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POTENTIAL OF GLYCOWITHANOLIDES FROM *WITHANIA SOMNIFERA* (ASHWAGANDHA) AS THERAPEUTIC AGENTS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is the most common cause of dementia. As of 2014, there were an estimated nearly 36 million people have Alzheimer's disease or a related dementia worldwide. This number will increase to an estimated 75.6 million in 2030, and 135.5 million in 2050. Today, someone develops AD every 67 seconds. By 2050, one new case of AD is expected to develop every 33 seconds in United States. Already 62% of people with dementia live in developing countries, but by 2050 this will rise to 71%. Currently FDA approved drugs such as acetylcholinesterase inhibitors (AChEI): Donepezil, Rivastigmine and Galantamine and N-methyl-daspartate (NMDA) antagonist: Memantine are prescribed for the treatment of AD. Meanwhile less than 20% of AD patients are responding moderately to these drugs with an average benefit for six to twelve months, often with serious side effects. Therefore there is an urgent need to develop and evaluate more effective pharmacological interventions with fewer side effects. Plant *Withania somnifera* which is used as herb in Ayurvedic medicine contain steroidal lactones glycowithanolides (Withaferin A, Withasomniferin-A) which has potential as therapeutic agent for AD. In this review, we compare that immunomodulatory, antioxidative, anticholinesterase and anti-inflammatory properties of glycowithanolides with the market available drugs.

KEYWORDS: Acetylcholinesterase inhibitor; N-methyl-daspartate antagonist; *Withania somnifera;* Withaferin A; Withasomniferin A; Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease which is characterized by brain atrophy, loss of neurons and synaptic function, caused by extracellular amyloid β (Aβ) aggregation and tau hyperphosphorylation which are hallmarks of AD pathogenesis. The asymmetrical cleavage of the amyloid precursor protein substrate by γ- secretase or altered catabolism of the Aβ peptides effects the relative amounts of Aβ 42 and Aβ 40 and formation of neurotoxic oligomers. Similarly, studies indicate that soluble Aβ oligomers are more responsible for both the neurodegeneration and impairment of synaptic function in AD rather than Aβ- aggregation.[1-4] These soluble Aβ oligomers increase tau phosphorylation and also cause disturbance in glutamatergic neurotransmission.[5] But the exact pathogenic role of deposited versus soluble forms of Aβ- oligomers is still controversial.[6] Additional histopathological characteristic feature of AD is the deposition of neurofibrillary tangles (NFTs) within neurons. In AD brain, the imbalance between phosphorylation protein kinase and dephosphorylation of protein phosphatase, leading to tau hyperphosphorylation (at up to 21 epitopes), microtubules instability, disturbed axoplasmic flow and, consequently cell death. [7] In addition, two isoform of Glycogen synthase kinase 3(GSK3), GSK3α and GSK3β and cyclin protein kinase 5 (Cdk5) have pivotal roles in Aβ- aggregation and tau hyperphosphorylation.[8] Cholinergic abnormalities and activation of microglial to secrete neurotoxic pro-inflammatory molecules which increases the oxidative stress play important role in the onset of AD.[9] Oxidative stress is also influenced by the by Fenton and Haber-Weiss Reactions which have catalytic activities in the survival and pathological signaling pathways, neural plasticity, and neuroprotection in AD.[10] Brain imaging studies have demonstrated reduction of glucose utilization in AD brain [11-13] may precede the onset of cognitive deficits. [14, 15] The cause of hypometabolism of glucose is not well understood.[16] Mainly glucose metabolism is regulated by insulin signaling, but the role of insulin in the brain is unrevealed.[17] The cause of this defect is not well understood. It is possible that impaired mitochondrial function[11, 18] or defective glucose transportation[19] might have contributed of this observation.

At present there are no permanent therapeutic or protective treatments available for AD. Typically there are five drugs approved by FDA for the treatment of AD. Due to high

prevalence of hepatotoxicity, acetylcholinesterase inhibitor (AChEI): Tacrine (1993) is no longer used. Other AChEI: Donepezil (1996), Rivastigmine (2000) and Galantamine (2001) frequently used to treat patients with mild to moderate AD and N-methyl-daspartate (NMDA) antagonist: Memantine (2003) is used to treat patients with moderate to severe AD. AChEI delays acetylcholine degradation and enhance cholinergic transmission. But long-term efficacy of AChEIs remains controversial[20, 21], continuing treatment will be useful if it can be tolerated.[22] Similarly, protecting effect of AChEI in mild cognitive impairment (MCI) from converting into AD remains inconclusive.[23] NMDA open channel blocker Memantine can also protect neurons from excitotoxicity. The benefit of these medicines extended for an average of six to twelve months and are more expensive.[24] Therefore, we need to explore alternative medicinal approach to develop cheaper and more effective compounds for the delay and treatment of AD. This review aims to explore the benefits of extracted steroidal lactones from *Withania somnifera* or Ashwagandha, as possible treatment or delay in the progression of AD. The molecular structures of FDA approved medicines and glycowithanolides from *W. somnifera* are given in figure 1.

MARKET-AVAILABLE MEDICINES

Acetylcholinesterase Inhibitors

Donepezil

Donepezil [(RS)-2-[(1-benzyl-4-piperidyl) methyl]-5, 6-dimethoxy-2, 3 dihydroinden-1-one] is a noncompetitive, reversible inhibitor of Acetylcholinesterase (AchE) but not butyrylcholinesterase (BuChE) activity. It has an N- benzylpiperidine and an indanone moiety with high specificity for the central nervous and peripheral cholinergic system.[25, 26] It is now marketed under the trade name of “Aricept” which is developed by “Eisai Ltd.” and Partner “Pfizer”.

Since donepezil acts as an AchE inhibitor, it reduces the hydrolysis of acetylcholine and improve the AD pathogenesis. Donepezil neither directly interact with catalytic triad of AchE nor oxyanion hole. Donepezil also can’t form any direct hydrogen bond with AchE or by electrostatic interactions, it interacts through aromatic stacking and solvent mediated interaction. Primarily, donepezil interact with AchE through Glu 199, His 440, Phe 330, Trp 84, Tyr334, Tyr 121, Phe 331, Phe 288, Ser 286, Phe 290, Arg289, Trp 279 and Leu 282.[27] Although donepezil acts as a neuroprotective agent at the neurotransmitter level, but it also has anti-oxidative properties.[28] It reduces the expression of inflammatory cytokines[29],

inhibits GSK-3 activity, enhances the Akt phosphorylation and reduces tau phosphorylation (Thr 231, ser 199, and ser 396). [30-32] Therefore, Donepezil can prevent Aβ-42 –induced neurotoxicity through the activation of phosphoinositide 3 kinase (PI3 kinase) / Akt and inhibition of GSK-3 as well as through the activation of nicotinic acetylcholine receptors. [33] Donepezil protects neurons from glutamate cytotoxicity through the stimulation of α7 nAChR (nicotinic acetylcholine receptor) and a Src family tyrosine kinase and it reduces glutamate-induced caspase-3 activation. Therefore donepezil decreases the glutamate toxicity through down-regulation of NMDA receptors, through stimulation of α7nAChRs and up-regulation of PI3k-Akt cascade or defensive system.[34]

Donepezil is well absorbed with 100% oral bioavailability, reaches peak plasma concentrations within 3 to 4 hours. The plasma half-life of Donepezil is about 70 hours and approximately 96% it can bound to human plasma protein. Donepezil is metabolized by cytochrome P450 (CYP) isoenzymes CYP2D6 and CYP3A4 and undergoes glucuronidation to become water soluble and less toxic.[29] It excretes through urine and faces. Normally the side effects of Donepezil are nausea, diarrhea, vomiting and insomnia.

Rivastigmine

Rivastigmine [(S)-3-[1-(dimethylamino) ethyl] phenyl N-ethyl-N-methylcarbamate] is a derivative of physostigmine formed by using a miotine template of carbamates. It is a noncompetitive inhibitor of both AchE and BuChE and acts by the carbamoylation of serine residues of the active sites of both estrases.[35] In market, it is known as “Exelon” which is marketed by “Novartis Pharmaceuticals Ltd.”

Due to small molecular size (< 300Da) of Rivastigmine, it has Blood-Brain-Barrier (BBB) permeability and it also converted to a patch delivery system. It has both lipophilic and hydrophilic properties. Since Rivastigmine has dual acetyl and butyrylcholinesterase inhibitory activity, it is a novel drug for the treatment of AD. But the mode of action of Rivastigmine is not completely understood. However, *in vivo* studies indicated that Rivastigmine can preserve or enhance neuronal and synaptic terminal markers APP and Aβ processing. Rivastigmine can protect neurons from degeneration by the formation of low molecular weight sAPP, which suggests a stronger neuronal contribution to sAPP secretion by alternative splicing. Rivastigmine increases sAPPα and decreases Aβ secretion which can prevent AD pathogenesis. Rivastigmine enhances the production of sAPP and shifts APP processing through α- secretase pathway.[36]

Rivastigmine is well absorbed with 60%-70% of oral bioavailability. The plasma half-life of Rivastigmine is about 1-2 hours and it can bind approximately 40% to human plasma protein for a 3 mg dose. It is metabolized by cytochrome P450 system. It excretes through renal system. Rivastigmine has been associated with side effects such as nausea, diarrhea, vomiting and anorexia.

Galantamine

Galantamine [(4aS,6R,8aS)-5,6,9,10,11,12- hexahydro-3-methoxy-11-methyl-4aH-[1] benzofuro [3a,3,2-ef] [2] benzazepin -6-01] is a natural alkaloid, isolated from bulbs and aerial parts of plants from the family Amaryllidaceae: Galanthus woronowi Losinsk, Galanthus nivalis L., Leucojum aestivum L., Lycoris aurea L., Lycoris radiata L., Narcissus tazetta L. It is a cholinergic potentiator, competitive cholinesterase inhibitor with antioxidant [37-39] and neuroprotective properties.[40, 41] It has more than 50-fold of acetylcholinesterase selectivity with respect to butyrylcholinesterase.[42] Galantamine is also an allosteric potentiator of nicotinic acetylcholine receptors (nAChRs) such as $\alpha_4\beta_2$, $\alpha_3\beta_4$, $\alpha_6\beta_4$ and $\alpha_7/5HT_3$ (chimeras of serotonin-ACh receptors) in brain. It is marketed by "Shire pharmaceuticals Ltd." under the trade name "Razadyne".

Galantamine also acts as a free radical scavenger of reactive oxygen species (ROS) by preventing the activation of P2X7 receptors and the membrane fluidity disturbances and by the protection of mitochondrial membrane potential.[43, 44] In addition, Galantamine decreases the overproduction of reactive oxygen species by the inhibition of acetylcholinesterase and allosteric potentiation of α7-subtype of nicotinic acetylcholine receptors.[45-47] Galantamine protects against a variety of cytotoxic agents such as Aβ- aggregation, glutamate, hydrogen peroxide, oxygen and glucose deprivation by induction of phosphorylation of serine-threonine protein kinase[45] stimulation of phosphoinositide 3-kinase and the expression of protective protein Bc1-2.[48, 49] *In vivo* studies indicates that Galantamine enhances dopaminergic neurotransmission as to increase dopamine concentration through allosteric potentiation of α7-subtype of nicotinic acetylcholine receptors.[50] Galantamine also increases the acetylcholine levels by dopamine-D1 receptor mediated mechanism.[51] It activates the muscarinic receptors and increases the levels of acetylcholine.[52] Therefore, Galantamine protects against several neurotoxic stimuli and elevates the neurotransmitter release of dopamine, noradrenaline, glutamate and γ Aminobutyric acid by acetylcholinesterase

inhibition and allosterically potentiation of α7- subtype of nicotinic acetylcholine receptors.[51]

The oral bioavailability, half-life and plasma protein binding of Galantamine are 80-100%, seven hours and 18% respectively. Approximately 75% of a dose of Galantamine is metabolised in liver. *In vitro* studies indicated that hepatic CYP2D6 and CYP3A4 substrate are partially involved in Galantamine metabolism. It excretes through renal (95%, of which 32% unchanged) and fecal (5%) system. The common side-effect of Galantamine are nausea, vomiting, diarrhea, weight loss and loss of appetite.

N- methyl-D-aspartic acid receptor -antagonist

Memantine

Memantine (3, 5-dimethyltriccyclo [3.3.1.$1^{3,7}$] decan-1 amine or 3,5- dimethyladamantan-1-amine) is a low- affinity voltage–dependent uncompetitive antagonist at glutamatergic N-methyl-D-aspartate (NMDA) receptors.[53] Memantine also acts as a non-competitive antagonist at the 5-HT3 receptor with a potency similar to that for the NMDA receptor [54] and acts as a non-competitive antagonist at α7-nicotinic acetylcholine receptors (nAChRs) too.[55] It is marketed under the brand name of "Namenda", "Axura and Akatinol" , "Ebixa and Abixa" and " Memox" by " Forest", "Merz" , "Lundbeck" and "Unipharm" respectively.

Memantine prevent the influx of Ca^{2+} and oxidative stress in postsynaptic neurons. It preserves the transmission of strong transient physiological signals.[56] *In vivo* studies indicates that Memantine prevents neuronal cell loss and apoptosis induced by Aβ 1-40 peptide[57] through decreasing lesions observed [58] and rescues the neocortical cholinergic fibers originating from nucleus basalis of Meynert (nbM). It also attenuates microglial activation around the intracerebral lesions and improves attention and memory[59] protects against various other toxic insults to the cholinergic fibers and NMDA receptors.[60] The rat septal neuronal cells studies indicated that neuroprotective effect of donepezil against Aβ (1-42) toxicity is not mediated by interference with the NMDA- mediated excitotoxic process, and that donepezil may be more effective than Memantine against cholinergic neuronal damage induced by Aβ (1-42) exposure.[61] *In vitro* studies indicated that Memantine reduces the Aβ 40 aggregation [62,63] and inhibits extra-synaptic NMDAR induced Aβ42 production[64] protects the tau phosphorylation[65] at S262/S356 and S396 through calmodulin-dependent protein kinase β (CaMKKβ) activation of AMPK[66] and inhibition of protein phosphatase

(PP)-2A[67], completely protects against Aβ- induced ROS.[68] It was shown that memantine increased the sAPPα without affecting the levels of total intracellular APP which indicates that memantine may enhance the α-secretase for non-amyloidogenic pathway. [69] It decreases the level of Aβ 40 and Aβ 42.[70, 71] Overall mechanism of memantine is still unclear about its effects on the levels of Aβ and related peptides[69] and protects cholinergic neurons from inflammatory process[72] but memantine has neither effect in p-GSK3β in hippocampus or prefrontal cortex (PFC) nor total GSK3β levels.[73] Memantine prevents neuronal oxidative stress and memory impairment which is caused by high-molecular-weight (HMW) oligomers, but can't prevent induced by low-molecular-weight (LMW) oligomers.[74] Memantine increases the levels of brain-derived neurotrophic factor (BDNF) - mRNA in the limbic cortex and this effect increases with increase in dose, as a result, it increases the production of BDNF which enhances hippocampal synaptic transmission by increasing NMDA receptor activity.[75, 76] At the same time, memantine induces isoforms of the BDNF receptor trkB. [77] Therefore, memantine protect the oxidative stress, Aβ 40/42 aggregation, tau hyperphosphorylation in AD brain.

The bioavailability of Memantine is about 100%. The plasma half–life is 60-100 hours. It is metabolized by hepatic (< 10%) and excretes through renal systems. Memantine has been associated with the side effects of fatigue, pain, hypertension, headache, and constipation.

BIOCHEMICAL CONSTITUENTS OF *W. SOMNIFERA*

Major parts of *W. somnifera* such as roots, fruits and leaves contain high level of polyphenols and lactones so ashwagandha has beneficial role in human health.[78, 79] Major biochemical constituents such as Withanolide A ,Withanolide D, 3-β-hydroxy-2, 3- dihydro-withanolide F, Withaferin A, Isopelletierine, Anaferine, Cuseohygrine, Anahygrine, Withanoside I, Withanoside II , Withanoside III, Withanoside IV, Withanoside V, Withanoside VI, Withanoside VII , Sitoindoside IX , Sitoindoside X and Withasomniferin-A are found in Ashwagandha (**Figure 2**). Steroidal lactones glycowithanolides (Withaferin A, Withasomniferin-A) also has potential as therapeutic agent for AD.

GLYCOWITHANOLIDES FROM *W. SOMNIFERA*

Glycowithanolides (Withaferin A, withasomniferin A) which are isolated from *W. somnifera* seems to have therapeutic properties for AD. Withaferin A exhibits a vital role in ibotenic acid induced cognitive defects and produces an increase in the cholinergic markers such as Ach, ChAT. [80] Withaferin A reduces the inflammatory mediators such as IL-1 and TNF-α

[81] in plaque formation and neurodegeneration.[82] Ashwagandha has anti-inflammatory effect due to presence of Withaferin A.[83] Withaferin A have anti-tumor, anti- inflammatory, and immunomodulatory activities.[84] Withaferin A have anti-inflammatory activity through the blocking of IκB phosphorylation and degradation by inhibiting IκB kinase activation.[85] Withaferin A also inhibits lipopolysaccharide (LPS)–induced inducible nitric oxide synthase (iNOS) expression and NO production as well as TNF-α induced intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 by reducing Akt and NF-κB activation.[86] Withaferin A inhibits LPS-induced cyclooxygenase (COX)-2 mRNA and protein expression and prostaglandin E2 (PGE2) production in BV2 murine microglial cells. Withaferin A had no effect on LPS-induced Akt and ERK phosphorylation, but Withaferin A slightly decreases p38 phosphorylation and JNK pathway. Withaferin A inhibits LPS-induced STAT1 and STAT3 phosphorylation. Withaferin A inhibits nuclear translocation of STAT1 and interferon-gamma activated sequence (GAS)-promoter activity. Withaferin A significantly inhibit the microglial inflammatory response through inhibition of COX-2 protein expression and production of PGE2, one of the major COX-2 products .Therefore, withaferin A modulate the inflammatory response in microglia by reducing COX-2 expression and PGE2 production and by blocking STAT1 and STAT3 activation.[87] It can prevent lipid peroxidation and decrease Monoamineoxidase (MAO), GABA, 5-hydroxytrytophan, and glutamic acid levels.[88] Withaferin A have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum.[89]

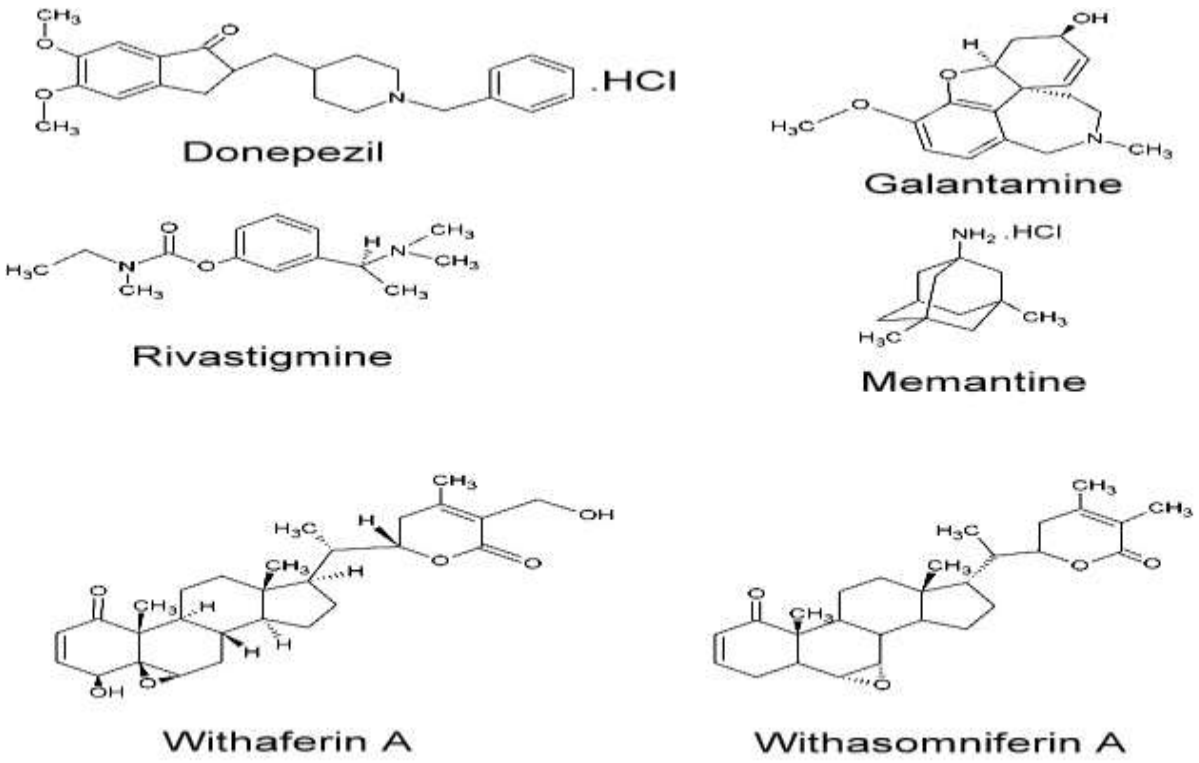
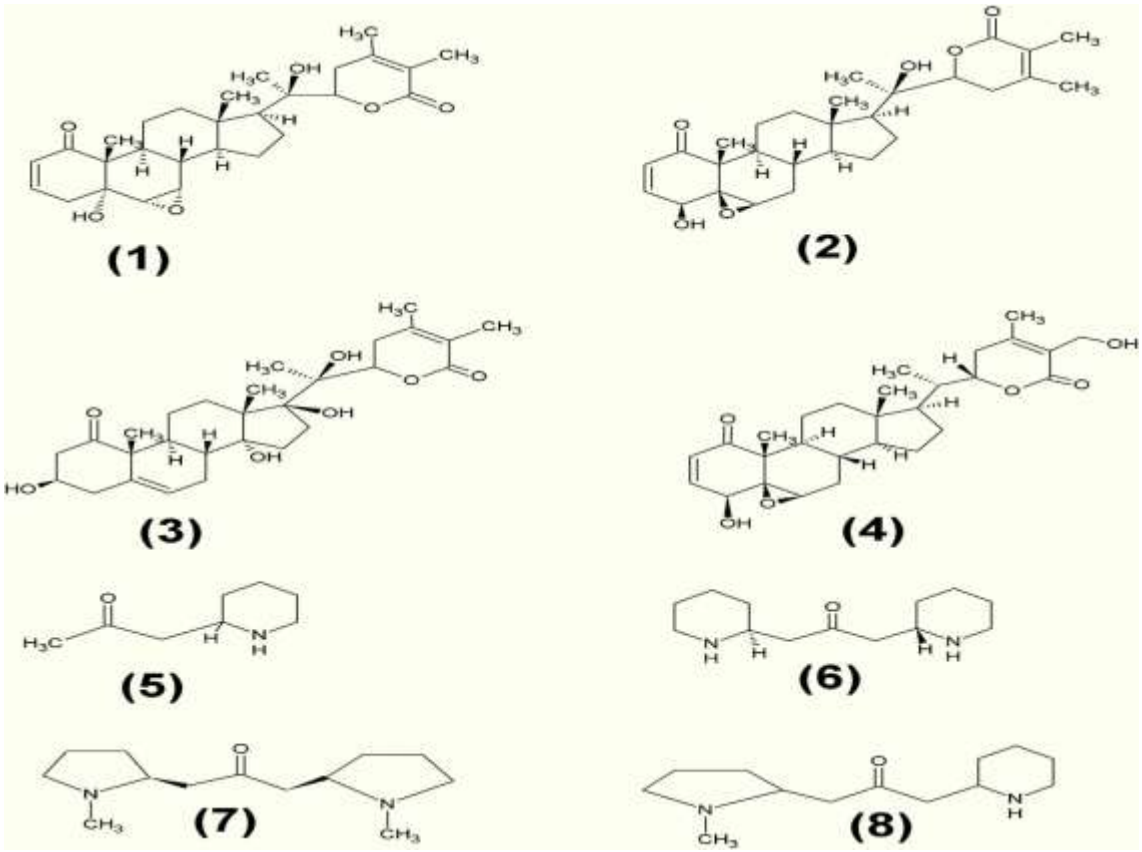
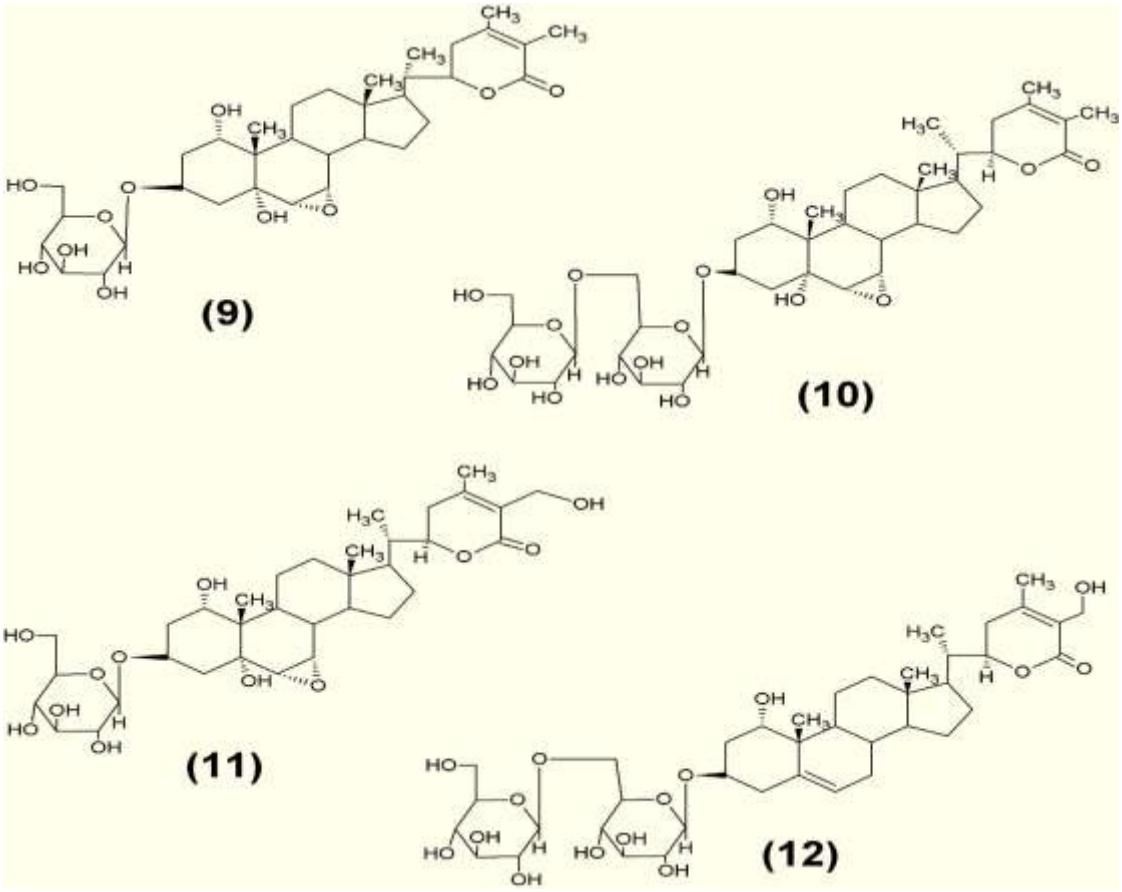


FIGURE 1: Molecular structures of Donepezil, Galantamine, Rivastigmine, and Memantine. Withaferin A and Withasomniferin A.

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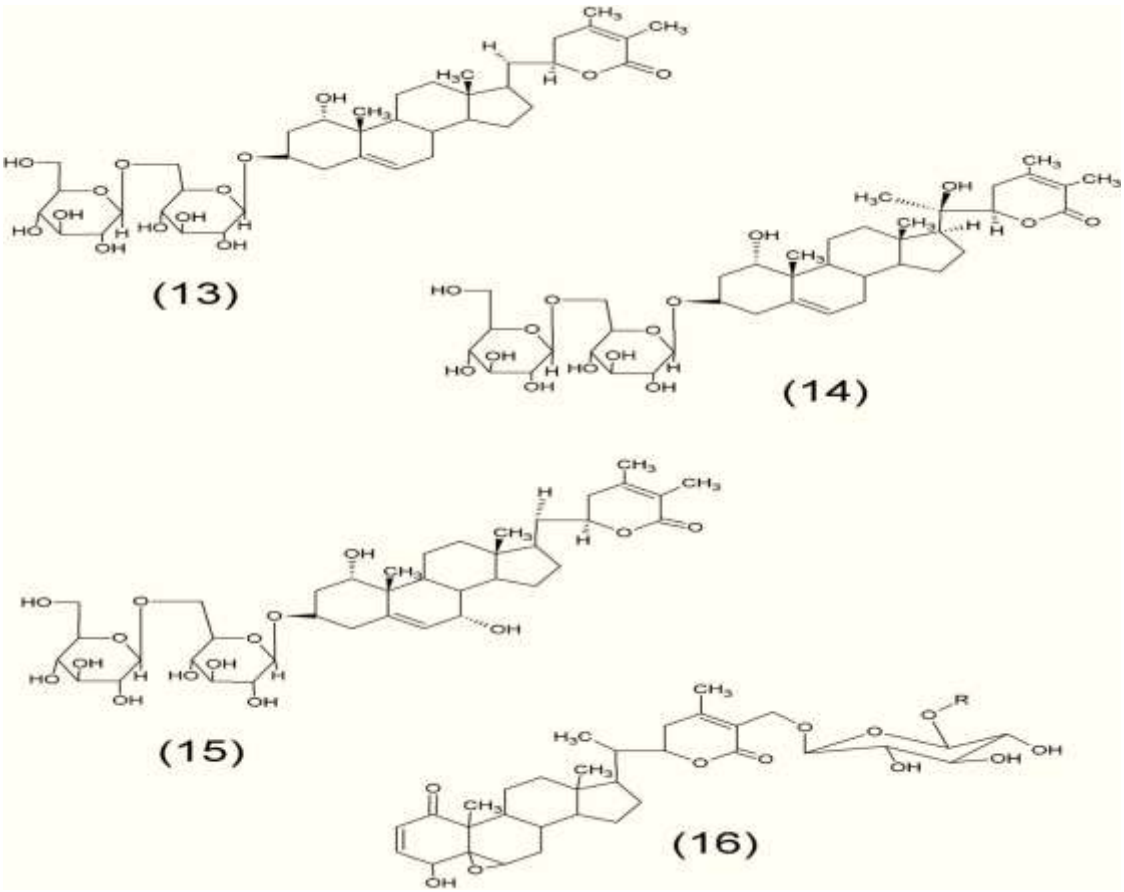
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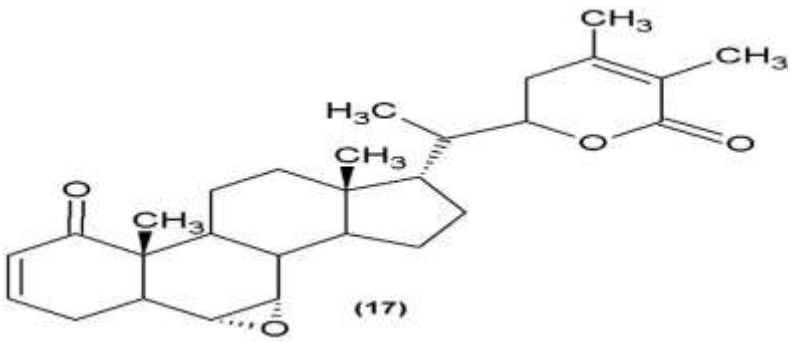

Figure 2: The structures of the major biochemical constituents of ashwagandha such as (1) Withanolide A (2) Withanolide D (3) 3-β-hydroxy-2, 3- dihydro-withanolide F(4) Withaferin A (5) Isopelletierine (6) Anaferine (7) Cuseohygrine (8) Anahygrine (9) Withanoside I (10) Withanoside II (11) Withanoside III (12) Withanoside IV (13) Withanoside V (14) Withanoside VI (15) Withanoside VII (16) Sitoindoside IX when R =H and Sitoindoside X when R= polmitoyl and (17) Withasomniferin-A. Among of these constituents Glycowithanolides (Withaferin A, withasomniferin A) has seems to have therapeutic properties for AD.

TABLE 1: Comparative study of Withaferin A and Withasomniferin A of *Withania somnifera* and available FDA approved market medicines for the treatment of Alzheimer's disease.

	***W. somnifera* Glycowithanolides**		**Cholinesterase inhibitor**			**NMDA antagonist**
Properties	Withaferin A	Withasomnifer in A	Donepezil (Aricept)	Rivastigmine (Exelon)	Galantamine (Razadyne)	Memantine (Namenda)
Mode of action	increases the cholinergic markers such as Ach, ChAT [92]	increases the cholinergic markers such as Ach, ChAT [92]	Non-competitive reversible cholinesterase inhibitor, AChE not BuChE	Noncompetitive pseudo irreversible cholinesterase inhibitor, Both AChE & BuChE	Reversible, competitive cholinesterase inhibitor, AChE more than BuChE	uncompetitive / noncompetitive , NMDA antagonist, NMDA and 5-HT3 receptors
Anti-oxidative properties	+++ [89,93,94]	+++ [89,93,95]	+++ [96,97]	++ [98]	+++ [41, 99]	++ [100,101]
Anti- inflammatory properties	+++ [102,103,104]	+++ [89,93,95,105]	+ [106]	++ [107]	+++ [41,99]	++ [108]
Free radical scavenger property	+++ [109,110]	+++ [89,93,95,105]	+++ [111]	++ [98]	+++ [41,99]	++ [112]
GSK3 activity	- [87]	Not found	--- [113,114]	- [115]	- [115,116]	--- [117,118]
Dosage	3-6 grams daily of the dried root, 300- 500 mg of the extract [119,120]		5 mg, 10 mg, 23mg. Once daily	1.5 mg, 3 mg, 4.5 mg, 6 mg, Twice daily/once daily patch expected soon	4 mg, 8 mg, 12 mg (standard); 8 mg, 16 mg, 24 mg (prolonged release). Twice daily/once daily (prolonged release formulation)	5mg, 7mg, 10mg, 20mg and 28mg. Once/ twice daily.
Metabolism by	Cytochrome (Cyp) P450 enzymes and P-glycoprotein (P-gp) [119,121]		cytochrome P450 (CYP) isoenzymes 2D6 and 3A4 and undergoes glucuronidation [29]	cytochrome P450 (CYP) system [29]	Hepatic CYP2D6 and CYP3A4 [29]	Hepatic [29]

Plasma half-life (hours)	Not found	70	1-2	7	60-100
Bioavailability	Not found	100%	60-70%	80-100%	100%
Plasma protein binding	Not found	96%	40%	18%	47%
Excretion	Urine	Urine/ fecal	Urine	Urine / fecal	Renal
Warning and Contraindications	Pregnancy, avoid alcohol, sedative and other anxiolytics while taking ashwagandha [119,120]	Allergic to another cholinesterase inhibitor ,severe liver disease, stomach, ulcers, seizures, asthma, hepatitis, kidney disease	Allergic to another cholinesterase inhibitor, severe or persistent skin irritation.	Allergic in another cholinesterase inhibitor, atrioventricular, asthma, pulmonary disease, ulcer, kidney disease.	Allergies to other NMDA-receptor antagonists, epileptic seizures, myocardial infarction, uncontrolled hypertension, kidney problems, including renal tubulary acidosis, severe infections of the urinary tract.
Drug interaction potential	sedative effects of barbiturates and some benzodiazepines [121]	Antibiotics. Anti-fungal, beta-blockers Muscle relaxants medicines	Muscle relaxants (succinylcholine-type), Nicotine.	Erythromycin, Piperaquine, Clarithromycin, Ketoconazole, Oxybutynin, Paroxetine, Quinidine	Amantadine, Quinidine, Quinine, Dopaminergic agonists, Neuroleptics, Oral anticoagulants
Side effect	gastrointestinal upset, diarrhea and vomiting [119,120]	nausea, diarrhea, vomiting and insomnia	nausea, diarrhea, vomiting and anorexia	Nausea, vomiting, diarrhea, weight loss and loss of appetite	dizziness, headache, constipation and confusion

DISCUSSION AND CONCLUSION

It has been estimated that less than 20 % of AD patients are responding moderately to the FDA approved drugs.[90] These drugs have little or no neuroprotection, effective only for short duration, often produce serious side effects, and expensive.[91] On the other hand, medicinal plant *W. somnifera* shows great potential in alleviating anxiety, inflammation, cognitive and neurological disorders such as Alzheimer's disease. Comparative studies of the properties of glycowithanolides extracted from *W. somnifera* with the available medicines are shown in **table 1**. More research is needed to understand the mechanism of the action of these molecules as therapeutic agents for Alzheimer's disease.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests regarding the publication of this paper.

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