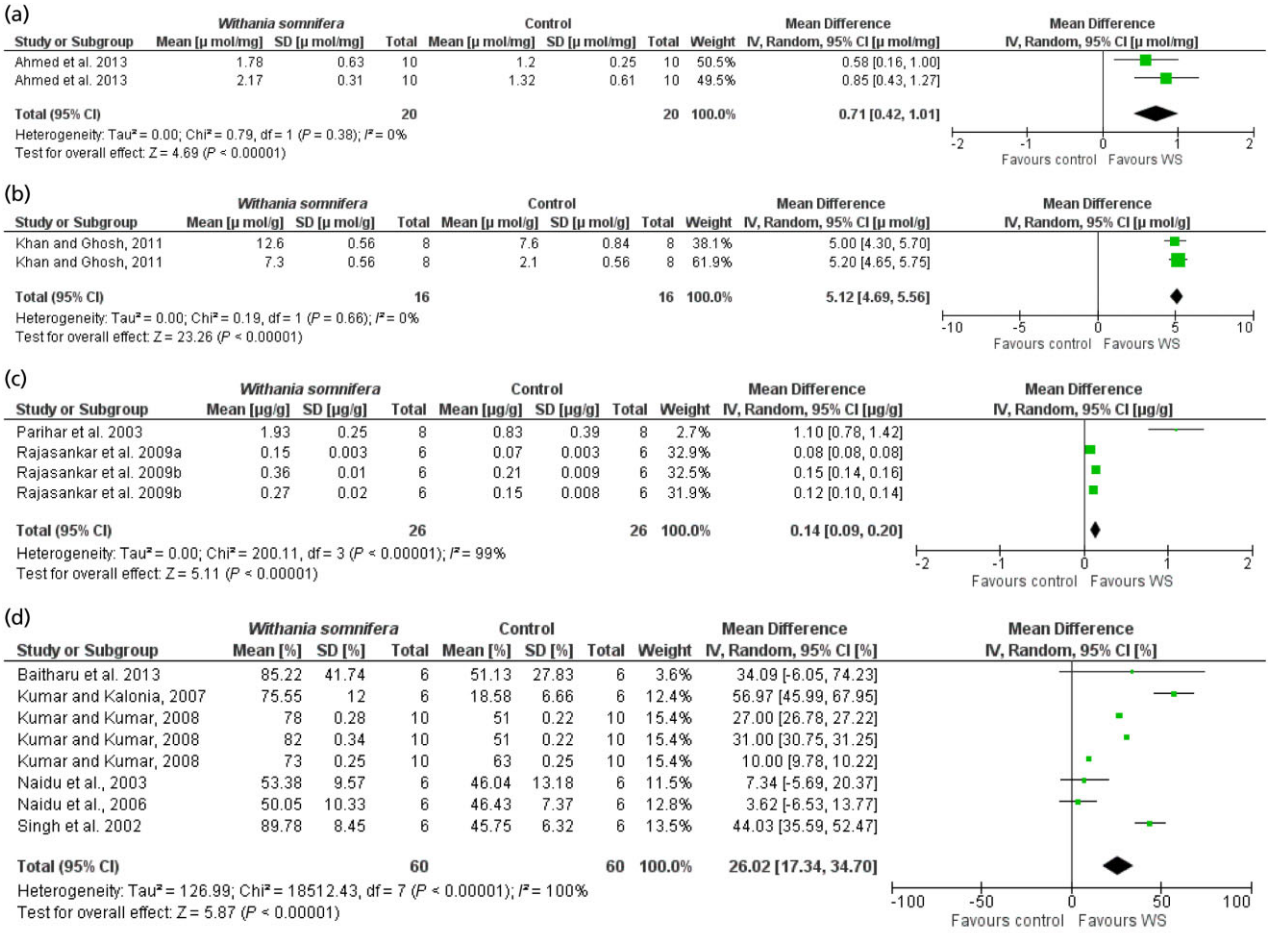


Figure 5 Forest plots showing glutathione levels in (a) hippocampus and cerebral cortex of rat brain; (b) rat brain homogenate; (c) striatum, mid-brain and frontal cortex of mice brain; and (d) hippocampus, cortex, striatum and brain homogenate of mice and rat (percentage change).

26.02, 95% CI, 17.34 to 34.70, $I^2 = 100\%$, $P < 0.00001$; Figure 5d).

Lipid peroxidation

Increased levels of malondialdehyde (MDA), thiobarbituric acid reactive substances, lipid peroxides and hydroperoxides were reported in the rodent neurological conditions. *W. somnifera* extract and its biomarkers reduced the augmented LPO content in hippocampus, cerebral cortex, forebrain and rat brain homogenate (IV; −2.28, 95% CI, −3.03 to −1.54, $I^2 = 94\%$, $P < 0.00001$, Figure 6a). Some studies expressed LPO in terms of unit per gram tissue in different parts of rat brain; the meta-analysed data of the same showed significant outcomes (IV; −2.34, 95% CI, −3.33 to −1.35, $I^2 = 0\%$, $P < 0.00001$, Figure 6b). The LPO content in different parts of mice brain also significantly decreased with *W. somnifera* coadministration, Figure 6c (IV; −1.73, 95% CI, −2.37 to −1.08, $I^2 = 93\%$, $P < 0.00001$) and Figure 6d (IV; −1.38, 95% CI, −1.67 to −1.08, $I^2 = 18\%$, $P < 0.00001$). In the studies where the findings of LPO expressed as percentage change; a significant reversal was noted with *W. somnifera* pretreatment (IV; −100.82, 95% CI, −121.96 to −79.68, $I^2 = 54\%$, $P < 0.00001$, Figure 6e) compared with disease control animals.

In some others studies, chemical inducers like kainic acid (647 ± 56.40 pmol/mg protein in mice brain homogenate),[24] Maneb–Paraquat (581.69 ± 96.60 nmol MDA/mg protein in nigrostriatal tissue of mice brain)[34] and copper (237.73 ± 12.62 nmol MDA/mg protein in spinal cord of rat),[28] as well as MCA occlusion model of stroke (690 ± 96.88 nmol MDA/g wet tissue of rat brain),[22] exhibited significant elevation of LPO levels as compared with vehicle-treated groups. Supplementation of *W. somnifera* significantly (46.48%, 32.20%, 84.28% and 47.82%, respectively) reduced the elevated levels of LPO.

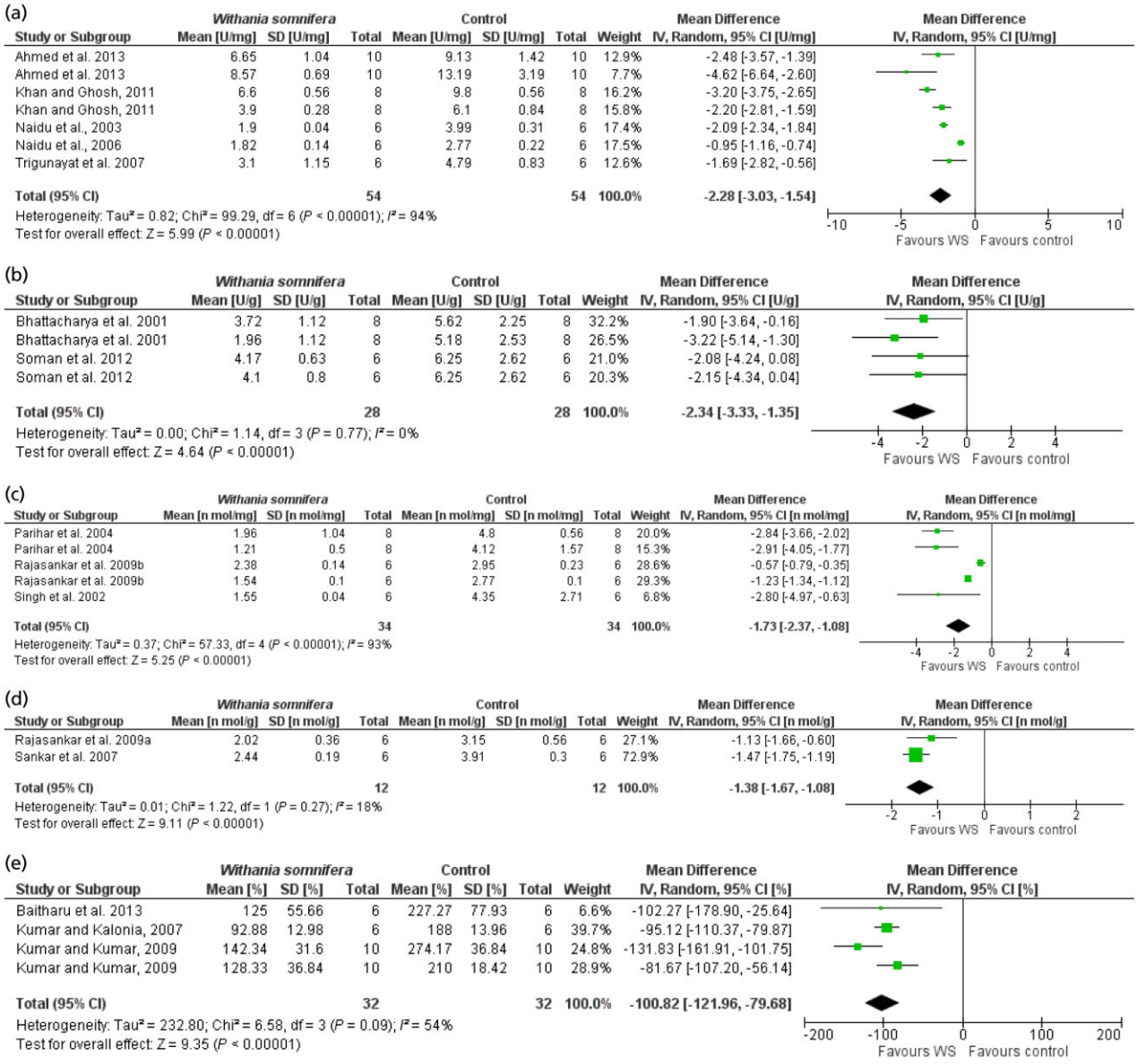


Figure 6 Forest plots showing lipid peroxidation levels in (a) hippocampus, cerebral cortex, forebrain and brain homogenate of rat (U/mg protein); (b) striatum, frontal cortex and hippocampus of rat brain (U/g tissue); (c) striatum, hippocampus, cerebral cortex and midbrain of mice (n mol/mg protein); (d) striatum and midbrain of mice brain (nmol/g tissue); and (e) hippocampus, striatum, cortex and midbrain of mice and rat (percentage change).

Nitrite concentration

Systemic administration of 3-NP (10 mg/kg) or sleep loss for 48 h caused a marked increase in nitrite levels as compared with naive group or baseline. Chronic *W. somnifera* (100 and 200 mg/kg, p.o.) administration attenuated nitrite (IV; −116.33, 95% CI, −151.21 to −81.45, $I^2 = 36\%$, $P < 0.00001$, Figure 7) levels in striatum and cortex of 3-NP-treated rats and brain homogenate of sleep-deprived mice.

Similarly, Parkash and colleagues (2013) also showed augmented level of nitrite in the nigrostriatum of the PD mice (59.75 ± 7.52 μmol/ml) induced by Maneb–Paraquat. The treatment of PD mice with *W. somnifera* significantly (28.56%) reduced (42.68 ± 6.27 μmol/ml) the elevated levels of nitrite in the nigrostriatum.[34]

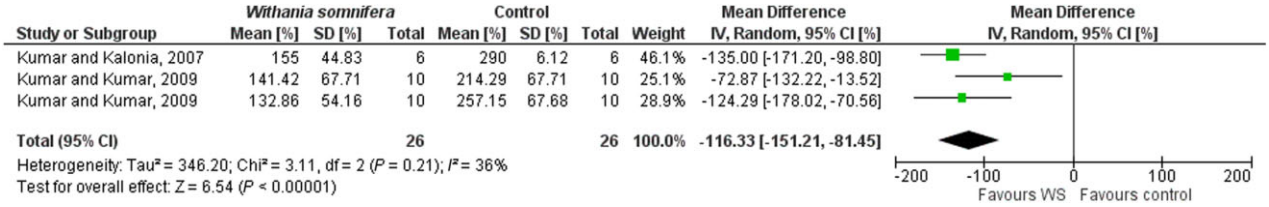
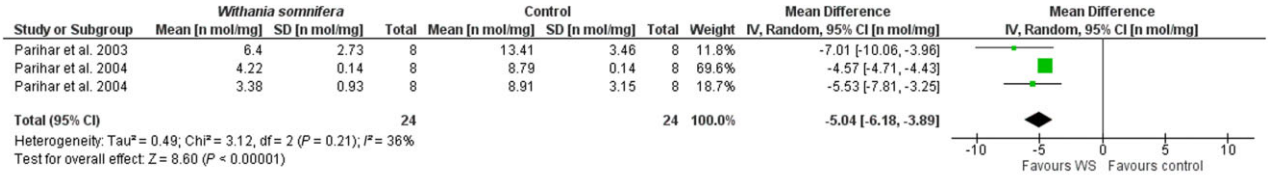


Figure 7 Forest plot showing nitrite level in striatum, cortex and brain homogenate. Where WS, *Withania somnifera*; IV, inverse variance; CI, confidence interval.



where WS - *Withania somnifera*; IV - inverse variance; CI - confidence interval

Figure 8 Forest plot showing protein carbonyl level in hippocampus, cerebral cortex and brain homogenate.

Khan and Ghosh (2010 and 2011) studied the effect of restraint stress on brain nitric oxide metabolites (NOx) and their modulation by withaferin A. The rat brain NOx level (0.61 ± 0.14 and 2.3 ± 0.56 nmol/mg, respectively) was significantly increased by restraint stress. Treatment with withaferin A reduced (22.95 and 56.52%, respectively) the brain NOx levels (0.47 ± 0.09 and 1.0 ± 0.28 nmol/mg, respectively) as compared with the vehicle-treated group.[12,13]

Protein carbonyl

The excitotoxic neuronal damage was measured by assaying protein carbonyl content. Kainic acid and streptozotocin administration demonstrated a significant increase in protein carbonyl content in mice brain as compared with control. *W. somnifera* pretreatment significantly reduced the elevated protein carbonyl content in hippocampus, cerebral cortex and whole brain homogenate (IV; −5.04, 95% CI, −6.18 to −3.89, $I^2 = 36\%$, $P < 0.00001$, Figure 8) when compared with pathological control mice.

AchE, AchT and Ach

3-NP challenge and hypobaric hypoxia significantly increased acetyl cholinesterase (AchE) enzyme level in the striatum, cortex and hippocampus region of the rat brain. Supplementation of *W. somnifera* root extract before and during exposure to 3-NP and hypoxia significantly decreased AchE (IV; −24.93, 95% CI, −29.20 to −20.65, $I^2 = 86\%$, $P < 0.00001$) activity compared with vehicle only treated group (Figure 9a). Augmented levels of AchE were also observed in the cerebellum (16.17 ± 1.64 μmol/mg protein) and hippocampus (1.54 ± 0.18 μmol thiocholine/min/mg protein) of the rotenone- and streptozotocin-induced oxidative damage, respectively, in the mice and rat brain.[8,29] *W. somnifera* prophylaxis significantly (23.06 and 33.76%, respectively) reduced the elevated levels of AchE in the cerebellum (12.44 ± 1.39 μmol/mg protein) and hippocampus (1.02 ± 0.15 μmol thiocholine/min/mg protein).

In another study, Bhattacharya and colleagues (1995) evaluated the effects of glycowithanolides from *W. somnifera* in an animal model of AD induced by ibotenic acid (IA) lesioning of the nucleus basalis magnocellularis. IA injection decreased choline acetyltransferase (ChAT) activity and acetyl choline (Ach) concentrations in frontal cortical and hippocampal of rat brain. *W. somnifera* (50 mg/kg) after 2 weeks of treatment significantly reversed IA-induced reduction in ChAT activity (IV; 8.69, 95% CI, 7.24 to 10.13, $I^2 = 0\%$, $P < 0.00001$, Figure 9b) and Ach concentration (IV; 9.57, 95% CI, 5.55 to 13.59, $I^2 = 84\%$, $P < 0.00001$, Figure 9c).

Discussion

Systematic review with meta-analysis is an essential tool to summarize evidence relating to effectiveness of health care interventions accurately and reliably.[36] This is the first systematic review with meta-analysis to our knowledge that evaluated the effects of *W. somnifera* (*Ashwagandha*) on brain oxidative stress in rodents. The present review consists of 28 research studies of *W. somnifera* which illustrated protective effects in body weight changes, motor- and cognition-related behaviours, and brain oxidative stress markers status in rodent neuropathology.

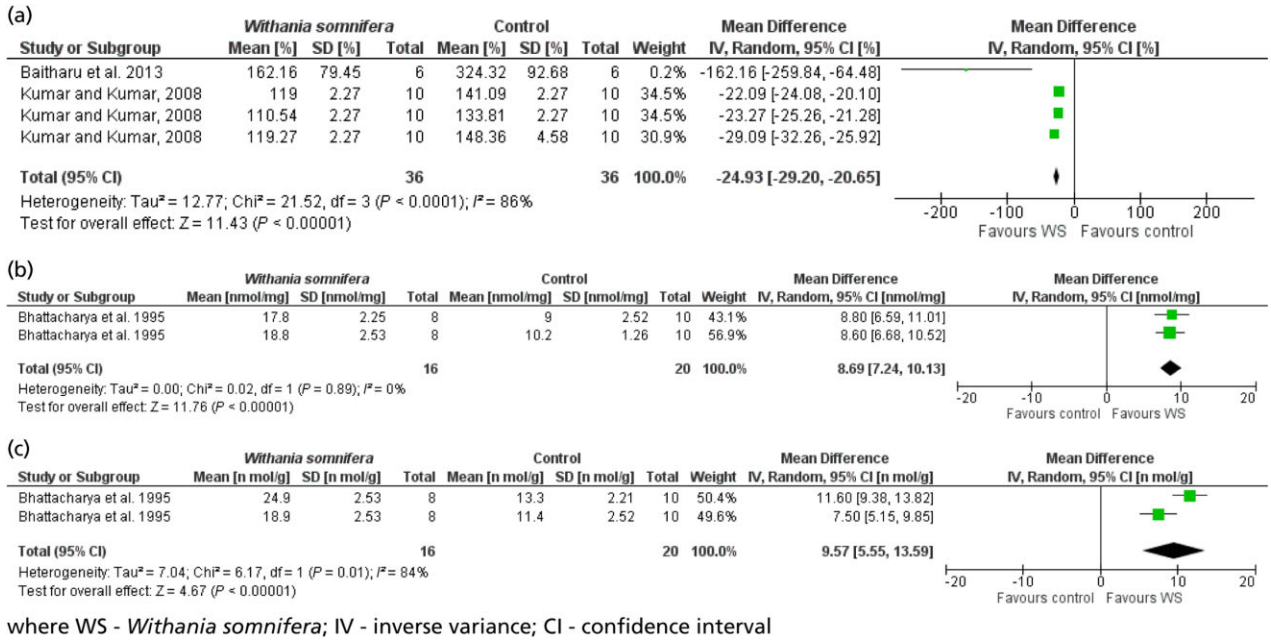


Figure 9 Forest plots showing (a) acetyl choline esterase concentration in hippocampus, cortex and striatum; (b and c) acetyl choline transferase and acetyl choline concentration in hippocampus and frontal cortex of rat brain.

Kumar and colleagues (2007 and 2009) presented marked reduction of body weight in 3-NP challenged rats, which could be due to 3-NP-induced metabolic impairment, i.e. impairment in energy metabolism and mobilization of energy stores.[19,25,37] 3-NP also decreases spontaneous locomotor activity and produces striatal lesions in rodents. Bradykinesia and striatal lesions may be the contributing factors for the observed weight loss in 3-NP toxicity.[37] *W. somnifera* (100 and 200 mg/kg) prevented the body weight reduction and increased spontaneous locomotor activity in 3-NP-treated rats, indicating its mitochondrial protection and anti-bradykinesia ability.

Treatment with *W. somnifera* significantly reversed the alterations induced by various CNS toxicants in rotarod[14,15,18,22] and hang tests,[14,15] indicating beneficial effect against reduced muscle grip strength in CNS pathology. Further, *W. somnifera* also significantly reduced memory loss and increased learning ability in rodents, which was evidenced by the Morris water maze test[8,9,18,21,32] and elevated plus maze test.[12,13,18,25]

Apart from the above-said motor skill tests, the effect of *W. somnifera* also tested for narrow beam walking and open field test,[12,16,35] catalepsy,[5] foot fault test,[22] limb withdrawal test,[19] T-maze,[27] and radial arm maze test.[23] *W. somnifera* supplementation demonstrated significant motor coordination improvement and recovery from locomotor abnormalities. The observed beneficial effects of *W. somnifera* have been shown to be attributed considerably due to its antioxidant activity.[2]

The brain oxidative stress results in excessive generation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and the hydroxyl radicals. These ROS function in concert to induce LPO of cell membrane lipids.[4] LPO, the toxic peroxide product, causes cellular and molecular damage and shows increased LPO levels in different parts of brain in neuropathological environment. The present meta-analysis findings of *W. somnifera* effect on brain LPO levels showed marked decrease and reversal to normal in most of the studies.

Meta-analysis was performed on SOD, catalase (a heme-containing enzyme) and GPx (a selenium group-containing enzyme), which are natural antioxidant enzymes of biological system. The role of SOD is to scavenge the superoxide ion by accelerating its dismutation. Meta-analysis of *W. somnifera* effect on SOD showed significant increase of its level in striatum, cerebellum, frontal cortex, hippocampus and other parts of the brain. However, in some pathophysiological conditions, the concentration of brain SOD increases significantly along with LPO.[38] Chronic foot shock and cold restraint stress induced augmentation of SOD activity in the brain (frontal cortex and striatum) and gastric mucosa, respectively.[4,39] Excess of SOD is believed to also increase harmful tissue effects and oxidative stress (SOD) in rodent brain associated with catalepsy.[5,38] It is interesting to note that in such environments, *W. somnifera* tends to normalize the elevated SOD level (in striatum and other parts of brain). The free radical scavenging action of SOD is helpful only when the enzymes catalase

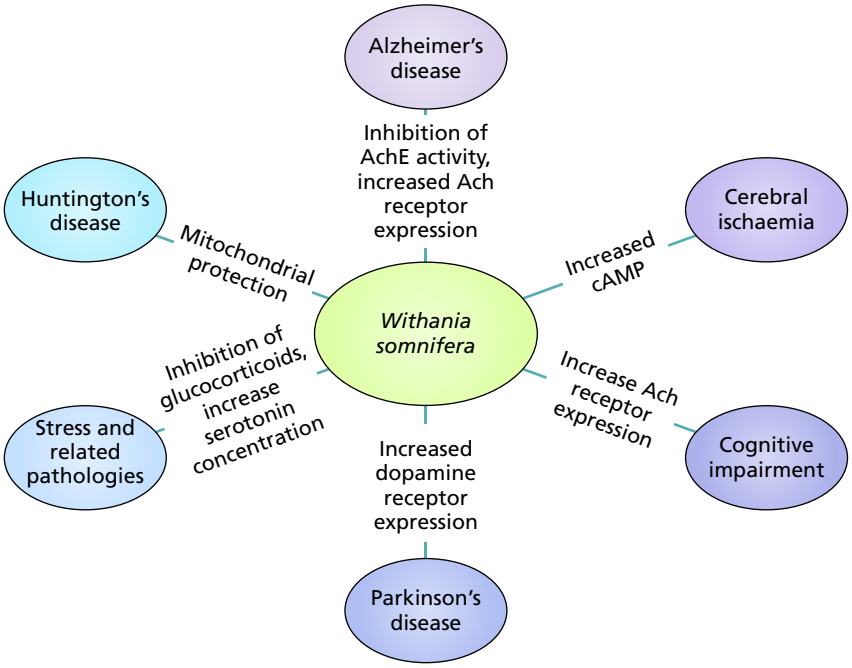


Figure 10 Proposed mechanism of *Withania somnifera* in brain oxidative stress other than antioxidant. Ach, Acetylcholine; AchE, Acetylcholinesterase; cAMP, Cyclic adenosine monophosphate.

and GPx activity follows the actions of SOD. Hydrogen peroxide and toxic ROS are produced by dismutase action of SOD. Hydrogen peroxide is more toxic than oxygen-derived free radicals and is further scavenged by catalase and GPx. In this study, the authors found significant activity of catalase in striatum, cortex and hippocampus part of the brain, as well as whole brain homogenate. GPx activity was also meta-analysed in striatum, cerebellum, hippocampus, cortex and other parts of the brain. The meta-analysis of all three natural cellular antioxidants activity showed low to high heterogeneity, which might be due to diversity of *W. somnifera* extract, as the selected studies used water and alcoholic extracts as well as the use of an isolated biomarker. The reviewing authors felt that cultivar variety of *W. somnifera* may have also contributed to the observed heterogeneity. Another important cause of heterogeneity might have been due to the use of different neuropathology-inducing agents in rodents between the studies, along with estimation of antioxidants in different parts of rodent brain and by different biochemical methods. Duration of studies and different strains of mice and rats also account for heterogeneity. Also, the selected dose for studies varied (1.7–1000 mg/kg/day), although most of the studies (16 studies) used 100 mg/kg/day. In a few studies, the route of administration was also different. These factors may also play important role in causing heterogeneity.

Apart from enzymatic antioxidant activity restoration, the meta-analysis of *W. somnifera* treatment also showed beneficial effect in maintaining non-enzymatic antioxidant level such as of GSH. GSH is an intracellular antioxidant and a member of endogenous antioxidant defence system, which reacts with oxygen free radicals and organic peroxides, and acts as a substrate for other detoxification enzymes such as GPx and glutathione-S-transferase. Astrocytes have an important role in the synthesis and maintenance of brain GSH levels either by supplying GSH or cysteine-containing precursor to the neurons.[40,41] The meta-analysis results showed that *W. somnifera* supplementation restored the brain GSH levels towards normal as compared with disease control animals.[8,9,13,15,16,18,24,26]

Certain studies, however, have also put supportive mechanism of actions besides antioxidant principle such as augmentation of reduced levels of catecholamines (Figure 10),[17] increased expression of serotonin and cholinergic markers like Ach and ChAT,[8,9,18,29,33] elevation of cyclic AMP,[35] decrease of nitrite and glucocorticoid levels,[9,12,19,25,33] diminution of protein carbonyl content,[24,27] and inhibition of nitric oxide.[9,12] The statistical analysis with literature review also showed significant reversal of oxidative stress impaired protein carbonyl, nitrite, AchE, ChAT and Ach concentrations with *W. somnifera* coadministration.

It is well reported that *W. somnifera* contains many steroidal alkaloids and lactones such as withanine, somniferine, withanone, withaferin A,[12,13] withanolide A, D and G, glyco-withanolides (sitoindosides IX or sitoindosides X),

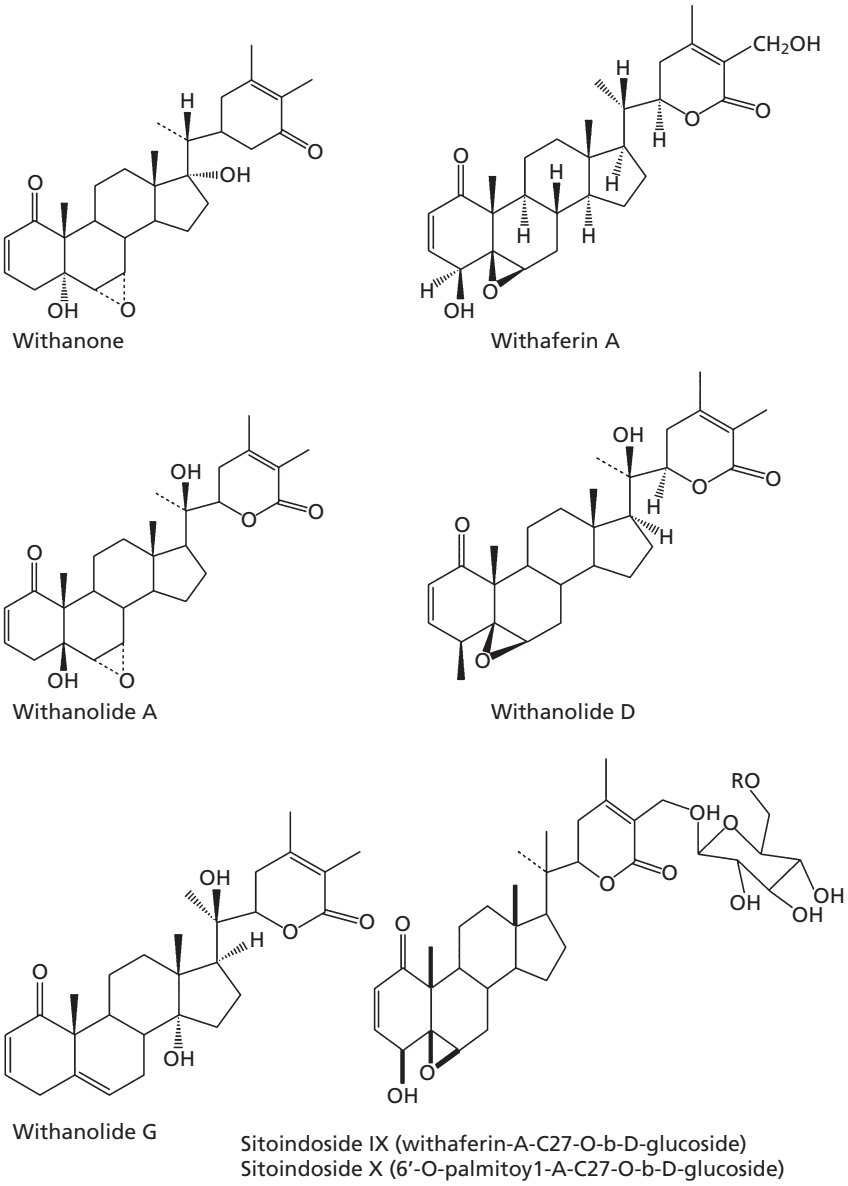


Figure 11 Major active biomarkers of *Withania somnifera*.

etc.[2,4,23] The presence of these active principles and metabolites (Figure 11) may be responsible for its beneficial action against various CNS toxic effects. The withanolides, which are steroidal lactones with ergostane skeleton, have the structural similarity with bioactive principles present in the plant species *Panax ginseng* known as ginsenosides. The withanolides exhibit acetyl and butyryl cholinesterase inhibition activity, indicating its protective role in AD and associated neurodegenerative disorders.[2] The bioactive constituents of *W. somnifera*, mainly sitoindosides VII-X and withaferin A (glyco-withanolides), have shown potent antioxidant and neuroprotective properties against ROS-scavenging enzymes, SOD, CAT and GPx.[4,6]

Lately many medicinal plants are being explored in neurological disorders for treating brain oxidative stress as ideal resource and to develop safe and effective treatment. Plants such as *Hypericum perforatum*, *Ginko biloba* and *Valeriana officinalis* have been documented to possess antioxidant activity against oxidative stress-induced damages.[42] Many preparations of *W. somnifera* are available in market for treatment of stress, insomnia, mental tension, depression, cognitive impairment, etc. (Table 2). The major barrier for the use of *W. somnifera* in oxidative stress and other neurological disorders in human beings is the lack of scientific and clinical data proving its safety and effectiveness. To generate clinical facts of herbal drugs, there is a necessity to

Table 2 *Withania somnifera* products and their claimed central nervous system therapeutic effects

Product name	Manufacturer	Stated central nervous system therapeutic claim
Arshadi Pills	Dehlvi Remedies	Stress, depression, cardiac tonic
Ashvagandha	Morpheme Remedies	Combating stress
Ashvagandha (anti-stress)	The Himalaya Drug Company	Chronic stress-related physiological abnormalities
Ashwagandha	Ayurceutics	Stress reliever
Ashwagandharista	Baidynath Ayurved Bhawan	Nerve tonic, memory and cognition improvement, better power of concentration, relieves mental tension, natural sleep induction, and recovery from nervous and general debility
Ashwagandhahills	Herbal Hills	Stress and revitalizer
Brento	Zandu Pharmaceutical Works Ltd	Nerve tonic
Dabur Ashwagandha Churna	Dabur	Combating stress
Geriforte (tablet and syrup)	The Himalaya Drug Company	Occupational stress, stress-related anxiety, chronic fatigue syndrome
Himalaya Massage oil	The Himalaya Drug Company	Stress relief and relief from insomnia
Mentat and Mentat DS	The Himalaya Drug Company	Memory and learning disorders, behavioural disorders, attention deficit hyperactivity disorder, anxiety and stress-related disorders, mental fatigue, as an adjuvant in Alzheimer's and Parkinson's disease
MindCare and MindCare Jr	The Himalaya Drug Company	Mental alertness, mental fatigue and occasional irritability
StressCare	The Himalaya Drug Company	Supports cortisol levels for peace of mind
Stresscom	Dabur India Ltd	Relieves anxiety neurosis, physical and mental stress, and relieves general debility and depression
Stresswin	Baidynath Ayurved Bhawan	Combating exertion, reduction in anxiety, strain and stress, improvement of stamina, relief from disturbed sleep, mental alertness

conduct clinical trials for documenting their pharmacological and toxicological profile. Further, it is important to authenticate and develop the active marker compounds from these herbal drugs to widen its scientific data.[43]

In conclusion of the systematic review, *W. somnifera* successfully inhibited the neurobehavioural abnormalities produced by different physical and chemical stimuli on oxidative stress in rodent brain. It also significantly decreased the increased LPO, protein carbonyl, AchE and nitrite levels in different parts of rodent brain. The natural cellular antioxidants (SOD, catalase and GPx) and the non-enzymatic antioxidant like GSH, ChAT and Ach alteration in the neuropathological environment were also considerably restored to normal by *W. somnifera*. These findings provide scientific evidence for the traditional claim of *W. somnifera* use in different neurological aliments. However, further study is recommended on human volunteers and also on the pharmacodynamic and pharmacokinetic profiles of the herb.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose. The authors alone are responsible for the content and writing of the paper.

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