ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Possible protective roles of tempol on amyloidosis and oxidative stress in experimentally induced-Alzheimer's like in mice

Mohammed Ragab Abdel-Aziz Ali[1], Amira Morad Hussein Abo-Youssef[1], Basim Anwar Shehata Messiha[1], Mahmoud Mohamed Khattab[2]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University

Manuscript Info

Manuscript History:

Received: 14 April 2015
Final Accepted: 15 May 2015
Published Online: June 2015

Key words:

Alzheimer's disease, β-amyloid, Oxidative stress, Lipopolysaccharide

Corresponding Author

Mohammed Ragab Abdel-Aziz Ali

Abstract

Alzheimer's disease is the most common cause of dementia where the loss of intellectual and social abilities is severe enough to interfere with daily functioning. This may be a warning sign to our economy as unfortunately, the disease is growing up especially in the developing countries . Deposition of β-amyloid in brain is one of the pathological hallmarks of AD that is often associated with oxidative stress response. In this study, we investigated the possible mechanisms of tempol (superoxide scavenger), in a lipopolysaccharide model of Alzheimer's disease. Mice were randomly divided into three groups each group consisted of 8 mice. Mice were injected with lipopolysaccharide (0.8 mg/kg, *i.p.*) once then divided into two groups: the first was remained injected with lipopolysaccharide only serving as Alzheimer's disease control and the second was injected with tempol (100 mg/kg/day, *i.p.*), in addition to the normal control mice injected with 1% tween 80. Brain oxidative stress markers namely malondialdehyde, reduced glutathione and superoxide dismutase in addition to β-amyloid levels were measured. Lipopolysaccharide increased oxidative stress burden and finally caused β-amyloid deposition in brain. These effects were reversed by using tempol. Tempol decreased parameters of Alzheimer's disease and oxidative markers serving as a good protective a gaint against Alzheimer's disease.

INTRODUCTION

As the population ages, Alzheimer disease (AD) is becoming more of a medical, social and public health issue. It is a massive neurodegenerative disorder that causes severe and permanent loss of intellectual function leading to complete incapability of even daily activities (Nowotny et al., 2001). The number of AD patients in the U.S. was presently about 4.5 million, with nearly 37 million worldwide (McKhann et al., 2011). By 2040 the worldwide number was predicted to reach 81 million with 4.6 million new patients diagnosed per year (Ferri et al., 2006).

There are two major mechanisms of AD; the amyloid cascade theory and the cholinergic hypothesis. β-amyloid peptide (Aβ) causes the neurodegeneration that lead to clinical AD (Kugaevskaya, 2011; Zhang et al., 2015). In the cholinergic theory, AD is also characterized by neuronal loss, especially those expressing nicotinic acetylcholine receptors (nAChR) (Buckingham et al., 2009; Lombardo and Maskos, 2014).

Today, the total view on AD progression is focused on the amyloid cascade theory, reporting that Aβ is the causative leading agent in AD and that mostly of the remaining AD characteristics are results of inappropriate deposition of the peptide (Ramberg, 2011). Moreover, Aβ (1-42) has become more interesting than Aβ (1-40), due to its higher ability to aggregate even in form of self aggregation and produce cytotoxic properties (Findeis, 2007; Ramberg, 2011). Aggregation of Aβ is thought to induce sequences of neurodegenerative actions, involving the intracellular accumulation of hyperphosphorylated tau (Hardy and Selkoe, 2002; Jack et al., 2013).

Deposition of Aβ in brain is also included in stimulation of oxidative stress pathway leading to generation of free radicals known as reactive oxygen species (ROS) through activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex which resulted in increase of malondialdehyde (MDA) as a product of increased amounts of lipid peroxidation due to increased oxygen free radicals in addition to decreasing defense enzymes against oxidative stress like reduced glutathione (GSH) and superoxide dismutase (SOD), these mechanisms caused pathological processes of AD which leads to direct cellular injury and death of neurons (Han et al., 2015; Hardy and Selkoe, 2002; Mota et al., 2015).

Release of ROS is also triggered via induction by extracellular iron and copper ions attachment to Aβ plaques (Collingwood et al., 2008; Graham et al., 2014). Microglial cells locate and attack Aβ plaques as immunological response, causing ROS formation and starting oxidative stress (Gallagher et al., 2012).

Oxidative stress was not only considered as a result of of Aβ deposition but also considered as a causative factor for Aβ deposition (Abdel Moneim, 2015; Nunomura et al., 2006). Oxidative stress is increasing with normal aging but it is further exaggerated in several neurodegenerative disorders such as AD and Parkinson's disease (Jomova et al., 2010). Several studies on how oxidative stress is triggering Aβ production have stated that oxidative stress is inhibiting the activity of alpha (α-secretase) enzyme involved in non amyloidegenic cleavage of APP while activating the expression of β- and γ -secretase, enzymes critical for the generation of Aβ from APP (Chen et al., 2008; Oda et al., 2010; Quiroz-Baez et al., 2009; Tamagno et al., 2002; Yoo et al., 2010).

Tempol, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl, is SOD mimetic, recognized to catalyze the detoxification of ROS, particularly superoxide (Mizera et al., 2015; Wilcox and Pearlman, 2008). Tempol was reported to cross the BBB (Zhelev et al., 2009) and has previously been shown to be neuroprotective as a free-radical scavenger in several models of brain injury and ischemia (Deng-Bryant et al., 2008; Kwon et al., 2003; Mustafa et al., 2010). It was selected in the present study based on its ROS-scavenging activity.

Lipopolysaccharides (LPS), gram negative bacterial endotoxin was reported to trigger production of ROS and also enhance permeability of BBB in rodents (Kitazawa et al., 2005; Takeda et al., 2013; Tweedie et al., 2012). LPS was found also to increase APP through activation of γ-secretase and β-secretase leading to Aβ (1-42) deposition in brain (Deng et al., 2014; Joshi et al., 2014; Lee et al., 2008). LPS was used for induction of AD like in the present study.

2. Material and methods

2.1. Material

Adult male mice weighing 22-25 g were purchased from the animal house of the National Research Center. Mice were kept in the laboratory animal center of the Faculty of Pharmacy, Beni-Suef University under a optimum temperature (22-23°C), on 12-hour light/dark cycles and supplied with standard chow and tap water *ad libitum*. The study was done according to the guidelines of the Ethics Committee, Faculty of Pharmacy, Beni-Suef University, which was under the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs, chemicals and reagent kits

Tempol, lipopolysaccharide (LPS), thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (MDA standard), 5,5`-dithio-bis-(2-nitrobenzoic acid) (DTNB), and GSH standard were purchased from Sigma Chemicals Co. (USA). Murine Aβ ELISA kits were obtained from MyBioSource Co. (China) and Murine superoxide dismutase (SOD) ELISA kits were obtained from Trevigen Co. (USA). All other chemicals, solvents and reagents used were obtained from certified sources and were of analytical grade.

2.3. Experimental design

Mice were randomly divided into 3 groups, namely a normal control group, an Alzheimer control group and a tempol treatment group. Tempol was injected through the *i.p.* route daily for seven days in a dose of 100 mg/kg/day (Hahn et al., 1992). twenty four hours later, after the last drug dose animals were sacrificed with brain tissue withdrawn as indicated for biochemical analysis.

2.4. Methodology

2.4.1. Alzheimer induction

Induction of AD was done by a single *i.p.* dose injection of LPS (0.8 mg/kg), in 1% tween 80 solution in saline, which causes Alzheimer symptoms after 7 days of injection (Arai et al., 2001; Sheng et al., 2003). Tempol was administered on a daily basis for seven days starting from the day of LPS injection.

2.4.2. Biochemical analysis

2.4.2.1. Alzheimer marker

The level of Aβ (1-42) in brain tissue was measured using ELISA diagnostic kit as described by manufacturer's instructions according to the principles described by Verges et al. (2011).

2.4.2.2. Oxidative stress markers

The level of SOD in brain tissue was measured using assay diagnostic kit as described by manufacturer's instructions according to the principles described by Robak and Gryglewski (1988)**.** The levels of MDA and GSH levels in brain tissue were assessed colorimetrically according to the principles of Ohkawa et al. (1979) and Ellman (1959), respectively.

2.4.3. Statistical analysis

All data are presented as means ± SEM (standard error of the mean). Statistical analysis was done using Statistical Package for Social Science (SPSS) software (version 22). One-way analysis of variance (ANOVA) test was used to detect significance among group means, followed by Tukey's post-hoc test for pair-wise comparison between means of groups. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Alzheimer marker

Injection of LPS to mice significantly showed increase of brain tissue level of Aβ compared with normal control values. Treatment of mice with tempol showed significant decrease of Aβ levels compared with LPS mice (Table 1) & (Figure 1).

3.2. Oxidative stress markers

Injection of mice with LPS showed significant decrease in SOD and GSH levels in addition to significant increase of MDA levels in brain tissue compared to normal control mice (Table 2) & (Figure 2,3 and 4 respectively). Treatment of mice with tempol showed significant increase in brain tissue SOD activity and GSH levels in addition to significant decrease of brain tissue MDA levels compared with LPS mice.

Table (1): Effect of tempol on brain tissue Alzheimer markers in mice with LPS-induced AD

Group / Parameter	Normal Control	LPS Control	Tempol (100 mg/kg)
Aβ (pg/g tissue)	2.04 ± 0.14	14.10 ± 0.45[a]	5.53 ± 0.22[ab]

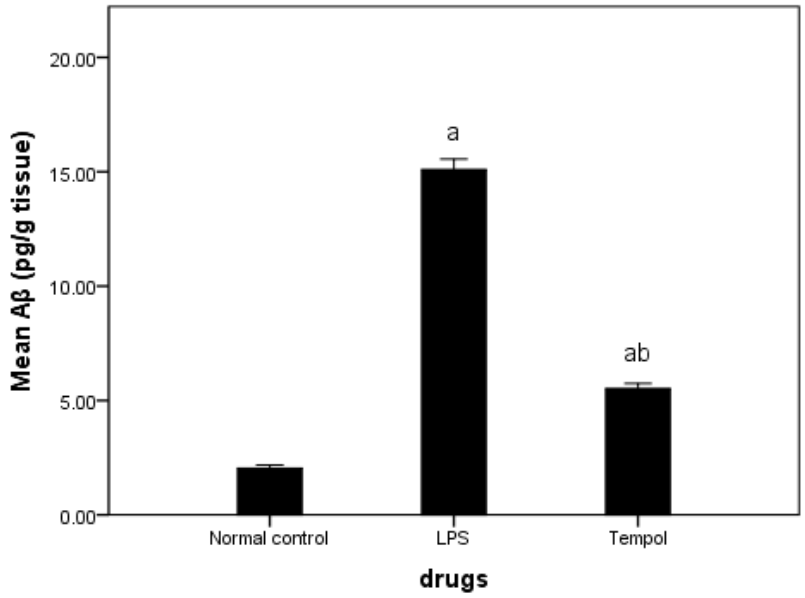
Each value represents a mean of 8 values ± SEM.
[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
Aβ: beta amyloid, AD: Alzheimer's disease, LPS: lipopolysaccharide, SEM: standard error of the mean.

Table (2): Effect of tempol on brain tissue oxidative stress markers in mice with LPS-induced AD

Group / Parameter	Normal Control	LPS Control	Tempol (100 mg/kg)
SOD (U/g tissue)	7.13 ± 0.38	2.40 ± 0.73[a]	4.63 ± 0.21[ab]
MDA (nmol/g tissue)	10.40 ± 0.61	65.49 ± 2.67[a]	26.56 ± 1.11[ab]
GSH (µmol/g tissue)	4.51 ± 0.24	0.73 ± 0.03[a]	1.37 ± 0.05[ab]

Each value represents a mean of 8 values ± SEM.
[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
AD: Alzheimer's disease, GSH: glutathione reduced, LPS: lipopolysaccharide, MDA: malondialdehyde, lipopolysaccharide, SEM: standard error of the mean, SOD: superoxide dismutase.

Figure (1): Effect of tempol on brain tissue Alzheimer markers Aβ in mice with LPS-induced AD



Each bar represents a mean of 8 values ± SEM.
[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
Aβ: beta amyloid, AD: Alzheimer's disease, LPS: lipopolysaccharide, SEM: standard error of the mean.

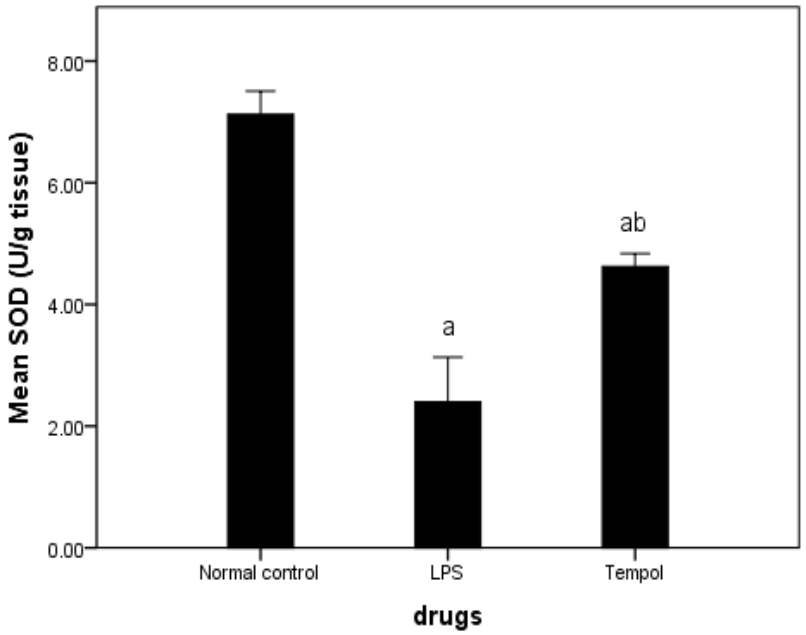
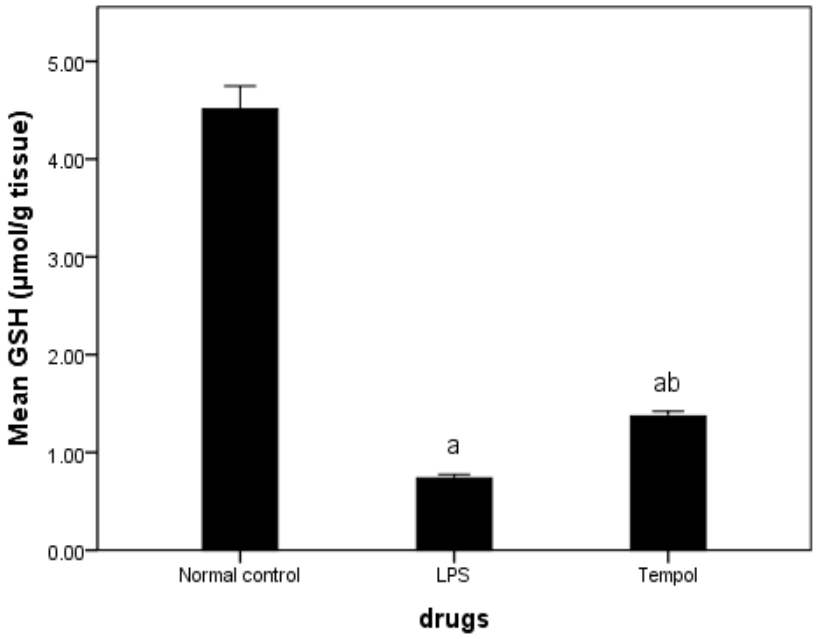


Figure (2): Effect of tempol on brain tissue SOD in mice with LPS-induced AD
Each bar represents a mean of 8 values ± SEM.

[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
AD: Alzheimer's disease, LPS: lipopolysaccharide, lipopolysaccharide, SEM: standard error of the mean, SOD: superoxide dismutase.

Figure (3): Effect of tempol on brain tissue GSH in mice with LPS-induced AD



Each bar represents a mean of 8 values ± SEM.
[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
AD: Alzheimer's disease, GSH: glutathione reduced, LPS: lipopolysaccharide, lipopolysaccharide, SEM: standard error of the mean.

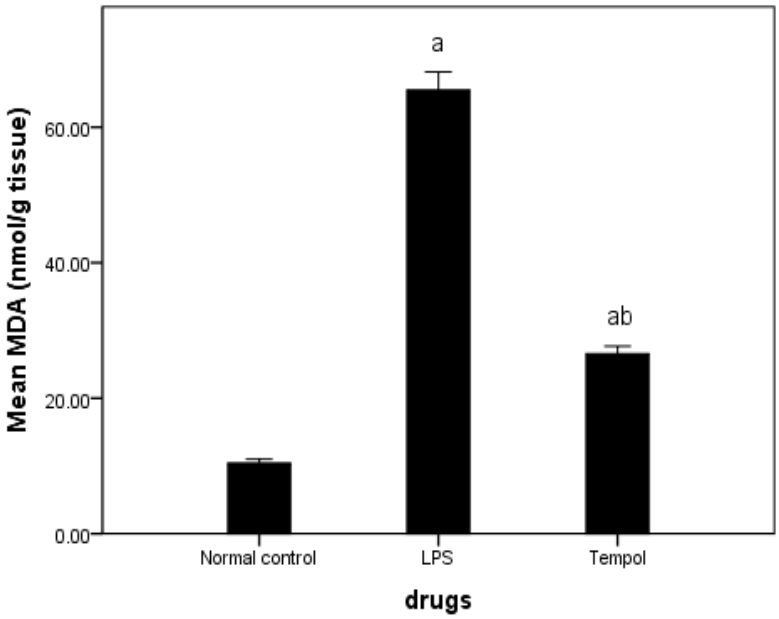


Figure (4): Effect of tempol on brain tissue MDA in mice with LPS-induced AD
Each bar represents a mean of 8 values ± SEM.
[a]Significantly different from normal control value at $p < 0.05$.
[b]Significantly different from LPS control value at $p < 0.05$.
AD: Alzheimer's disease, LPS: lipopolysaccharide, MDA: malondialdehyde, lipopolysaccharide, SEM: standard error of the mean

4. Discussion

The aim of the present work is to investigate the possible protective roles of tempol in management of AD and understand its underlying mechanisms.

Results of the present study showed that single dose of LPS adminstration caused a significant deposition of Aβ in brain tissues of mice (Table 1, Figure 1). In agreement, Jaeger et al. (2009) stated that LPS injection to experimental rodents enhanced Aβ deposition in brain through affecting brain permeability or through pro-inflammatory potential and ROS release as a result.

Tempol showed significant decline in Aβ compared with LPS mice (Table 1, Figure 1). Little research results are available regarding the effect of tempol on AD, but Rao et al. (2013) revealed that tempol could suppress expression of the matrix proteins thrombopondins in cultured astrocytes, while Han et al. (2015) reported that tempol could decrease cerebral amyloid angiopathy and Aβ deposition in mice through ROS scavenging activity.

The oxidative stress in brain tissue evidenced in the present study by the significant decreased levels of SOD (Table 2, Figure 2) and GSH (Table 2, Figure 3) coupled with significant increased levels of MDA (Table 2, Figure 4) in brain upon administration of mice with LPS compared to normal control mice. This is in agreement with the studies that was reported by Dubinina et al. (2015) and Sharma et al. (2014).

High oxidative stress burden including elevated levels of MDA in addition to decreased levels of the protective defense antioxidant enzymes SOD and GSH was demonstrated to be as a result of Aβ deposition (Kim et al., 2015) and also as a pathogenic factor enhancing neuronal damage and AD progression (Dubinina et al., 2015).

As we mentioned before oxidative stress was once claimed not only to be a result of Aβ deposition but also a causative factor. So, exposure of the brain to oxidative stress, caused by LPS, may result in Aβ formation as a compensatory response and vice versa (Abdel Moneim, 2015; Nunomura et al., 2006).

Regarding oxidative stress markers in the present study, tempol as SOD mimetic significantly increased SOD (Table 2, Figure 2) activity and GSH levels (Table 2, Figure 3), coupled with decreased MDA levels (Table 2, Figure 4) compared with LPS control mice, indicating amelioration of oxidative stress. The results of tempol was in agreement with the studies conducted by Luan et al. (2012) who reported effects on MDA, GSH and SOD.

As mentioned above, oxidative stress played a significant pathogenic roles in neuronal damage and AD progression, and this may explain, at least partly, the protective effect of tempol in AD (Dubinina et al., 2015).

Support of antioxidant status was evidenced by many authors to suppress AD progression in different animal models (Braidy et al., 2015; Leirós et al., 2015; Persichilli et al., 2015).

So the present study focused on relation between oxidative stress and Aβ in AD to evaluate novel therapies as tempol as a protective antioxidant in AD as the current standard therapies of AD did not achieve a great progress in this field, so the need for new strategies for dealing with AD had grown up in the last years. This study which is in our hand provides a new approach for dealing with AD, a study which needs further clinical trials for confirmation of our study.

5. Conclusion

In conclusion, tempol is a promising protective agent against AD progression able to suppress Aβ deposition and ameliorate oxidative stress markers as MDA in addition to enhancement of defense protective antioxidant enzymes as SOD and GSH.

6. References

Abdel Moneim, A.E. (2015). Oxidant/Antioxidant Imbalance and the Risk of Alzheimer's Disease. Current Alzheimer Research. 12**,** 335-349.

Arai, K., Matsuki, N., Ikegaya, Y., Nishiyama, N. (2001). Deterioration of spatial learning performances in lipopolysaccharide-treated mice. The Japanese Journal of Pharmacology. 87**,** 195-201.

Braidy, N., Zarka, M., Welch, J., Bridge, W. (2015). Therapeutic Approaches to Modulating Glutathione Levels as a Pharmacological Strategy in Alzheimer's Disease. Current Alzheimer Research. 12**,** 298-313.

Buckingham, S.D., Jones, A.K., Brown, L.A., Sattelle, D.B. (2009). Nicotinic acetylcholine receptor signalling: roles in Alzheimer's disease and amyloid neuroprotection. Pharmacological reviews. 61**,** 39-61.

Chen, L., Na, R., Gu, M., Richardson, A., Ran, Q. (2008). Lipid peroxidation up- regulates BACE1 expression in vivo: a possible early event of amyloidogenesis in Alzheimer's disease. Journal of neurochemistry. 107**,** 197-207.

Collingwood, J.F., Chong, R.K., Kasama, T., Cervera-Gontard, L., Dunin-Borkowski, R.E., Perry, G., Posfai, M., Siedlak, S.L., Simpson, E.T., Smith, M.A. (2008). Three-dimensional tomographic imaging and characterization of iron compounds within Alzheimer's plaque core material. Journal of Alzheimer's Disease. 14**,** 235-245.

Deng-Bryant, Y., Singh, I.N., Carrico, K.M., Hall, E.D. (2008). Neuroprotective effects of tempol, a catalytic scavenger of peroxynitrite-derived free radicals, in a mouse traumatic brain injury model. Journal of Cerebral Blood Flow & Metabolism. 28**,** 1114-1126.

Deng, X., Meili Li, W.A., He, L., Lu, D., Patrylo, P.R., Cai, H., Luo, X., Li, Z., Yan, X. (2014). Lipolysaccharide-induced neuroinflammation is associated with Alzheimer-like amyloidogenic axonal pathology and dendritic degeneration in rats. Advances in Alzheimer's disease. 3**,** 78.

Dubinina, E., Schedrina, L., Neznanov, N., Zalutskaya, N., Zakharchenko, D. (2015). [Oxidative stress and its effect on cells functional activity of alzheimer's disease]. Biomeditsinskaia khimiia. 61**,** 57-69.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 82**,** 70-77.

Ferri, C.P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., Hall, K., Hasegawa, K., Hendrie, H., Huang, Y. (2006). Global prevalence of dementia: a Delphi consensus study. The Lancet. 366**,** 2112-2117.

Findeis, M.A. (2007). The role of amyloid β peptide 42 in Alzheimer's disease. Pharmacology & therapeutics. 116**,** 266-286.

Gallagher, J.J., Finnegan, M.E., Grehan, B., Dobson, J., Collingwood, J.F., Lynch, M.A. (2012). Modest amyloid deposition is associated with iron dysregulation, microglial activation, and oxidative stress. Journal of Alzheimer's disease. 28**,** 147-161.

Graham, S.F., Nasaruddin, M.B., Carey, M., Holscher, C., McGuinness, B., Kehoe, P.G., Love, S., Passmore, P., Elliott, C.T., Meharg, A.A. (2014). Age-associated changes of brain copper, iron, and zinc in Alzheimer's disease and dementia with Lewy bodies. Journal of Alzheimer's Disease. 42**,** 1407-1413.

Hahn, S.M., Tochner, Z., Krishna, C.M., Glass, J., Wilson, L., Samuni, A., Sprague, M., Venzon, D., Glatstein, E., Mitchell, J.B. (1992). Tempol, a stable free radical, is a novel murine radiation protector. Cancer research. 52**,** 1750-1753.

Han, B.H., Zhou, M.-l., Johnson, A.W., Singh, I., Liao, F., Vellimana, A.K., Nelson, J.W., Milner, E., Cirrito, J.R., Basak, J. (2015). Contribution of reactive oxygen species to cerebral amyloid angiopathy, vasomotor dysfunction, and microhemorrhage in aged Tg2576 mice. Proceedings of the National Academy of Sciences. 112**,** E881-E890.

Hardy, J., Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 297**,** 353-356.

Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D. (2013). Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. The Lancet Neurology. 12**,** 207-216.

Jaeger, L.B., Dohgu, S., Sultana, R., Lynch, J.L., Owen, J.B., Erickson, M.A., Shah, G.N., Price, T.O., Fleegal-Demotta, M.A., Butterfiled, D.A. (2009). Lipopolysaccharide alters the blood–brain barrier transport of amyloid β protein: A mechanism for inflammation in the progression of Alzheimer's disease. Brain, behavior, and immunity. 23**,** 507-517.

Jomova, K., Vondrakova, D., Lawson, M., Valko, M. (2010). Metals, oxidative stress and neurodegenerative disorders. Molecular and cellular biochemistry. 345**,** 91-104.

Joshi, Y.B., Giannopoulos, P.F., Chu, J., Praticò, D. (2014). Modulation of lipopolysaccharide-induced memory insult, γ-secretase, and neuroinflammation in triple transgenic mice by 5-lipoxygenase. Neurobiology of aging. 35**,** 1024-1031.

Kim, E.-A., Cho, C., Kim, D., Choi, S., Huh, J.-W., Cho, S.-W. (2015). Antioxidative effects of ethyl 2-(3-(benzo [d] thiazol-2-yl) ureido) acetate against amyloid β-induced oxidative cell death via NF-κB, GSK-3β and β-catenin signaling pathways in cultured cortical neurons. Free radical research. 49**,** 411-421.

Kitazawa, M., Oddo, S., Yamasaki, T.R., Green, K.N., LaFerla, F.M. (2005). Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. The Journal of neuroscience. 25**,** 8843-8853.

Kugaevskaya, E. (2011). Angiotensin Converting Enzyme and Alzheimer's Disease. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry. 6**,** 11-22.

Kwon, T.-H., Chao, D.L., Malloy, K., Sun, D., Alessandri, B., Bullock, M.R. (2003). Tempol, a novel stable nitroxide, reduces brain damage and free radical production, after acute subdural hematoma in the rat. Journal of neurotrauma. 20**,** 337-345.

Lee, J.W., Lee, Y.K., Yuk, D.Y., Choi, D.Y., Ban, S.B., Oh, K.W., Hong, J.T. (2008). Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation. 5**,** 37.

Leirós, M., Alonso, E., Rateb, M.E., Houssen, W.E., Ebel, R., Jaspars, M., Alfonso, A., Botana, L.M. (2015). Gracilins: Spongionella-derived promising compounds for Alzheimer disease. Neuropharmacology. 93**,** 285-293.

Lombardo, S., Maskos, U. (2014). Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. Neuropharmacology.

Luan, J., Li, W., Han, J., Zhang, W., Gong, H., Ma, R. (2012). Renal protection of in vivo administration of tempol in streptozotocin-induced diabetic rats. Journal of pharmacological sciences. 119**,** 167.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Jr, C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R. (2011). The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & Dementia. 7**,** 263-269.

Mizera, R., Hodyc, D., Herget, J. (2015). ROS scavenger decreases basal perfusion pressure, vasoconstriction and NO synthase activity in pulmonary circulation during pulmonary microembolism. Physiological research/Academia Scientiarum Bohemoslovaca.

Mota, S.I., Costa, R.O., Ferreira, I.L., Santana, I., Caldeira, G.L., Padovano, C., Fonseca, A.C., Baldeiras, I., Cunha, C., Letra, L. (2015). Oxidative stress involving changes in Nrf2 and ER stress in early stages of Alzheimer's disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1852**,** 1428-1441.

Mustafa, A.G., Singh, I.N., Wang, J., Carrico, K.M., Hall, E.D. (2010). Mitochondrial protection after traumatic brain injury by scavenging lipid peroxyl radicals. Journal of neurochemistry. 114**,** 271-280.

Nowotny , P., Kwon, J.M., Goate, M, A. (2001). Alzheimer Disease. eLS.

Nunomura, A., Castellani, R.J., Zhu, X., Moreira, P.I., Perry, G., Smith, M.A. (2006). Involvement of oxidative stress in Alzheimer disease. Journal of Neuropathology & Experimental Neurology. 65**,** 631-641.

Oda, A., Tamaoka, A., Araki, W. (2010). Oxidative stress up- regulates presenilin 1 in lipid rafts in neuronal cells. Journal of neuroscience research. 88**,** 1137-1145.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 95**,** 351-358.

Persichilli, S., Gervasoni, J., Di Napoli, A., Fuso, A., Nicolia, V., Giardina, B., Scarpa, S., Desiderio, C., Cavallaro, R.A. (2015). Plasma Thiols Levels in Alzheimer's Disease Mice under Diet-Induced Hyperhomocysteinemia: Effect of S-Adenosylmethionine and Superoxide-Dismutase Supplementation. Journal of Alzheimer's disease: JAD. 44**,** 1323-1331.

Quiroz-Baez, R., Rojas, E., Arias, C. (2009). Oxidative stress promotes JNK-dependent amyloidogenic processing of normally expressed human APP by differential modification of α-, β-and γ-secretase expression. Neurochemistry international. 55**,** 662-670.

Ramberg, V. (2011). Neuroinflammation in Alzheimer's disease: Focus on NF-κB and C/EBP transcription factors.

Rao, K.V.R., Curtis, K.M., Johnstone, J.T., Norenberg, M.D. (2013). Amyloid-β inhibits thrombospondin 1 release from cultured astrocytes: effects on synaptic protein expression. Journal of Neuropathology & Experimental Neurology. 72**,** 735-744.

Robak, J., Gryglewski, R.J. (1988). Flavonoids are scavengers of superoxide anions. Biochemical pharmacology. 37**,** 837-841.

Sharma, N., Kapoor, M., Nehru, B. (2014). Spinacea oleracea l. extract protects against LPS induced oxidative stress, inflammatory response and ensuing biochemical, neurochemical and neurobehavioral impairment in mice. International Journal of Pharmacy & Pharmaceutical Sciences. 6.

Sheng, J.G., Bora, S.H., Xu, G., Borchelt, D.R., Price, D.L., Koliatsos, V.E. (2003). Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid β peptide in APPswe transgenic mice. Neurobiology of disease. 14**,** 133-145.

Takeda, S., Sato, N., Ikimura, K., Nishino, H., Rakugi, H., Morishita, R. (2013). Increased blood–brain barrier vulnerability to systemic inflammation in an Alzheimer disease mouse model. Neurobiology of aging. 34**,** 2064-2070.

Tamagno, E., Bardini, P., Obbili, A., Vitali, A., Borghi, R., Zaccheo, D., Pronzato, M.A., Danni, O., Smith, M.A., Perry, G. (2002). Oxidative stress increases expression and activity of BACE in NT 2 neurons. Neurobiology of disease. 10**,** 279-288.

Tweedie, D., Ferguson, R.A., Fishman, K., Frankola, K.A., Van Praag, H., Holloway, H.W., Luo, W., Li, Y., Caracciolo, L., Russo, I. (2012). Tumor necrosis factor-alpha synthesis inhibitor 3, 6′-dithiothalidomide attenuates

markers of inflammation, Alzheimer pathology and behavioral deficits in animal models of neuroinflammation and Alzheimer's disease. J Neuroinflammation. 9**,** 163-177.

Verges, D.K., Restivo, J.L., Goebel, W.D., Holtzman, D.M., Cirrito, J.R. (2011). Opposing synaptic regulation of amyloid-β metabolism by NMDA receptors in vivo. The Journal of Neuroscience. 31**,** 11328-11337.

Wilcox, C.S., Pearlman, A. (2008). Chemistry and antihypertensive effects of tempol and other nitroxides. Pharmacological reviews. 60**,** 418-469.

Yoo, M.-H., Gu, X., Xu, X.-M., Kim, J.-Y., Carlson, B.A., Patterson, A.D., Cai, H., Gladyshev, V.N., Hatfield, D.L. (2010). Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage and in Alzheimer's disease. Antioxidants & redox signaling. 12**,** 819-827.

Zhang, X.-Y., Cao, J.-B., Zhang, L.-M., Li, Y.-F., Mi, W.-D. (2015). Deferoxamine attenuates lipopolysaccharide-induced neuroinflammation and memory impairment in mice. Journal of neuroinflammation. 12**,** 20.

Zhelev, Z., Bakalova, R., Aoki, I., Matsumoto, K.-i., Gadjeva, V., Anzai, K., Kanno, I. (2009). Nitroxyl Radicals for Labeling of Conventional Therapeutics and Noninvasive Magnetic Resonance Imaging of Their Permeability for Blood− Brain Barrier: Relationship between Structure, Blood Clearance, and MRI Signal Dynamic in the Brain. Molecular pharmaceutics. 6**,** 504-512.