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Resveratrol Treatment Attenuates Amyloid Beta, Tau Protein and Markers of Oxidative Stress, and Inflammation in Alzheimer's disease Rat Model

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ABSTRACT

Background/aim: Extracellular Amyloid beta plaque formation and intracellular tau hyperphosphorylation in brain are hallmarks of AD pathology. Studies have indicated protective effects of resveratrol against AD. However, the underlying mechanisms are poorly understood. Here we examined the effect of resveratrol on amyloid beta (Aβ), tau protein, acetylcholine esterase (AchE), oxidative stress and inflammation to understand mechanisms in protective effects of resveratrol using aluminum chloride ($AlCl_3$) induced AD inrat model.
Materials and method: Sixty Rats were divided into six groups (ten rats each). Rats were treated orally with $AlCl_3$to induced AD. Rats were treated before and after $AlCl_3$ with resveratrol as protective and therapeutic effect.
Results: AlCl3 treatment significantly induced the pathological characteristics of AD in the rats as evident from the significantly increased serum Aβ, tau protein, AchE, CRP, IL-6, TNF-α, TGF-β, and MDA levels and significantly decreased catalase and SOD activities. Marked histological alterations were noticed in brain tissues of AD rats. Oral treatment with resveratrol for 45 days before or after induction of AD resulted in a significant reversal of studied parameters. Moreover, AD rats treated with pharmacological drug, ebixa had comparable effects to that of resveratrol. The underlying mechanism in the protective effects of resveratrol against AD appears to involve its modulatory effects on Aβ, tau, oxidative stress and inflammation. This is the first study to simultaneously measure the multiple pathological markers to confirm the therapeutic and preventive potential of resveratrol against AD.
Conclusion: Our finding shed the light on the promising therapeutic and protective effect of resveratrol in attenuation AD complications through its antioxidant and anti-inflammatory effect.

Key words: *Alzheimer's disease, Resveratrol, Oxidative Stress, Inflammation, Tau protein.*

INTRODUCTION

Alzheimer's disease (AD) is probably the most common chronic neurodegenerative disease affecting 40-60% of dementia cases (1). AD is characterized by dementia, behavioral abnormalities and disability. Formation of neuriticplaques and neurofibrillary tangles in the brain is considered to be the hallmark of AD pathology (2). Neuriticplaques result from the accumulation of insoluble amyloid-beta peptide (Aβ) in the brain tissue, while the formation of neurofibrillary tangles is linked to hyper phosphorylation and deposition of microtubule-bound tau protein in neuron (3, 4). The Aβ is not only the major constituent of neuritic senile plaques; it also participates in the

pathophysiology of AD by augmenting oxidative stress, neuroinflammation and disturbing the integrity of blood brain barrier (5). Neuroimaging of neuritic plaques and measurement of Aβ and tau isoforms in cerebrospinal fluid (CSF) are regarded as gold standard in the diagnosis of AD (6, 7). Despite the high world over prevalence, the effective treatment and intervention of AD remains elusive, presumably due to the complex nature of disease pathology. Several drugs such as choline esterase inhibitors revstigmine and memantine have produced relatively modest beneficial effects in the reversal of AD symptoms (8). These drugs act by the inhibition of AchE enzyme responsible for termination of excitatory transmission at cholinergic synapses by hydrolyzing neurotransmitter acetylcholine or by blocking over activation of N-methyl-d-aspartate (NMDA)-type glutamate receptors (8, 9). Further, the use of these drugs was marred by prolonged side effects. Due to shortcomings associated with the use of pharmaceutical drugs, there is an increased interest in exploring the plant based natural products for their beneficial properties against AD. Natural products such resveratrol (trans-3, 5, 4'-trihydroxystilbene), a major polyphenolic component of grapes, red wine (10), curcumin (11), olive oil (12), and apigenin (11) have shown promising therapeutic and preventive effects against AD in humans and animal models. Neuroprotective effects of these plant based products are suggested to be mediated by their ability to blunt the oxidative stress, inflammation and to potentiate antioxidant mechanism (12, 14). Resveratrol has been extensively investigated for its potential to regress and prevent AD using *in vitro* and *in vivo* systems in humans and animal models. For instance, resveratrol has been shown to cross the blood brain barrier, decrease the insoluble Aβ1-42 levels in hippocampus and protect the blood brain barrier in AD rats (15). Resveratrol has been shown to exert its neuroprotective effects through the modulation of Aβ levels, oxidative stress and inflammatory mediators (16, 17). Additionally, in vitro treatment with resveratrol attenuated tau mediated neurodegeneration (18). Here we measured the effects of resveratrol on Aβ, tau, oxidative stress and inflammatory markers, and AchE activity to confirm the beneficial effects of resveratrol in lowering the AD risk using a well-established $AlCl_3$ induced ADmodel in rats (19, 20).

MATERIALS AND METHODS

Animals

This study was conducted in accordance with the set guidelines for animal experiments and approved by the Ethical Committee of King Fahd Medical Research Center, King Abdulaziz University. Jeddah, Saudi Arabia.Adult male albino rats weighing 120-130 g were obtained from the King Fahad Medical Research Center. Rats were maintained at 12 h light/dark cycle and left for acclimatization to animal house facility for 10 days. Rats had free access to food and water. Rats were randomly divided into different groups namely; C, Res, AD, AD+Res, Res+AD and AD+Ex with each group having 10 rats. The "C' group rats were normal healthy rats and served as control. The "AD" group rats were administered orally with aluminum chloride ($AlCl_3$) (17 mg/kg body weight) (LobaChemie, Mumbai, India) daily for 45 days to induce AD characteristics and served as AD rat model (19-21). The "Res" group rats were gavage administered daily with resveratrol (20 mg/kg body weight) (Sigma, St. Lois, MO, USA) for 45days according to the previously described method (22). Rats in "AD+Res" group were treated with $AlCl_3$ as described above to develop AD followed by the daily treatment with resveratrol (20 mg/kg body weight) for 45 days (Therapeutic effect of resveratrol). The "Res+AD" rats were initially treated with resveratrol for 45 days as described above followed by the induction of AD with $AlCl_3$ treatment (Protective effect of resveratrol). The "AD+Ex" group animals were treated with $AlCl_3$ for 45 days to induce AD followed by oral treatment of ebixa (Drumsheugh Gardens, Edinburgh. UK) (1mg/kg/day) according to the procedure described previously (23). The Ebixa is a prescription drug to alleviate the symptoms of AD in humans and acts via blocking the over activation of N-methyl-d-aspartate (NMDA)-type glutamate receptors in neurons (9). At the end of all the treatments, blood samples were collected from rats and were anesthetized by diethyl ether and sacrificed by cervical decapitation. Brains were dissected out and the brain tissue was fixed in 10% formalin for histopathology. Serum was separated and used for the measurement of Aβ, tau, AchE activity, oxidative stress and inflammatory markers.

Measurement of Aβ, tau, acetylcholine esterase, MDA, catalase activity and inflammatory markers

The Aβ, tauprotein, acetylcholine esterase, MDA, catalase activity and inflammatory markers were measured according to the manufacturer's instructions (Glory Science Co. Ltd. TX. USA).

Estimation of total protein

Total protein content in the serum was determined according to the supplier's instructions (Choronolab, Spain).

SOD

The SOD activity was measured by adding10 µl of sample to reaction mixture containing 0.4 ml tris buffer (pH7.4),20 µl of NADH (7.5 mM) and 25µl of EDTA /$MnCl_2$ (100 mM / 50 mM). The reaction was initiated by adding 50 µl of mercaptoethanol and the initial absorbance was recorded and NADH oxidation was followed by measuring the absorbency at 340 nm for 3 minutes.The activity of SOD was expressed as unit / mg protein. One unit is determined as the amount of enzyme that inhibited the oxidation of NADH by 50 %.

Histopathology

Brain tissue was fixed in10% formalin and embedded in paraffin block. Sections of 4 to5 µm thick were cut and stained with hematoxylin and eosin. Stained sections were visualized under light microscope for possible histological alterations. Fifteen sections were randomly selected and 12 different view fields were counted in each section.

Statistical analysis

Statistical analysis was carried out using SPSS v.20. One-way ANOVA was used to compare the significance between different treatment groups.Numerical data were expressed as mean ± SE. Differences between groupsare considered significant at $p \leq 0.05$.

RESULTS

3.1 Serum Aβ and Tau protein

The concentrations of Aβ and tau protein levels in control and different treated groups are presented in Table 1. Resveratrol alone treatment of normal healthy rats had no effect on Aβ and tau levels compared to control rats as no significant change inAβ or tau levels was observed between control and resveratrol treated control rats ($p \leq 0.05$). A significant increase in Aβ and tau was noted in the AD rats compared to control or resveratrol treated control rats. After resveratrol treatment, a significant decline in Aβ and tau levels was registered in rats treated with resveratrol before the induction of AD and in those treated with resveratrol after AD induction ($p \leq 0.05$).Additionally rats treated with ebixa had a significantly lowered Aβ and tau levels. These data suggest that resveratrol was able to exert both therapeutic and preventive effects against AD.

3.2 AchE and total protein

Data of serum AchE levels and serum total protein contentin control and different treated groups are provided in Table 2. AchE activity was significantly elevated in AD rats compared to control and resveratrol treated control rats. However, a significant reduction in AchE activities was noticed in AD rats treated with resveratrol before or after AD induction ($p \leq 0.05$). Interestingly, AD rats treated with ebixa also displayed a significantly reduced AchE activity compared to that in AD rats.These data indicate the inhibitory effect of resveratrol on AchE activity as evident by a decrease in enzyme activity in resveratrol treated rats.No significant change in total serum protein levels was observed in AD rats or in rats treated with resveratrol or ebixa compared to control rats.

Inflammatory markers

The level of inflammatory mediators in serum of control, AD and resveratrol treated groups are given in Table 3. The studied markers including IL-6, CRP, TNF-α and TGF-β were all significantly upregulated in $AlCl_3$ treated AD rats compared to control or resveratrol treated control rats, indicating the increased systemic inflammatory activity in AD rats presumably due to AD pathology. All these markers were significantly down modulated after resveratrol treatment either before or after the induction of AD. Rats treated with ebixa also showed a marked decrease in these markers.

Oxidative stress indices

Changes in oxidative stress markers in control, AD and resveratrol treated rats are given in Table 4. The MDA levels between control and resveratrol treated normal healthy rats were comparable suggesting the lack of any adverse effect of resveratrol on redox homeostasis. The MDA levels significantly elevated in AD rats compared to control or resveratrol treated control rats ($p \leq 0.05$) demonstrating the role of oxidative stress in AD pathogenesis. In rats fed with resveratrol before or after the induction of AD significantly truncated the MDA levels showing antioxidant capacity of resveratrol. In contrast, in AD rats, antioxidant markers, catalase and SOD activities were significantly down modulated, while in resveratrol treated rats, the activities of these enzymes were found to be significantly restored. These data indicate that resveratrol possesses the antioxidant potential to negate the cellular oxidative stress. Consistent with the effect of resveratrol, ebixa treatment also led to significant reduction in MDA and a significant increase in catalase and SOD activities.

Histological alterations

Histological changes in brain tissue of control, AD and resveratrol treated rats are presented in Figure 1. Control and resveratrol fed rats displayed normal histological features in cerebral cortex and hippocampus. Contrastingly AD rats demonstrated a significant nuclear pyknosis in hippocampus region and focal hemorrhage in striatum of cerebrum. In rats treated with resveratrol before the induction of AD, a significant congestion in blood vessels of cerebellum was noted, while rats fed with resveratrol after the induction of AD displayed normal histological structure of neuronal cells of hippocampus.

Table 1: Aβ, and tau protein levels in serum of control, AD and resveratrol treated rats

Parameters	C (N=8)	Res (N=8)	AD (N=8)	Res+AD (N=8)	AD+Res (N=8)	AD+Ex (N=8)
Aβ (pg/ml)	134.3±. 1.5	136.9±1.6	183.2±1.4**	148.2±0.6*	160.5±2.9*	155.0±2.3*
Tau protein (pg/ml)	17.7±0.5	24.5±0.8	66.4±1.0**	45.4±0.3*	50.6±0.8*	48.8±2.5*

Aβ; amyloid beta, Res; resveratrol, AD; Alzheimer's disease, Ex; ebixa. Each data point represents mean ± SE.*p<0.01, **p<0.001.

Table 2: Serum acetylcholine esterase and total protein levels in control and different treatedGroups

Parameters	C (N=8)	Res (N=8)	AD (N=8)	Res+AD (N=8)	AD+Res (N=8)	AD+Ex (N=8)
Serum AchE (U/mg)	38.4±1.1	35.8±2.3	61.4±2.4**	45.35±0.7*	56±2.3*	47.8±0.7*
Total serum protein	7.4±0.2	6.7±0.2	7.0±0.3	8.5±0.1	7.2±0.2	7.1±0.3

AchE; acetylcholine esterase, Res; resveratrol, AD; Alzheimer's disease, Ex; ebixa. Each data point is representative of 3 independent experiments, *p<0.01, **p<0.001. Each experiment represents mean ±SE.

Table 3: Serum inflammatory markers in control, AD and resveratrol treated rats

Parameters	Control (n=8)	Res (n=8)	AD (n=8)	Res+AD (n=8)	AD+Res (n=8)	AD+Ex (n=8)
CRP (µg/l)	387±25.0	303±22.3	632±52.6**	409±20.5*	514±23*	438.5±28*
IL-6 (ng/l)	145±20.6	149±21.3	178±25.6*	156±22*	168.5±4.1*	157.7±3.2*
TNF-α (ng/l)	13±.2.1	12.5±3.2	24.5±4.5**	18.0±2.8*	19.6±2.5*	20.3±3.4*
TGF-β (ng/l)	13.4±2.4	18.1±2.0	44.36±5.3**	25.23±4.3*	35.98±4.6*	33.7±3.8*

CRP; C-reactive protein, IL-6; interleukin-6, TNF-α; tumor necrosis factor-alpha, TGF-β; transforming growth factor-beta, Res; resveratrol, AD; Alzheimer's disease, Ex; ebixa, each data point presented as mean ±SE.*p<0.01, **p<0.001

Table 4: Oxidative stress markers of control, AD and resveratrol treated rats

Parameters	Control (n=8)	Res (n=8)	AD (n=8)	Res+AD (n=8)	AD+Res (n=8)	AD+Ex (n=8)
Catalase (U/g)	112±12.5	107±11.8	58±7.2**	84±8.6*	65±7.0*	72.5±8.7*
SOD (U/mg)	33.0±4.5	32.7±3.6	14.8±2.3**	27±4.7*	18.37±5.2*	25.3±6.1*
MDA (nmol/ml)	28.6±3.8	30 ±3.1	48.3±6.4**	34.5±3.2*	43±4.3*	40.4±4.6*

SOD; superoxide dismutase, MDA; malodialdehyde, Res; resveratrol, AD; Alzheimer's disease, Ex; ebixa, Mean ± SE *p<0.01, **p<0.001

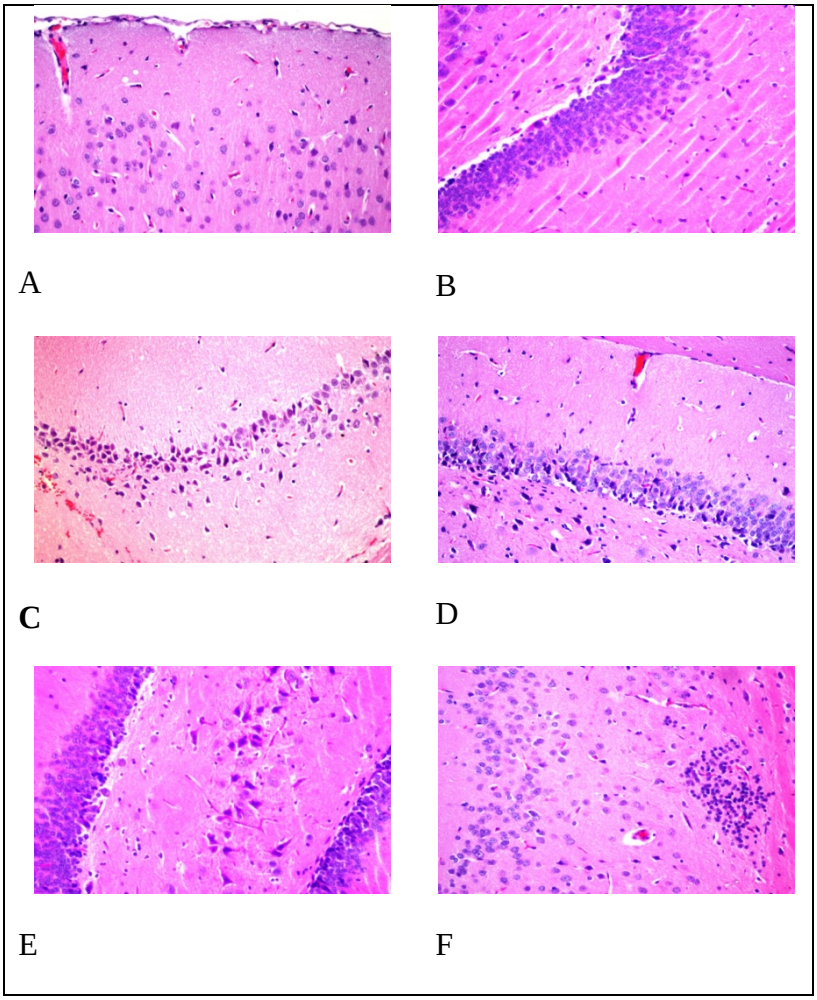

Figure 1: H&E (x 40) staining of brain tissues of control, AD and resveratrol treated rats. (A) showing normal histological structure of the meninges and cerbral cortex (B) A photomicrograph of brain tissue in healthy treated with resveratrol group showing normal histological structure of hippocampus. (C) A photomicrograph of brain tissue in AD induced group showing nuclear pyknosis in some neurons in hippocampus. (D) A photomicrograph of brain tissue in AD induced group and treated with resveratrol as protective effect (Resv + AD) showing nuclear pyknosis in some neurons in the hippocampus. (E) A photomicrograph of brain tissue in AD induced group s + Resveratrol (therapeutic) showing normal histological structure of neuronal cells of hippocampus. (F) A photomicrograph of brain tissue in AD induced group + Epixa showing focal gliosis in cerbral cortex.

DISCUSSION

In the present study, we evaluated the therapeutic and preventive potentials of resveratrol in AD rats. Disease specific biomarkers such as amyloid β (Aβ) and tau proteins, as well as oxidative stress and inflammatory indices were measured to examine the underlying mechanism involved in the protective effects of resveratrol. Aluminum chloride is an established chemical agent for the induction of pathological characteristics typical of AD (19, 20). Consistently, in the present study, rats treated with $AlCl_3$ exhibited significant increase in Aβ and tau protein levels, confirming the etiology of AD. In addition, these rats had significantly increased oxidative stress and inflammatory markers. These observations also support the major role of oxidative stress and inflammation in the pathogenesis of AD (5, 25). Further, we found a significant histopathological changes in the $AlCl_3$ treated rat brains confirming the AD etiology.

A number of studies have demonstrated the therapeutic and preventive potential of plant derived resveratrol against AD in human and animal systems. In our observation, we found that treatment of AD rats with resveratrol significantly blunted the Aβ and tau protein levels compared to untreated AD rats. Importantly, resveratrol was able to markedly reduce these pathological markers in both the experimental conditions where the rats were treated with resveratrol prior to the induction of AD and in the rats treated with resveratrol after the induction of AD. This clearly demonstrates that resveratrol is able to attenuate the progression of AD and also to prevent the onset of AD pathogenesis through the down modulating effects on Aβ, suggesting the therapeutic as well as preventive potential of resveratrol against AD. In contrast resveratrol exhibited no effect on Aβ and tau protein in normal healthy mice, where the levels of these molecules were comparable to those in untreated control mice. Further, resveratrol had no significant effects on total protein, body weight, and behavior of the animals, indicating the lack of any adverse effects of resveratrol on normal physiology and activities in the treated rats. Our findings are consistent with several studies where the resveratrol has shown to decline the Aβ peptide levels. For example, resveratrol significantly truncated both the intracellular and secreted Aβ levels in KEK293 and mouse neuroblastoma N2a cells (17). Importantly, the Aβ reducing effect of resveratrol was shown to be mediated by the degradation of Aβ by proteasome and the effect was reversed by the selective proteasomes inhibitors. Likewise, AD mice fed with clinically feasible doses of resveratrol showed reduction in the Aβ plaques in cortex, striatum and hypothalamus regions of brain (26). Resveratrol also lowered the Aβ levels in neuronal and non-neuronal cells including mouse primary neurons by activating AMP-activated protein kinase (AMPK) signaling (27). Consistently, in an age related AD mouse model resveratrol significantly declined the Aβ formation (28). Although studies indicated the reduction of tau protein hyper phosphorylation in resveratrol treated AD mice, none of the studies have investigated the effected of resveratrol on tau protein levels (28, 29). In the present study, we found that resveratrol significantly reduced the tau protein content of AD rats. As in the case of Aβ, resveratrol, whether treated before or after the induction of AD, was able to lower the tau protein levels. This suggests the effect of resveratrol on multiple molecular targets in the prevention and stalling the progression of AD.

It has been reported that neurotransmitter acetylcholine (Ach) levels are reduced in AD and this contributes to insufficient signal transmittance leading to AD pathology (30). The deficiency of ACh results due to its degradation by acetylcholine esterase (AChE), the activity of which has been shown to be upregulated in AD (31). Consistent with the modulating effects of AchE on Ach, the EchE inhibitors including donepezil, rivastigmine and galantamine are shown to reverse the effect of enzyme (32). To further explore the possible underlying mechanisms mediating the favorable effects of resveratrol, we examined for a possible down modulating effect of resveratrol on AChE. We found that there is a significant decrease in AChE activity. Thus the mechanism underlying the favorable effects of resveratrol may also involve its ability to inhibit the AchE activity which might result in the improved neurotransmission, possibly due to increased levels of neurotransmitter, acetylcholine. Interestingly, we found a significant decrease in AchE activity in rats treated with ebixa, which is a specific inhibitor of over activation of N-methyl-d-aspartate (NMDA)-type glutamate receptors in neurons. This observation needs further investigation.

A number of studies have examined the effect of resveratrol on oxidative stress and inflammation in the context of AD. For instance, rat hippocampal H19-7 neuronal cells treated with resveratrol exhibited a significant decrease in

Aβ induced lipid peroxidation and a significant increase in enzymatic and non-enzymatic antioxidants as well as the expression of memory associated proteins (33). Likewise, resveratrol treatment prevented inflammation, oxidative stress, and improved antioxidant status in AD rats (15, 34). Consistent with the above observations, we found a significant decline in MDA levels in resveratrol treated AD mice. Besides, resveratrol also augmented the levels of antioxidants including catalase and SOD in AD mice. We also found a significant down modulation of pro-inflammatory cytokines, IL-6, CRP, TNF-α and TGF-β in resveratrol treated AD mice. These data support the anti-inflammatory and antioxidant capacity of resveratrol. Histopathological analysis of brain tissue of rats treated with resveratrol either before or after the induction of AD revealed a significant restoration of tissue architecture as evident from the normal meninges and cerebral cortex.

Several possible mechanisms have been put forth in the neuroprotective effects of resveratrol in AD. For example, resveratrol has been shown to inhibit Aβ induced microglial activation via NADPH oxidase to attenuate inflammation (35). Resveratrol treatment of rat pheochromocytoma (PC-12) cells resulted in the reduction of cell apoptosis, stabilization of intercellular Ca (2+) homeostasis and restoration of Aβ suppressed silent information regulator 1 (SIRT1) activity, eventually to attenuate neurotoxicity (36). Resveratrol also inhibited the production of reactive oxygen species in PC12 cells to inhibit oxidative stress (16).

CONCLUSIONS

Collectively, in the present study we showed the neuroprotective capacity of resveratrol which appears to be mediated through its ability to suppress AchE activity which possibly resulted in the attenuation of Aβ, tau protein, oxidative stress and inflammation. To the best of our knowledge, this is the first study to simultaneously examine the multiple pathological makers as well as brain tissue architecture to establish the beneficial effects of resveratrol use in lowering the AD risk. The findings here will advance our understanding of the therapeutic and preventive mechanisms through which the resveratrol exerts its protection against AD.

Conflicts of interest

The Authors declare that there is no conflict of interest.

Ethical approval: All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

REFERENCES

1. H.W. Querfurth and F.M. LaFerla, “Alzheimers’ Disease”, N. Eng. J. Med., 362, 329-44 (2010).
2. J. Kalra and A. Khan, “Reducing Aβ load and tau phosphorylation: Emerging perspective for treating Alzheimer's disease”, Eur. J. Pharmacol., 764, 571-581 (2015).
3. X. Sun, W.D. Chen, and Y.D. Wang, “β-Amyloid: the key peptide in the pathogenesis of Alzheimer's disease”, Front. Pharmacol., 6, 221-227 (2015).
4. V.J. De-Paula, M. Radanovic, B.S. Diniz, and O.V. Forlenza, “Alzheimer's disease”, Subcell. Biochem., 65, 329-352 (2012).
5. A. Malhotra, S. Bath, and F. Elbarbry, “An Organ System Approach to explore the antioxidative, Anti-Inflammatory, and Cytoprotective Actions of Resveratrol”, Oxid. Med. Cell. Longev., 803971 (2015).
6. T.J. Montine, C.H. Phelps, T, G. Beach, et al., “National Institute on Aging; Alzheimer’s Association. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach”, ActaNeuropathol., 123, 1-11 (2012).
7. J.B. Toledo, J. Brettschneider, M. Grossman, et al., “CSF biomarkers cutoffs: the importance of coincident neuropathological diseases”, ActaNeuropathol., 124, 23-35 (2012).
8. H. Allain, D. Bentué-Ferrer, O. Tribut et al., “Alzheimer’s disease: the pharmacological pathway”, Fundam. Clin. Pharmacol.,17, 419-428 (2003).
9. S.A. Lipton, “The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism”, Curr. Alzheimer Res., 2, 155-165 (2005).
10. E. Tellone, A. Galtieri, B. Russo, et al., “Resveratrol: A Focus on Several Neurodegenerative Diseases”, Oxid. Med. Cell. Longev., 392169 (2015).

11. M. Venigalla, E. Gyengesi, and G. Münch, "Curcumin and Apigenin - novel and promising therapeutics against chronic neuroinflammation in Alzheimer's disease". Neural. Regen. Res., 10, 1181-1185 (2015).
12. S. Rigacci, "Olive Oil Phenols as Promising Multi-targeting Agents against Alzheimer's disease", Adv. Exp. Med. Biol., 863, 1-20 (2015).
13. M. Moniruzzaman, M. Asaduzzaman, M.S. Hossain, et al., "In vitro antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of Phyllanthusacidus", BMC Complement. Altern. Med., 15, 403-409 (2015).
14. Y. Okada and M. Okada, "Protective effects of plant seed extracts against amyloid β-induced neurotoxicity in cultured hippocampal neurons", J. Pharm. Bioallied Sci., 5,141-147 (2013).
15. H.F. Zhao, N. Li, Q. Wang, et al., "Resveratrol decreases the insoluble Aβ1-42 level in hippocampus and protects the integrity of the blood-brain barrier in AD rats" Neuroscience, 310, 641-649 (2015).
16. J.H. Jang and Y.J. Surh, "Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death" Free Radic. Biol. Med., 34, 1100-1110 (2003).
17. P. Marambaud, H. Zhao and P. Davies, "Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides", J. Biol. Chem., 280, 37377-373782 (2005).
18. S.W. Min, S.H. Cho, Y. Zhou, et al., "Acetylation of tau inhibits its degradation and contributes to tauopathy" Neuron, 67, 953-966 (2010).
19. B.V. Lakshmi, M. Sudhakar, and K.S. Prakash, "Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: behavioral and biochemical alterations in rats", Biol. Trace. Elem. Res., 165, 67-74 (2015).
20. A. Justin Thenmozhi, T.R. Raja, U. Janakiraman, and T. Manivasagam, "Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats" Neurochem. Res., 40, 767-76 (2015).
21. G.N. Krasovskiĭ, L.Y. Vasukovich, and O.G. Chariev, "Experimental study of biological effects of leads and aluminum following oral administration", Environ. Health Perspect. 30, 47-51 (1979).
22. D. Wang, T. Hang, C. Wu, and W. Liu, "Identification of the major metabolites of resveratrol in rat urine by HPLC-MS/MS", J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci., 829, 97-106 (2005).
23. S.W. Bihaqi, M. Sharma, A.P. Singh, and M. Tiwari, "Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain", J. Ethnopharmacol., 124, 409-415 (2009).
24. G.L. Ellman, K.D. Courtney, V. Andres Jr, and R.M. Feather-Stone, "A new and rapid colorimetric determination of acetylcholinesterase activity", Biochem. Pharmacol., 7, 88-95 (1961).
25. P. Agostinho, R.A. Cunha, and C. Oliveira, "Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease", Curr. Pharm. Des.,16, 2766-2778 (2010).
26. S.S. Karuppagounder, J.T. Pinto, H. Xu, et al., "Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease", Neurochem. Int., 54, 111-118 (2009).
27. V. Vingtdeux, L. Giliberto, H. Zhao, et al., "AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism" J. Biol. Chem. 285, 9100-9113 (2010).
28. D. Porquet, G. Casadesús, S. Bayod, et al., "Dietary resveratrol prevents Alzheimer's markers and increases life span in SAMP8", Age (Dordr), 35, 1851-1865 (2013).
29. L.L. Du, J.Z. Xie, X.S. Cheng, et al., "Activation of sirtuin 1 attenuates cerebral ventricular streptozotocin-induced tau hyperphosphorylation and cognitive injuries in rat hippocampi", Age (Dordr) 36, 613-623 (2014).
30. T. Rees, P.I. Hammond, H. Soreq, et al., "Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex", Neurobiol. Aging, 24, 777-787 (2003).
31. M.S. García-Ayllón, D.H. Small, J. Avila, J. Saez-Valero, "Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with P-tau and β-amyloid", Front. Mol. Neurosci. 4, 22-27 (2011).
32. M. Mehta, A. Adem, M.S. Kahlon, and M.N. Sabbagh, "The nicotinic acetylcholine receptor: smoking and Alzheimer's disease revisited", Front. Biosci., (Elite Ed) 4, 169-180 (2012).
33. S.D. Rege, T. Geetha, T.L. Broderick, and J.R. Babu, "Resveratrol protects β amyloid-induced oxidative damage and memory associated proteins in H19-7 hippocampal neuronal cells" Curr. Alzheimer Res., 12, 147-156 (2015).
34. H. Zhao, Q. Niu, X. Li, et al., "Long-term resveratrol consumption protects ovariectomized rats chronically treated with D-galactose from developing memory decline without effects on the uterus, Brain Res., 1467, 67-80 (2012).
35. Y. Yao, J. Li, Y. Niu, et al., "Resveratrol inhibits oligomeric Aβ-induced microglial activation via NADPH oxidase", Mol. Med. Rep. 12, 6133-6139 (2015).
36. X. Feng, N. Liang, D. Zhu, Q.Gao, et al., "Resveratrol inhibits β-amyloid-induced neuronal apoptosis through regulation of SIRT1-ROCK1 signaling pathway" PLoS One, 8, e59888 (2013).