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Withania somnifera against glutamate excitotoxicity and neuronal cell loss in a scopolamine-induced rat model of Alzheimer's disease

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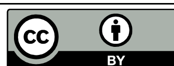

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ABSTRACT: Alzheimer's disease, a chronic and progressive neurodegenerative disorder with no prevention and cure, affecting nearly 50 million people worldwide. Glutamate is the principal excitatory neurotransmitter in the central nervous system involved in 50% of basic brain functions, especially cortical and hippocampal regions, like memory, cognition, and learning. The glutamate-mediated toxicity is termed as excitotoxicity. The present study was aimed to determine whether the methanolic and water extracts of root from the medicinal plant, *Withania somnifera*, could decrease the glutamate excitotoxicity and its related neuronal cell loss in a scopolamine-induced animal model of Alzheimer's disease. The rats were randomly divided into different groups of 5 in each: normal control - treated orally with saline; AD model - injected intra peritoneally with scopolamine (2 mg/Kg body wt) alone to induce Alzheimer's disease; AD model rats treated orally with the methanolic extract (AD+ME-WS) (300 mg/Kg body wt), water extract (AD+WE-WS) (300 mg/Kg body wt), and donepezil hydrochloride, a standard control (AD+DZ) (5 mg/Kg body wt) for 30 consecutive days. Increased glutamate (Glu) levels and decreased glutamate dehydrogenase (GDH) activity were reversed with *Withania somnifera* root extracts in both the cerebral cortex and hippocampus regions in scopolamine-induced Alzheimer's disease model rat brain. The histopathological studies of the same treatment also showed protection against neuronal cell loss in both regions. These results support the idea that these extracts could be effective for the reduction of brain damage by preventing glutamate excitotoxicity generated neuronal cell loss in the scopolamine-induced Alzheimer's disease model.

Keywords: *Withania somnifera*; Scopolamine; Alzheimer's disease; Glutamate; Excitotoxicity; Neuronal cell loss.

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic age-related, irreversible brain disorder characterized clinically by dementia, an over-time deterioration from early forgetfulness to gradual worsening in language, orientation, behavior, and late severe loss of memory with some bodily functions until the ultimate death. Neuropathologically, AD is characterized by the presence of anatomical lesions in the brain due to the

extracellular amyloid protein amassing as senile plaques, intracellular phosphorylated tau distortion as neurofibrillary tangles causes synaptic profile depletion and neuronal cell loss [1-3] which leads to personality change and downfall in the AD patients [4, 5]. During AD progression, beta-amyloid senile plaques become toxic to neurons by interceding in neuron-to-neuron communication at synapses. While on the other hand, tau tangles intercept the intraneural transit of essential molecules and nutrients, which causes dysfunctional axonal transport and neuronal loss [6, 7] in the hippocampus and neocortex, the vulnerable areas used for memory and cognition in the brain [8, 9].

Glutamate is an excitatory neurotransmitter particularly abundant in the mammalian central nervous system (CNS) [10], plays an essential role in neural development, excitatory synaptic transmission, and plasticity [11, 12]. Although normal brain physiology depends on an optimal glutamate level, its low and high levels trigger neurotoxic or excitotoxic cascades [13, 14] in acute neurological disorders such as stroke [15], traumatic brain injury [16] as well as chronic neurological disorders including multiple sclerosis, Huntington's disease, Parkinson's disease and Alzheimer's disease [17]. Glutamate mediated excitotoxicity is a complex process by glutamate receptor activation that results in the excessive Ca^{2+} influx across the cell membrane lead to reactive oxygen species (ROS) inducing oxidative stress, degeneration of dendrites, and cell death [18]. In AD patients, particularly in the hippocampus and cortical regions of the brain, Glutamate excitotoxicity disrupts glutamatergic neurotransmission, a process linked to decreased neuronal regeneration and dendritic branching, an important action in learning and memory [19-21]. Hence, the tight regulation of Glutamate function or disruption of glutamate uptake at the synaptic cleft significantly related to reduced sensitivity to depression, a symptom of 40% of AD patients which leads to worsening cognitive decline [22, 23].

Withania somnifera (*W. somnifera*) frequently known as "Ashwagandha" or "Indian Ginseng" belongs to Solanaceae, is one of the most esteemed medicinal plants with a long history of wide use in herbal medicine and indigenous medical systems of India [24, 25]. It is also well known as 'Queen of Ayurveda' because of its vital use for over 3000 years as an adaptogenic, analgesic, anti-stress, immunomodulatory, and immunostimulant effects [26, 27]. The therapeutic use of *W. somnifera* roots in Rasayanas promotes health and longevity by stimulating defense against disease, seizing the aging process, augmenting the capability of the individual to resist adverse environmental factors [28, 29]. The various constituents present in *W. somnifera* extracts, including leaves, shoots, and roots, are familiar with their anticancer, immunomodulatory and neuroprotective activities [26, 30]. On the other hand, the phytochemicals present in *W. somnifera* could also scavenge free radicals with its antioxidant properties [31, 32].

Although there is long-term research happening in the area of Alzheimer's targeting the beta-amyloid (Aβ) and the cholinergic system, it was failing to generate efficacious treatment for AD. So, the current research is focusing on the other targets like tau pathology and glutamatergic system in both *in vivo* and *in vitro* to find an effective treatment [33]. On the other hand, increasing therapeutic benefits of plants attracting the attention of pharmacologists and researchers continuously for biomedical investigations on their extracts and isolated compounds [27, 34]. In view of that, the present study focused to investigate that whether the administration of methanolic and water extracts of *W. somnifera* root has any synergistic protecting action against scopolamine-induced glutamate excitotoxicity and neuronal cell loss in male Wistar albino rats.

2. MATERIALS AND METHODS

2.1. Collection of plant material

W. somnifera, commonly known as Ashwagandha, is an evergreen shrub with colorful berries grows in the drier sectors of India. The roots of *W. somnifera* used in the present study was purchased from the distributor of Ayurveda products, Bangalore, Karnataka, India and authenticated by a botanist from the Department of Botany, S.V. University, Tirupati, Andhra Pradesh, India.

2.2. Extraction of plant material

The cleaned and dried roots of *W. somnifera* were ground into a fine powder through a mechanical grinding machine. The powdered root was suspended in methyl alcohol for a whole day and night at room temperature with constant stirrings. After 24 hours of suspension, the solvent was filtered by Whatman Grade 1 filter paper. The suspension with the same was repeated until the extract has no color. The residual solvent collected was undergone through the distillation and concentration processes in the Buchi Rotavapor R-114 yielding a gum-like residue called methanolic extract (ME). The same was repeated with distilled water and formed residue called as water extract (WE). The residues thus obtained were treated as 100% methanolic extract (ME) and water extract (WE), refrigerated at 4°C for further use to treat the animals.

2.3. Drugs and chemicals

Scopolamine hydrobromide (Scopolamine), donepezil hydrochloride (Donepezil) were obtained from Sigma, methanol HPLC grade, paraformaldehyde, hematoxylin-eosin, triethanolamine, perchloric acid, potassium phosphate, NAD, INT, diaphorase, GDH, sodium glutamate, phosphate buffer, glacial acetic acid, and toluene were analytical reagent grade.

2.4. Experimental animals and treatment

All experiments were performed with Wistar strain male albino rats having body weights 300-350 g. The animals were divided into separate groups of 5 animals in each. All the animals had free access to food and water under controlled temperature (25 ± 2°C) and humidity conditioned with 12:12 h light/dark cycle. The animal study was performed as per the guidelines provided by the Institutional Animal Ethics Committee of National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India (Reg No. 12/GO/ac/99/CPCSEA).

2.5. Experimental design

The rats were randomized into five groups and treated for one month consecutively:

Group 1: Control (Normal Control) - normal saline administered orally

Group 2: AD model (Scopolamine-induced AD model) - Scopolamine injected intraperitoneally (2 mg/Kg b.w.) and normal saline administered orally

Group 3: AD+ME-WS - Scopolamine injected intraperitoneally (2 mg/Kg b.w.) and methanolic extract of *W. somnifera* (300 mg/Kg b.w.) administered orally

Group 4: AD+WE-WS - Scopolamine injected intraperitoneally (2 mg/Kg b.w.) and water extract of *W. somnifera* (300 mg/Kg b.w.) administered orally

Group 5: AD+DZ (Standard Control) - Scopolamine injected intraperitoneally (2 mg/Kg b.w.) and Donepezil hydrochloride (5 mg/Kg b.w.) administered orally.

All the rats were decapitated after one month of treatment. The brains were quickly removed from the rats and washed in ice-cold saline. Different regions such as the cerebral cortex (CC) and hippocampus (HC) were quickly separated from the brains on an ice-cold petri-dish, thoroughly washed with ice-cold saline and stored at -80^0C till further use of glutamate (Glu) and glutamate dehydrogenase (GDH) biochemical assays.

2.6. Glutamate determination

L-glutamate levels were estimated according to Beutler and Michal [35]. The 7% (w/v) homogenates of CC and HC were prepared using deionized water. The homogenates were cooled and centrifuged after keeping for 15 min. in a hot water bath at 80°C. The supernatants obtained were decanted and filtered. The pH of the filtrates was adjusted to 10 with potassium hydroxide. The samples were deproteinized using 1 M perchloric acid and placed in a refrigerator for 20 min. After deproteinization, each sample was mixed with triethanolamine (57 mM), potassium phosphate (14 mM, pH 8.6), NAD (0.38 mM), INT (0.068 mM), diaphorase (0.14 IU/ml) and GDH (14 IU/ml). In the presence of NAD and GDH, L-glutamic acid present in the sample was oxidatively deaminated to 2-oxoglutarate whereas the iodonitrotetrazolium chloride (INT) was converted into formazan in the presence of diaphorase and NADH. Thus, resultant formazan color intensity was read at 492 nm in a spectrophotomer.

2.7. Glutamate dehydrogenase activity

The method of Lee and Lardy [36], as modified by Pramilamma and Swami [37], was followed to determine the GDH, EC 1.4.1.3 activity. The 4% (w/v) homogenates of CC and HC were prepared with 2.25 M sucrose solution. The homogenates were centrifuged for 15 minutes at 2500 rpm. The supernatants obtained after centrifugation were used for enzyme assay. The reaction mixture was prepared using 50 μmoles of substrate (sodium glutamate), 100 μmoles of phosphate buffer (pH 7.4), 2 μmoles of INT, 0.1 μmole of NAD and distilled water. The reaction was started by the addition of crude enzyme extract after half an hour of incubation of the samples at 37°C. Then the reaction was stopped by the addition of glacial acetic acid. The formazan formed was extracted overnight in toluene in cold. The color intensity of the formed formazan was measured at 495 nm against a toluene blank.

2.8. Histopathological studies

The brain regions, CC and HC of different groups were perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The samples were removed and post-fixed in the same fixative for overnight at 48°C. After post-fixation, the samples were then routinely embedded in paraffin and stained with Hematoxylin-Eosin. The lesions present in the CC and HC regions of different groups were examined microscopically at 10x magnification [38, 39].

2.9. Statistical analyses

All the parameters were carried out 5 times independently. The values of the measured parameters were expressed as Mean ± SEM. One-way ANOVA followed by Dunnett's Multiple Range Test (DMRT) has been employed for statistical analysis in order to determine significance among the different groups. The results were regarded as statistically significant different at $p<0.05$.

3. RESULTS

3.1. Glutamate levels

Glutamate levels in the CC and HC of control and treated rats were represented in graphs (Figure 1 and 2). From these, it was observed that there was a significant increase in glutamate levels of AD models CC (Figure 1) as well as HC (Figure 2). The elevated glutamate levels were rebound to almost normal with ME and WE of *W. somnifera* treatment. Standard controls, treated with Donepezil hydrochloride (DZ), an Acetylcholine Inhibitor, also showed a decrease in glutamate levels, but it was non-significant both in the CC and HC regions when compared to *W. somnifera* treatment.

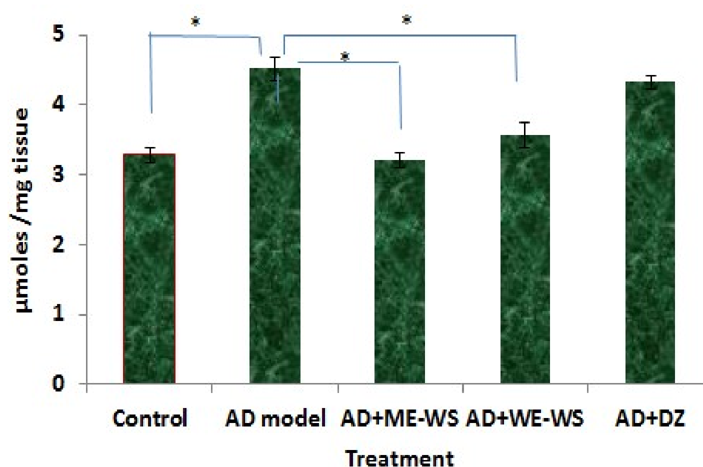

Figure 1. Effect of *W. somnifera* on the levels of glutamate in cerebral cortex (CC) of control and experimental groups of rats. Values are mean ± SEM (n=5), *P<0.05.

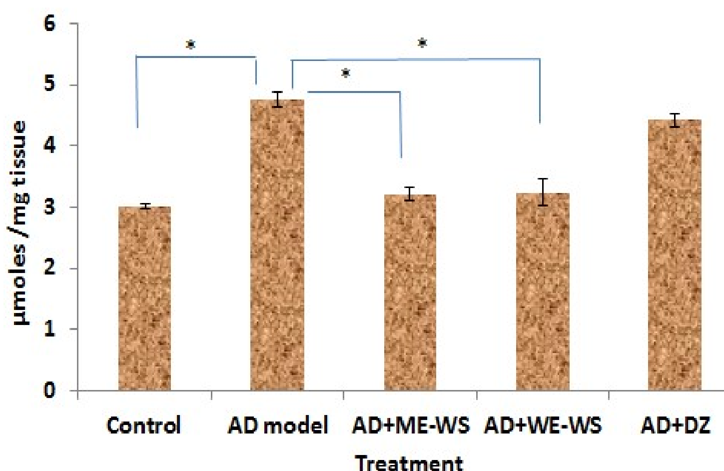

Figure 2. Effect of *W. somnifera* on the levels of glutamate in hippocampus (HC) of control and experimental groups of rats. Values are mean ± SEM (n=5), *P<0.05.

3.2. GDH activity

The quantitative evaluations of GDH activity were represented in graphs (Figure 3 and 4). From these, it was concluded that the GDH activity significantly decreased in AD models CC (Figure 3) and HC regions (Figure 4). The same was significantly increased with ME and WE of *W. somnifera* treatment. GDH activity

in CC and HC, treated with ME and WE of *W. somnifera* was found almost equal to control rats. But the standard controls, treated with DZ showed no significant changes in their GDH activity in both the regions when compared to *W. somnifera* treatment.

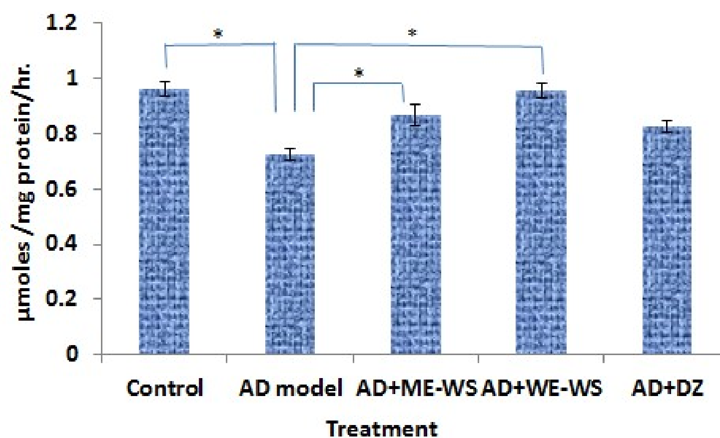

Figure 3. Effect of *W. somnifera* on the glutamate dehydrogenase activity in cerebral cortex (CC) of control and experimental groups of rats. Values are mean ± SEM (n=5), *P<0.05.

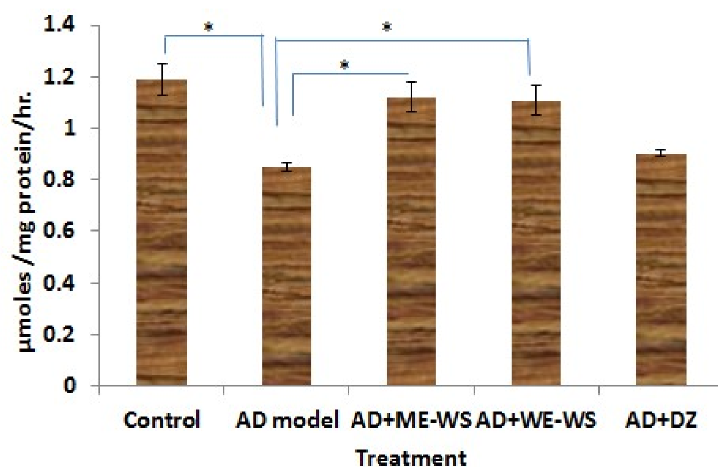

Figure 4. Effect of *W. somnifera* on the glutamate dehydrogenase activity in hippocampus (HC) of control and experimental groups of rats. Values are mean ± SEM (n=5), *P<0.05.

3.3. Histopathological studies

The results of present histopathological examinations demonstrated that scopolamine-induced AD models rat brain regions such as the CC and HC showed significant neuronal cell loss after 30 days of treatment. At the same time, in the ME and WE of *W. somnifera* treated animals, the reversal of neuronal cell loss was observed after 30 days. From Figure 5 and 6, it was visible that the degenerative cells are more visible in the AD model group when compared with other groups. This was indicated by the gaps in slides.

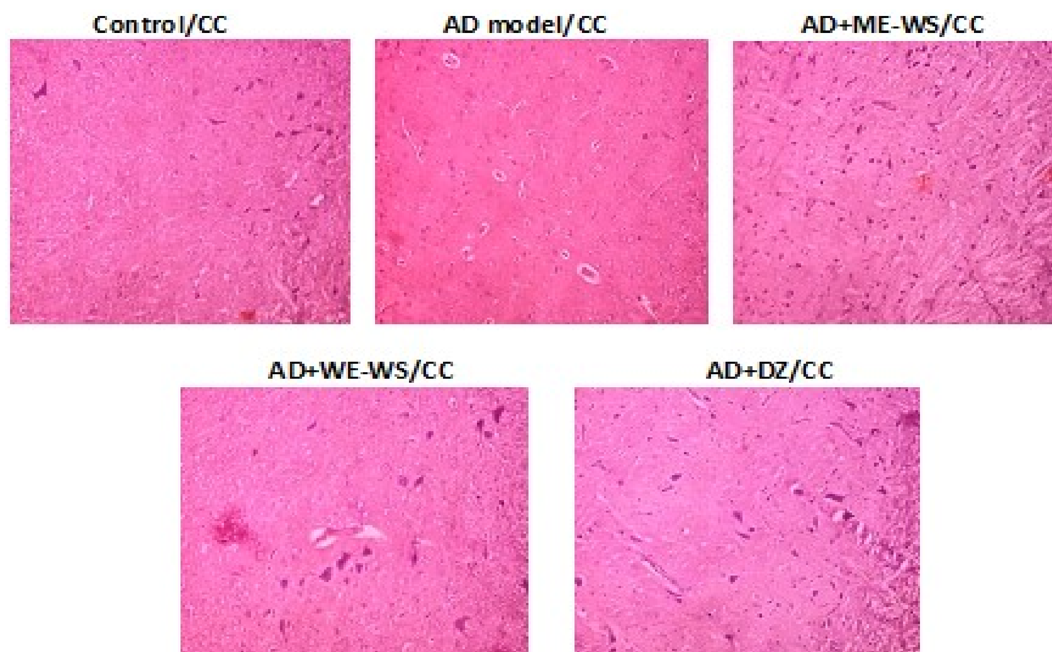

Figure 5. Sections of cerebral cortex (CC) of brain from different experimental groups of rats against scopolamine-induced AD after 30 days of treatment. These figures are normal control/CC, AD model/CC, AD+ME-WS/CC, AD+WE-WS/CC and AD+DZ/CC (standard control) respectively, representing neuronal cell loss after treatment.

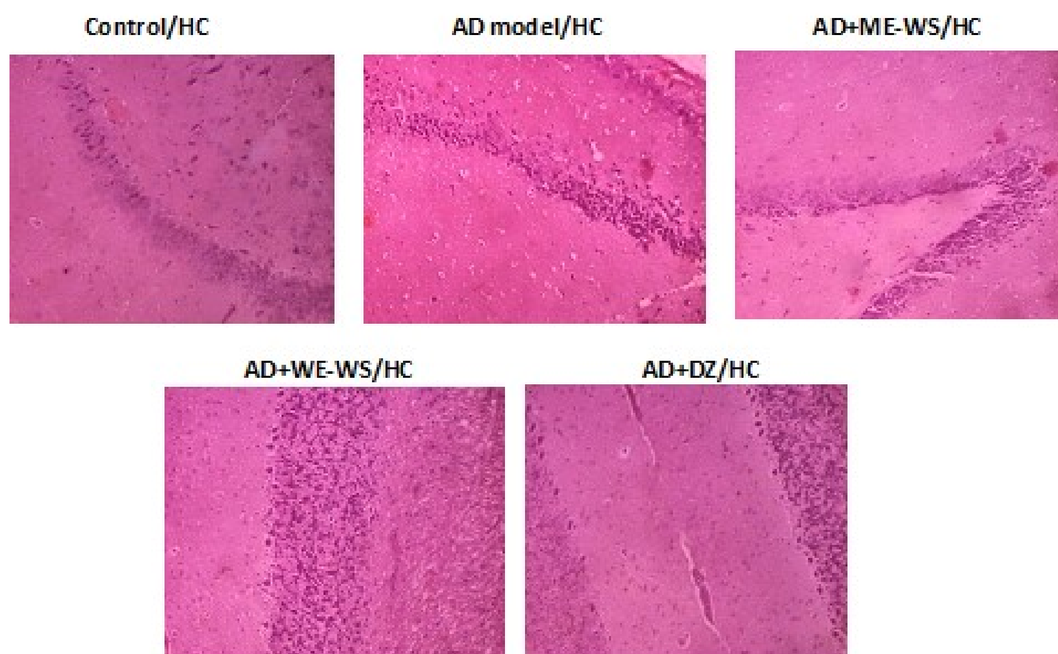

Figure 6. Sections of hippocampus (HC) of brain from different experimental groups of rats against scopolamine-induced AD after 30 days of treatment. These figures are normal control/HC, AD model/HC, AD+ME-WS/HC, AD+WE-WS/HC and AD+DZ/HC (standard control) respectively, representing neuronal cell loss after treatment.

4. DISCUSSION

Glutamate is the predominant amino acid that acts as a key intermediate metabolite for all the neurons. It is involved in many fundamental brain functions and communicating mechanisms responsible for fast

neuronal communication in the CNS [40, 41]. In normal states, it plays a central role in the development of the CNS, synaptic plasticity, an essential mechanism of cognition, learning and memory, as well as synapse induction and elimination, cell migration, differentiation, and death [42]. In an abnormal state, normally defined as an excitotoxic state, extracellular glutamate, causes neuronal dysfunction and degeneration, a pathological process for neuronal killing in the mammalian CNS [43, 44]. During excitotoxicity, inactivation of synaptic glutamate transporters and overactivation of NMDA receptors disrupts synaptic glutamate normal signaling which impairs long-term potentiation and synaptic plasticity, a major pathway towards neurodegenerative disorders such as AD [9].

Since, the currently available pharmacological options for AD, only have a limited effect and poor control over the disease-causing neurons linked with Alzheimer's symptoms and associated complications [7], research in this area has a vital role. Although many research groups have already explored the potential of using natural products as neuroprotective agents against AD, the anti-excitotoxic effect of *W. somnifera* concerning neuronal cell loss in a rat model of scopolamine-induced AD is not examined. Our present study, using scopolamine-induced rat as an AD model supported by earlier reports describing scopolamine-induced animal models could be used as an experimental model for AD [45-47].

The results of the present study indicated a significant increase in glutamate content and a decrease in GDH activity in both CC and HC regions in the scopolamine-induced AD rat model. The increased glutamate content in AD models after scopolamine administration was supported in the earlier study of scopolamine-induced extracellular glutamate elevation in the striatum of freely moving rats [48]. Elevated glutamate levels in the AD model indicate the inhibition of glutamate receptors and the deficient functioning of glutamate transporters failing to clear the excess glutamate at the synaptic cleft [49] after scopolamine administration. This causes many changes in neuronal cells, including impairment of calcium buffering, secondary excitotoxicity promoting oxidative damage which leads to a rise in the tissue peroxide levels and cell death, supporting the possibility that abnormal functioning of this system might be involved in the pathogenesis of AD [39, 50]. Significantly decreased GDH activity with increased Glutamate content in the AD model suggests that during scopolamine-induced AD, there is a lesser mobilization of glutamate for the synthesis of α-ketoglutarate which plays a major role in energy metabolism. This condition signifies the failure of the brain to opt for a protective mechanism to maintain low concentrations of glutamate and related excitotoxicity causing neuronal death during scopolamine-induced AD. In support of this, earlier studies have reported the elevated glutamate levels with decreased GDH activity causing excitotoxicity and glutamatergic neuronal damage in the brains with different neurodegenerative disorders such as AD [51] and epilepsy [52]. In the recent study, it was also observed that how the deficiency or overexpressed GDH activity could regulate whole-body energy metabolism and affect the early onset of AD in the brain of mutant mice [53].

The ME and WE of *W. somnifera* treatment for 30 days reversed the elevated glutamate content in both the regions of AD models significantly. At the same time, the significant decrease in GDH activity in AD models also increased significantly after *W. somnifera* treatment. The donepezil hydrochloride (DZ), an acetylcholine inhibitor also reversed the glutamate levels and GDH activity insignificantly in both the regions of the AD model rat brain. The activity of donepezil was also supported by some *in vitro* studies showing its neuroprotection against glutamate excitotoxicity [54].

The current results of *W. somnifera* supported by the earlier studies that Ashwagandha leaves derived water extract and its active components like Withanolide A pre-treatment could inhibit glutamate-induced cell-death and can reverse glutamate-induced changes in differentiated neuronal cells [55, 56]. Even pre-

treatment with aqueous extract of *W. somnifera* root also protected the differentiated PC12 cells against H_2O_2 and Aβ(1-42)-induced cytotoxicity significantly [57]. In the other study, water and alcoholic extracts, as well as its bioactive components of Ashwagandha leaves, were highly potent against H_2O_2- and glutamate-induced oxidative stress and cytotoxicity in both glial and neuronal cells [58]. Despite of all the above, the hot water extract of Ganoderma lucidum also proved its medicinal value by ameliorating AD symptoms in $A\beta_{1-42}$ induced AD models [59].

In the present study, the neuroprotective effect of *W. somnifera* was confirmed by the histopathological examination of the brain CC and HC of both control and treated animal models. The control rats showed no distinctive neuronal changes in both areas. Conversely, the AD model group showed more compressed cells in both the areas validating the neuronal cell loss with scopolamine-induced AD. The histopathological profile of the groups treated with ME and WE of *W. somnifera* showed no visible neuronal changes in both the areas confirming the safety and protective role of these extracts. Earlier studies confirmed that glutamate excitotoxicity could cause damage to the neurons present in the cortex, hippocampus, and basal forebrain regions [39, 51]. On the other hand, in SH-SY 5Y neuroblastoma cells and in mice, it was also confirmed that scopolamine could cause cytotoxicity to the neurons after its administration [60, 61]. At the same time, it was reported that how the water extract of Ashwagandha could show prevention against the LPS-induced neurodegeneration, neuroinflammation in both *in vivo* and *in vitro* model systems [62]. Another *in vitro* study reported that *W. somnifera* root extract could also protect the model neurons from traumatic injury caused neuronal damage [63]. This reversal in neural cell loss suggests that *W. somnifera* treatment has the potential to act against glutamate excitotoxicity and its related neuronal damage.

5. CONCLUSION

From these results, it was concluded that the treatment with methanolic and water extracts of *W. somnifera* root for 30 days could decrease glutamate excitotoxicity and related neuronal death in scopolamine-induced AD models by activating the glutamate receptors and transporters to mobilize the excess glutamate from the cerebral cortex and hippocampus regions. These extracts could also increase the GDH activity where it forms α-ketoglutarate from glutamate, which will be utilized in energy metabolism to maintain cellular mitochondrial ATP levels, the main protection towards neuronal cell loss causing by excitotoxicity.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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REFERENCES

1. Buntup D, Chayasadom A, Surarit R, Chutabhakdikul N, Thangnipon W. Effects of amyloid - peptide on glutamine transporter mRNA expression and cell viability in cultured rat cortical cells. Sci Asia. 2009; 35: 156-160.
2. Perl DP. Neuropathology of Alzheimer's disease. Mt Sinai J Med. 2010; 77: 32-42.
3. Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's Disease. J Alzheimers Dis. 2017; 57: 1041-1048.

4. Parihar MS, Hemnani T. Alzheimer's disease pathogenesis and therapeutic interventions. J Clin Neurosci. 2004; 11: 456-467.
5. Kulkarni KS, Kasture SB, Mengi SA. Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. Indian J Pharmacol. 2010; 42: 168-173.
6. Ali T, Yoon GH, Shah SA, Lee HY, Kim MO. Osmotin attenuates amyloid beta-induced memory impairment, tau phosphorylation and neurodegeneration in the mouse hippocampus. Sci Rep. 2015; 5: 11708.
7. Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimers Dement. 2017; 13: 325-373.
8. Mota SI, Ferreira IL, Rego AC. Dysfunctional synapse in Alzheimer's disease - A focus on NMDA receptors. Neuropharmacology. 2014; 76 Pt A: 16-26.
9. Liu J, Chang L, Song Y, Li H, Wu Y. The Role of NMDA receptors in Alzheimer's Disease. Front Neurosci. 2019; 13: 43.
10. Khakpai F, Zarrindast MR, Nasehi M, Haeri-Rohani A, Eidi A. The role of glutamatergic pathway between septum and hippocampus in the memory formation. EXCLI J. 2013; 12: 41-51.
11. Lu YM, Mansuy IM, Kandel ER, Roder J. Calcineurin-mediated LTD of GABAergic inhibition underlies the increased excitability of CA1 neurons associated with LTP. Neuron. 2000; 26: 197-205.
12. Sheng M, Kim MJ. Postsynaptic signaling and plasticity mechanisms. Science. 2002; 298: 776-780.
13. Nakatsu Y, Kotake Y, Takishita T, Ohta S. Long-term exposure to endogenous levels of tributyltin decreases GluR2 expression and increases neuronal vulnerability to glutamate. Toxicol Appl Pharmacol. 2009; 240: 292-298.
14. Matute C. Glutamate and ATP signaling in white matter pathology. J Anat. 2011; 219: 53-64.
15. Hara MR, Snyder SH. Cell signaling and neuronal death. Annu Rev Pharmacol Toxicol. 2007; 47: 117-141.
16. Yi JH, Hazell AS. Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int. 2006; 48: 394-403.
17. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr. 2000; 130: 1007S-1015S.
18. Mattson MP. In: Stress: physiology, biochemistry, and pathology. Handbook of Stress Series, 2019; 3: 125-134.
19. Butterfield DA, Pocernich CB. The glutamatergic system and Alzheimer's disease: therapeutic implications. CNS Drugs. 2003; 17: 641-652.
20. Cortese BM, Phan KL. The role of glutamate in anxiety and related disorders. CNS Spectr. 2005; 10: 820-830.
21. Benussi A, Alberici A, Buratti E, Ghidoni R, Gardoni F, Luca MD, et al. Toward a glutamate hypothesis of frontotemporal dementia. Front Neurosci. 2019; 13: 304.
22. Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov. 2005; 4: 131-144.
23. Bechtholt-Gompf AJ, Walther HV, Adams MA, Carlezon WA Jr, Ongür D, Cohen BM. Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. Neuropsychopharmacology. 2010; 35: 2049-2059.
24. Verma SK, Kumar A. Therapeutic uses of *Withania somnifera* (Ashwagandha) with a note on withanolides and its pharmacological actions. Asian J Pharm Clin Res. 2011; 4: 1-4.
25. Jayanthi MK, Prathima C, Huralikuppi JC, Suresha RN, Murali Dhar. Anti-depressant effects of *Withania somnifera* fat (Ashwagandha ghrutha) extract in experimental mice. Int J Pharm Bio Sci. 2012; 3: 33-42.
26. Kulkarni SK, Dhir A. *Withania somnifera*: an Indian ginseng. Prog Neuropsychopharmacol Biol Psych. 2008; 32: 1093-1105.

27. Kataria H, Wadhwa R, Kaul SC, Kaur G. *Withania somnifera* water extract as a potential candidate for differentiation based therapy of human neuroblastomas. PLoS One. 2013; 8: 1.
28. Sharma V, Sharma S, Pracheta. Protective effect of *Withania somnifera* roots extract on hematoserological profiles against lead nitrate-induced toxicity in mice. Indian J Biochem Biophys. 2012; 49: 458-462.
29. Kiki SM, Mansour HH, Anis L. The modulatory role of Ashwagandha root extract on gamma-radiation-induced nephrotoxicity and cardiotoxicity in male Albino rats. Am J Phytomed Clin Ther. 2014; 2: 622-629.
30. Shah N, Singh R, Sarangi U, Saxena N, Chaudhary A, Kaur G, et al. Combinations of Ashwagandha leaf extracts protect brain-derived cells against oxidative stress and induce differentiation. PLoS One. 2015; 19: 10.
31. Alam N, Hossain M, Mottalib MA, Sulaiman SA, Gan SH, Khalil MI. Methanolic extracts of *Withania somnifera* leaves, fruits and roots possess antioxidant properties and antibacterial activities. BMC Complem Altern Med. 2012; 12: 175.
32. Jeyanthi T, Subramanian P. Protective effect of *Withania somnifera* root powder on lipid peroxidation and antioxidant status in gentamicin-induced nephrotoxic rats. J Basic Clin Physiol Pharmacol. 2010; 21: 61-78.
33. Tolar M, Abushakra S, Sabbagh M. The path forward in Alzheimer's disease therapeutics: Reevaluating the amyloid cascade hypothesis. Alzheimer's Dement. 2019; 16(1): 1553-1560.
34. Jayaprakasam B, Padmanabhan K, Nair MG. Withanamides in *Withania* somnifera fruit protect PC-12 cells from beta-amyloid responsible for Alzheimer's disease. Phytother Res. 2010; 24: 859-863.
35. Beutler HO, Michal G. In: Methods of enzymatic analysis. Bergmeyer HU, ed., 2nd edn., Verlag Chemie, Weinheim/Academic Press, Inc. New York and London, 1974: 1708-1713.
36. Lee YL, Lardy AA. Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat. J Bio Chem. 1965; 240: 1427-1430.
37. Pramilamma Y, Swami KS. Glutamate dehydrogenase activity in the normal and denervated gastrocnemius muscles of frog. Curr Sci. 1975; 44: 739.
38. Yu ZF, Cheng GJ, Hu BR. Mechanism of colchicine impairment of learning and memory, and protective effect of CGP36742 in mice. Brain Res. 1997; 750: 53-58.
39. Akina S, Thati M, Puchchakayala G. Neuroprotective effect of ceftriaxone and selegiline on scopolamine induced cognitive impairment in mice. Adv Biol Res. 2013; 7: 266-275.
40. Rossi DJ, Slater NT. The developmental onset of NMDA receptor-channel activity during neuronal migration. Neuropharmacology. 1993; 32: 1239-1248.
41. Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behav Brain Res. 2003; 140: 1-47.
42. Campos-Peña V, Meraz-Ríos MA. Alzheimer's disease: the role of AB in the glutamatergic system. http://dx.doi.org/10.5772/57367.
43. Butterfield DA, Perluigi M, Sultana R. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. Eur J Pharmacol. 2006; 545: 39-50.
44. Farooqui T, Farooqui AA. Curcumin: historical background, chemistry, pharmacological action, and potential therapeutic value. In: Curcumin for neurological and psychiatric disorders. Academic Press, 2019: 23-44.
45. Bajo R, Pusil S, López ME, Canuet L, Pereda E, Osipova D, et al. Scopolamine effects on functional brain connectivity: a pharmacological model of Alzheimer's disease. Sci Rep. 5. 2015; 9748.
46. Liu W, Rabinovich A, Nash Y, Frenkel D, Wang Y, Youdim MB, Weinreb O. Anti-inflammatory and protective effects of MT-031, a novel multitarget MAO-A and AChE/BuChE inhibitor in scopolamine mouse model and inflammatory cells. Neuropharmacology. 2017; 113: 445-456.

47. Bhuvanendran S, Kumari Y, Othman I, Shaikh MF. Amelioration of cognitive deficit by embelin in a scopolamine-induced Alzheimer's disease-like condition in a rat model. Front Pharmacol. 2018; 9: 665.
48. Rawls SM, Mcginty JF. Muscarinic receptors regulate extracellular glutamate levels in the rat striatum: an *in vivo* microdialysis study. J Pharmacol Exp Ther. 1998; 286: 91-98.
49. Kilian JG, Hsu HW, Mata K, Wolf FW, Kitazawa M. Astrocyte transport of glutamate and neuronal activity reciprocally modulate tau pathology in *Drosophila*. Neuroscience. 2017; 348: 191-200.
50. Yang Q, She H, Gearing M, Colla E, Lee M, Shacka JJ, et al. Regulation of neuronal survival factor MEF2D by chaperone-mediated autophagy. Science. 2009; 323: 124-127.
51. Albin RL, Greenamyre JT. Alternative excitotoxic hypotheses. Neurology. 1992; 42: 733-738.
52. Li Z, Yamamoto Y, Morimoto T, Ono J, Okada S, Yamatodani A. The effect of pentylenetetrazole-kindling on the extracellular glutamate and taurine levels in the frontal cortex of rats. Neurosci Lett. 2000; 282: 117-119.
53. Kim AY, Baik EJ. Glutamate dehydrogenase as a neuroprotective target against neurodegeneration. Neurochem Res. 2019; 44L 147-153.
54. Shen H, Kihara T, Hongo H, Wu X, Kem WR, Shimohama S et al. Neuroprotection by donepezil against glutamate excitotoxicity involves stimulation of α7 nicotinic receptors and internalization of NMDA receptors. Br J Pharmacol. 2010; 161: 127-139.
55. Kataria, H., Wadhwa R, Kaul SC, Kaur G. Water extract from the leaves of *Withania somnifera* protect RA differentiated C6 and IMR-32 cells against glutamate-induced excitotoxicity. PLoS One. 2012; 7: E37080.
56. Dar NJ, Satti NK, Dutt P, Hamid A, Ahmad M. Attenuation of glutamate-induced excitotoxicity by withanolide-A in neuron-like cells: role for PI3K/Akt/MAPK signaling pathway. Mol Neurobiol. 2018; 55: 2725-2739.
57. Kumar S, Seal CJ, Howes MJ, Kite GC, Okello EJ. *In vitro* protective effects of *Withania somnifera* (L.) dunal root extract against hydrogen peroxide and β-amyloid (1-42)-induced cytotoxicity in differentiated PC12 cells. Phytother Res. 2010; 24: 1567-1574.
58. Shah N, Singh R, Sarangi U, Saxena N, Chaudhary A, Kaur G, et al. Combinations of Ashwagandha leaf extracts protect brain-derived cells against oxidative stress and induce differentiation. PLoS One. 2015; 10(3): e0120554.
59. Rahman MA, Hossain S, Abdullah N, Aminudin N. Validation of *Ganoderma lucidum* against hypercholesterolemia and Alzheimer's disease. Eur J Biol Res. 2020; 10: 314-325.
60. Sohn E, Lim HS, Kim YJ, Kim BY, Jeong SJ. *Annona atemoya* leaf extract improves scopolamine-induced memory impairment by preventing hippocampal cholinergic dysfunction and neuronal cell death. Int J Mol Sci. 2019; 20: 3538.
61. Puangmalai N, Thangnipon W, Soi-Ampornkul R, Suwanna N, Tuchinda P, Nobsathian S. Neuroprotection of N-benzylcinnamide on scopolamine-induced cholinergic dysfunction in human SH-SY5Y neuroblastoma cells. Neural Regen Res. 2017; 12: 1492-1498.
62. Gupta M, Kaur G. *Withania somnifera* (L.) Dunal ameliorates neurodegeneration and cognitive impairments associated with systemic inflammation. BMC Complem Altern Med. 2019; 19: 217.
63. Saykally JN, Hatic H, Keeley KL, Jain SC, Ravindranath V, Citron BA. *Withania somnifera* extract protects model neurons from *in vitro* traumatic injury. Cell Transplant. 2017; 26: 1193-1201.